Russian Journal of Ecology, Vol. 34, No. 6, 2003, pp. 381–388. Translated from Ekologiya, No. 6, 2003, pp. 420–427.

Microscale Spatial Variation in Forest Litter Phytotoxicity

E. L. Vorobeichik and V. N. Pozolotina

Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences, ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia

Received January 26, 2003

Abstract—The spatial variation (within a 100×100 m plot) in the pollution of forest litter with heavy metals (Cu, Cd, Pb, and Zn), its acidity, and phytotoxicity (measured by the results of the root test using seedlings from a genetically homogeneous sample of common dandelion (*Taraxacum officinale* s.l.) have been estimated. Forest litter has been sampled in three zones differing in the toxic impact of long-term polymetal pollution by emissions from a copper-smelting plant emissions in the Middle Urals. The phytotoxicity variation is maximum in a moderately polluted plot, where both very high and very low pollution levels were observed, which determines a substantially nonlinear dose–effect relationship. The litter phytotoxicity is mainly accounted for by exchangeable forms of metals. Biological testing of samples from the most polluted plot has demonstrated marked antagonism between heavy metals and acidity.

Key words: heavy metals, acidity, forest litter, industrial pollution, biological testing, phytotoxicity, common dandelion, spatial variation, dose–effect relationships, the Middle Urals.

Heavy-metal toxicity in soil and forest litter may be modified by many factors, the most important of which are acidity, the concentrations of organic matter and clay particles, and the cation-exchange capacity (Alva *et al.*, 2000; Tyler and Olsson, 2001a, 2001b; Ladonin, 2002). Therefore, if the gross contents of metals in different localities are the same, their bioavailabilities and toxicities in these localities will substantially depend on the spatial variation in the aforementioned factors. Thus, the gross contents of heavy metals is uninformative in terms of the prediction of their deleterious effects on the biota. Data on the modification of pollutant toxicity have mainly been obtained in laboratory experiments, which demonstrated that the toxicity of metals for plants, animals, and microorganisms may vary within a range of several orders of magnitude, depending on the values of particular modifying factors (Kapustka *et al.*, 1995; Redente *et al.*, 1996). A question arises as to whether, and to what extent, the effects observed in laboratory experiments occur in natural ecosystems; in other words, whether the variation ranges of the values of modifying factors within relatively small areas are sufficiently wide to considerably affect the soil and litter toxicity.

In this study, we attempted to answer these questions. For this purpose, we studied a microscale (within an area of about 1 ha) spatial variation in the heavy-metal contamination, acidity, and phytotoxicity (estimated by plant growth responses) of the forest litter. Biological testing allowed us to estimate the combined effect of the numerous and widely varying natural factors (some of which might have opposite effects) and "automatically" detect the most important interactions.

MATERIAL AND METHODS

The substrate for biological testing was sampled near the Middle Ural Copper-smelting Plant (the town of Revda, Sverdlovsk oblast) located in the southern taiga zone of the Middle Urals. The main components of the smelter discharges were SO_2 and suspended polymetal particles (mainly consisting of the compounds of Cu, Pb, Cd, Zn, and As). Long-term pollution (beginning from 1940) has led to considerable acidification of the forest litter and an increase in its metal content (Table 1). Earlier, we described the regular patterns of the technogenic transformation of forest ecosystems in this region (Vorobeichik *et al.*, 1994).

Forest litter was sampled in green-moss spruce–fir forests on gray forest soils located in the background (20 km west of the smelter), buffer (4.5 km), and impact (1 km) zones of toxic contamination. In each zone, we set a 100×100 m plot divided into 25 cells by a square grid. The litter was sampled from 15×15 spots throughout the depth of the litter at the nodes of the grid. It was ground using a mechanical grinder and sifted through a sieve with a mesh size of 2 mm. The samples were kept in air-tight polyethylene packs in the laboratory before analysis. A total of 75 litter samples were analyzed.

The concentrations of heavy metals (Cu, Pb, Cd, and Zn) were measured using an AAS-3 atomic absorption spectrophotometer (Carl Zeiss) in extracts with a substrate to extracting agent ratio of 1 : 10 (the duration of extraction was one day). We used two extracting agents: 5% HNO_3 and 0.05 M $CaCl_2$. The former agent extracted the fraction of movable forms of metals,

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Table 1. Actual acidity of forest litter (pH) and contents of heavy metals (μg/g) in different zones of toxic contamination (25 samples were analyzed in each zone)

Element	Toxic contamination zone		
	background	buffer	impact
pH	5.36 ± 0.07	4.50 ± 0.04	4.03 ± 0.09
		Movable forms	
Cd	4.48 ± 0.21	8.84 ± 0.70	8.98 ± 0.94
Cu	113.23 ± 6.86	1886.62 ± 126.78	6107.66 ± 373.87
Pb	103.50 ± 6.34	664.82 ± 42.36	1438.52 ± 62.04
Zn	356.14 ± 18.21	475.29 ± 34.87	662.57 ± 71.31
		Exchangeable forms	
Cd	0.75 ± 0.06	3.83 ± 0.31	4.31 ± 0.43
Cu	4.55 ± 0.44	70.88 ± 5.97	622.97 ± 43.30
Pb	0.61 ± 0.08	4.51 ± 0.47	13.64 ± 1.50
Zn	37.22 ± 1.99	155.86 ± 13.47	280.55 ± 20.80

which, in the given case, is correlated with their gross content; and the latter agent extracts the pool of exchangeable forms (Ladonin, 2002). The acidity of the litter was measured in a water extract (with a litter to distilled water ratio of 1 : 25) using an ionometer.

We used seedlings of the common dandelion (*Taraxacum officinale* s.l.) as a test object for the estimation of toxicity. This plant is a perennial grassy polycarpous plant from the family Asteraceae. Common dandelion usually reproduces via unreduced parthenogenesis without pseudogamy (Poddubnaya-Arnol'di, 1976; Ermakova, 1990); i.e., embryos are developed from unfertilized, unreduced ova and do not carry paternal chromosomes. Therefore, the seed offspring of one plant is actually a clone or pure line. The use of this genetically homogeneous material makes it possible to minimize the individual (genetically determined) variation. The dandelion is sensitive to heavy metals (Pozolotina *et al.*, 2000). This species was absent in natural plant communities of the impact and buffer zones. For experiments, we used a sample of seeds of the third generation obtained from a common ancestor (the second generation was grown in a greenhouse under optimal conditions).

The toxicity of the forest litter was estimated using the root test, in which the relative growth rates of seedlings growing on substrates with different contamination levels are measured (*Rasteniya*…, 1983). Dandelion seeds were germinated by the roll-culture method in jars containing a suspension of litter in distilled water at a ratio 1 : 10 (the first experiment) or 1 : 50 (the second experiment). Each litter sample was tested once. The plants were cultivated for two weeks at a constant temperature under artificial illumination. Water was added to the jars daily to maintain the original litter concentration. At the end of the experiment, we measured the length of the root of each seedling to an accuracy of 1 mm and recorded the presence and type of necroses. We compared the lengths of the seedling roots in experiments with litter samples from the impact, buffer, and background (control) zones to estimate the suppression of growth, which indirectly reflected the substrate phytotoxicity. To obtain additional information, we determined the proportion of seedlings with necroses (with the experimental jar serving as a replicate). Individual seedlings served as replicates when analyzing frequency distributions; and the group of seedlings in one jar, when performing correlation and regression analyses. The sample sizes were 825, 768, and 736 seedlings for the background, buffer, and impact zones in the first experiment and 1466, 1358, and 1301 seedlings in the second experiment, respectively.

RESULTS AND DISCUSSION

The contamination of the forest litter close to the source of discharges estimated by the total pool of movable forms of metals was 2 times (Cd and Zn) to 50 times (Cu) higher than in the background zone; the contamination of these zones with exchangeable forms of metals differed even more, namely, from 6–7 times (Cd and Zn) to 137 times (Cu) (Table 1). The contents of exchangeable forms of metals 20 km away from the smelter was increased by factors of 1.2, 2.1, 2.7, and 3.0 for Zn, Cd, Cu, and Pb, respectively, compared to the mean regional background values (Vorobeichik, 2003) and by factors of 2.4, 4.0, 4.8 and 6.0, respectively, compared to the minimum background values. Therefore, the background plot used in this study may be considered to be moderately contaminated, compared to the regional background.

In the background zone, the exchangeable forms of metals constituted a small percentage of movable

Table 2. Indices of forest litter contamination with heavy metals (1, movable forms; 2, exchangeable forms) and concentrations of hydrogen atoms in it (μg/l) in different zones of toxic contamination

Parameter	Index 1			Index 2			H^+		
	background	buffer	impact	background	buffer	impact	background	buffer	impact
Arithmetic mean	1.72	10.66	28.59	2.12	18.76	103.62	5.84	34.64	122.24
Minimum	1.12	5.99	20.60	1.00	10.41	19.38	1.38	8.13	3.31
Maximum	2.69	21.24	50.88	3.54	41.88	183.96	23.99	79.43	295.12
Variation coefficient, %	21.35	30.68	26.10	29.99	40.70	34.22	87.97	46.27	58.25

forms, namely, 0.6, 4, 10, and 17% for Pb, Cu, Zn, and Cd, respectively. In contaminated zones, these proportions remained the same for some metals; however, the absolute proportions of exchangeable forms, especially those of zinc and cadmium, were increased. These proportions were 0.9, 10, 42, and 48% for Pb, Cu, Zn, and Cd, respectively, in the impact zone and 0.6, 4, 33, and 43%, respectively, in the buffer zone. The litter acidity in the impact zone was almost 1.5 pH higher than in the background zone.

When we pooled data for all zones into one sample, we found strong positive correlations between the contents of some elements. The linear correlation coefficients for the pairs Cu–Pb and Cd–Zn were 0.92 and 0.90, respectively; for other combinations, the correlation coefficients varied from 0.43 to 0.65 (in all cases, $p \ll 0.001$). Therefore, it was correct to use the aggregation index of toxic contamination to compact the information on the contamination of the substrate:

$$K_i = \frac{1}{n}\sum_{j=1}^{n}\frac{C_{ij}}{C_{jf}},$$

where K_i is the contamination index of the ith point, C_{ji} is the concentration of the jth element in the ith point, C_{jf} is the minimum concentration of the jth element in the background zone, and n is the number of elements analyzed. The index is the factor by which the average contamination with all metals is increased, compared to the background level. This index most strongly correlates with the concentrations of copper and lead (the correlation coefficients are 0.94–0.99, $p \ll 0.001$), which indicates that these metals made the greatest contribution into its variation.

The contamination indices calculated from the concentrations of movable and exchangeable forms close to the contamination source were 16.6 and 48.9 times higher than the respective background values. The variations in the contamination index and hydrogen ion concentration were maximum in the impact zone (Table 2). The maximum concentration of hydrogen ions and contamination indices for exchangeable and movable forms of metals were 89.2, 9.5, and 2.5 times higher than their respective minimum values. In the buffer and background zones, the contamination index varied less (the maximum-to-minimum ratio was 2.4–4.0), whereas the variation in hydrogen ion concentration remained considerable (the maximum-to-minimum ratio was 9.7–17.4). The variation coefficients of toxic contamination indices characterizing the average variations for all parts of frequency distributions, rather than their extreme values, were maximum in the buffer zone, where they were 1.4 times higher than the minimum values in the background zone. Both absolute indices of toxic contamination and variation coefficients were higher for exchangeable forms than for movable forms of metals. In contrast to the toxic contamination index, the variation coefficient of hydrogen ion concentration was maximum in the background zone and minimum in the buffer zone; in addition, it was substantially higher than the variation coefficients of the indices.

The results of biological testing indicate that plant growth responses considerably depended on the contamination zone and experimental conditions (Table 3). In the first experiment (with a more concentrated suspension), the roots of the plants grown on substrates from the impact and buffer zones were considerably shorter than the roots of control plants. The frequency distributions in these variants had high kurtoses and asymmetries (even to the extent of being practically one-sided) and narrow variation ranges (Fig. 1). Apparently, the extremely high toxicity of the substrate suppressed root growth at the earliest developmental stages in most plants. The tolerance index (the ratio of the mean length of the roots of plants grown on the tested substrate to the control value) was 0.45 and 0.12 for the buffer and impact zones, respectively. We observed necrosis of root meristems in almost all (81 to 100%) seedlings growing on the litter from the impact zone. This proportion for the litter from the buffer zone was, on average, 7.3% (from 0 to 51%), and only a few cases of necrosis were observed in control seedlings (mean frequency, 1.3%, variation range, from 0 to 17.6%).

In the second experiment (with a less concentrated litter suspension), the root growth test indicated more favorable conditions for plant growth: the mean root length in the control variant (litter from the background zone) was almost two times greater than in the first experiment. The variation ranges were increased in all samples due to the shift of the upper limit of distribu-

Table 3. Phytotoxicity of forest litter (mean root length; the record unit is an experimental jar) from different zones of toxic contamination and under different conditions of biological testing

Statistical parameter	Litter-to-water ratio					
	1 : 10			1 : 50		
	background	buffer	impact	background	buffer	impact
Arithmetic mean, mm	15.0	6.7	1.7	27.9	22.1	3.5
Asymmetry	1.62**	0.39	3.79**	–0.92*	–1.15**	4.12**
	(0.03)	(0.68**)	(4.8**)	(–0.56**)	(–0.20**)	(3.74**)
Kurtosis	4.53**	0.20	15.93**	0.29	0.78	18.51**
	(–0.08)	(–0.28)	(28.37**)	(–0.07)	(–0.67**)	(14.98**)
Minimum, mm	11.4	2.3	1.0	18.6	5.7	1.1
Maximum, mm	23.4	11.5	7.9	32.9	29.8	23.4
Variation coefficient, %:						
analytical	16.5	34.3	79.1	12.9	27.4	128.6
median	11.4	22.3	33.1	11.1	13.7	55.3

Note: The asymmetry and kurtosis values for the experiments where a single seedling served as a replicate are shown in parentheses. The significance of differences of asymmetry and kurtosis values from zero: * $p < 0.05$ and ** $p < 0.01$.

tion (Table 3). The tolerance index for the impact zone was still low (0.13); the distribution kurtosis and asymmetry also remained high. In the experiment with forest litter from the impact zone, even the sign of the asymmetry coefficient was different from that in the experiment with litter from the background zone, i.e., the distribution was left-sided versus a right-sided one in the background zone. Substrates from the buffer zone significantly suppressed root growth, compared to the substrates from the background zone (Student's *t* test was 4.64, $p < 0.001$); however, the suppression was not so strong as in the first experiment (the tolerance index was 0.79). In this range of metal concentrations, the diversity of the responses of plants to the toxic effect characteristic of the background zone was clearly visible (Fig. 1).

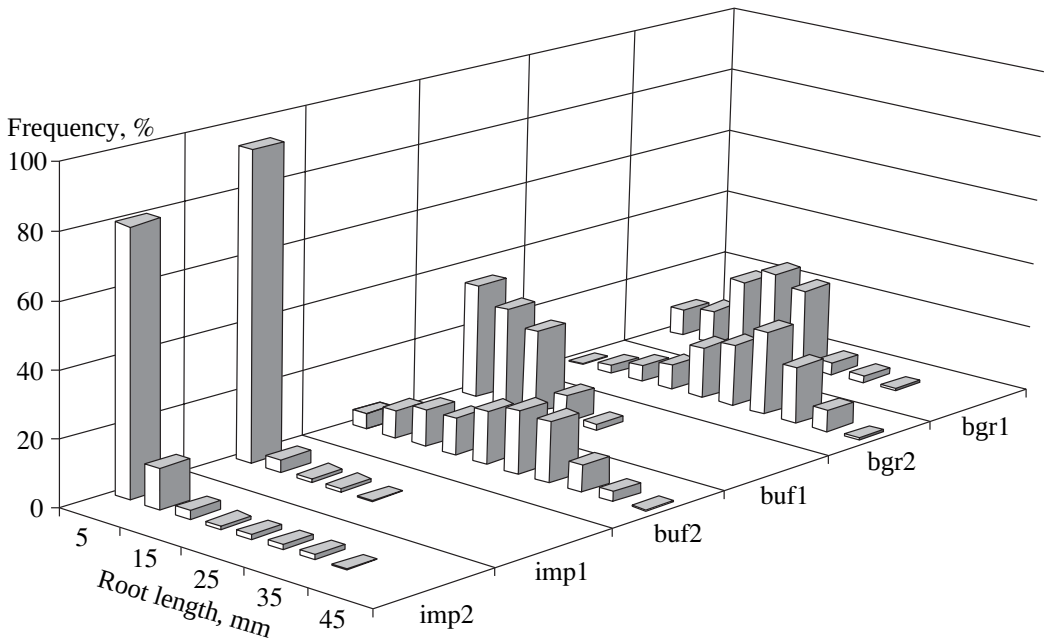

Fig. 1. Frequency distribution of root length (the record unit is a seedling) in plants growing on suspensions of forest litter from the background (bgr), buffer (buf), and impact (imp) zones. Substrate-to-water ratio: (1) 1 : 10; (2) 1 : 50.

In an additional experiment, we used the litter from the impact zone diluted to a substrate-to-water ratio of 1 : 100. In this case, the substrate toxicity was equally high, and the results were almost the same as in the second experiment.

In all experiments, the variation coefficients of root length were the highest for litter from the impact zone. The same relationship, i.e., an increase in variation of traits under extreme conditions, has been observed in various objects exposed to various factors (Bezel' *et al.*, 2001).

The relationship between litter contamination and the results of biological tests over the entire gradient fit an S-shaped logistic curve (Fig. 2). Figure 2 shows the results of the second experiment; the results of the first experiment were described by a similar curve. The maximum toxicity of forest litter from the buffer zone was comparable to the toxicity of litter from the impact zone, and the minimum toxicity was equal to the background level. Regarding the toxic contamination index calculated from the concentrations of movable forms of metals, a sample from the impact zone (with high metal concentrations) dropped out from the general pattern, because its phytotoxicity was very low (Fig. 2a). However, the acidity of this sample was unusually low for the impact zone (pH was 5.48 versus 3.68–4.45 for the remaining samples). The use of the toxic contamination index calculated from the concentrations of exchangeable forms restored the pattern: the point that dropped out in the previous case shifted to the cluster of points of litter samples from the buffer zone, and a logistic curve considerably better approximated the dose–effect relationship (Fig. 2b). This dropping-out sample explains the considerable differences between the analytical and median variation coefficients: the latter, which was calculated according to Zhivotovsky (1991), did not depend on dropping-out values (Table 3).

Analysis of the relationship between metal concentrations and the results of biological tests separately for each contamination zone showed that the relationship was practically absent for the background zone (Table 4). In the buffer zone, phytotoxicity negatively correlated with metal concentrations. This was especially distinct in the experiment with a less concentrated suspension and for exchangeable forms of metals. Biological testing of forest litter from the impact zone showed both negative and positive significant correlations between metal content and phytotoxicity. However, a more detailed analysis demonstrated that they were accounted for by the only the dropping-out point mentioned above. If we excluded this point, most correlation coefficients became insignificant. Similarly, the relationship between acidity and phytotoxicity "disappeared" in the variant with a less concentrated suspension In the variant with a more concentrated suspension, the correlation between pH and phytotoxicity remain significant even when the dropping-out sample was excluded. Thus, the strong, stable relationship

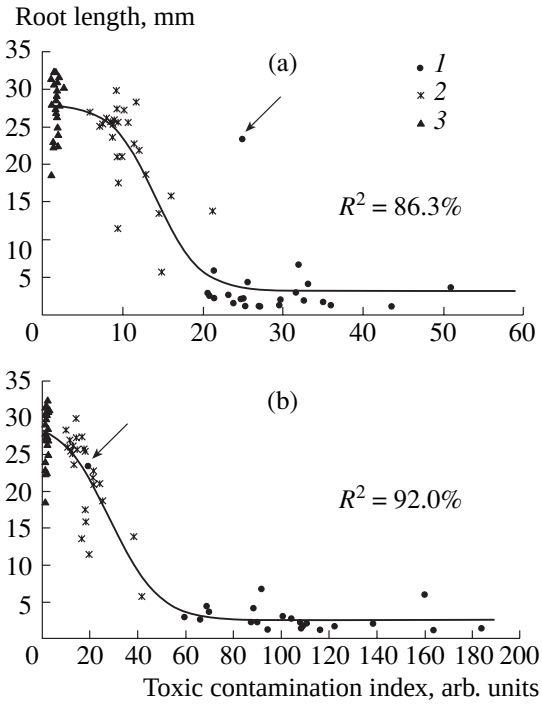

Fig. 2. The dependence of mean root length (the record unit is an experimental jar) on the heavy-metal content of forest litter (the substrate-to-water ratio is 1 : 50). The contamination indices have been calculated for (a) movable and (b) exchangeable forms of heavy metals. Zones from which forest litter was sampled: *1*, impact; *2*, buffer; *3*, background. The arrow indicates the dropping-out point (see the text for explanation). R^2 is the determination coefficient when the relationship is approximated by a logistic equation.

between metal concentration and phytotoxicity was found only in the buffer zone. In the background and impact zones, this relationship was unstable and, most probably, accidental.

Stepwise regression analysis allowed us to isolate the main determinants of phytotoxicity (Table 5). Taking into account the strong correlation between the explanatory variables, we used ridge regression specially developed for such analyses. In the buffer zone, the main determinant was the content of exchangeable forms of metals, which, in turn, depended on the content of movable forms and pH. For example, in the buffer zone, the corrected determination coefficient (R^2) of multiple regression (dependent variable, the concentration of the exchangeable form; explanatory variables, the concentration of the movable form and pH) for the four elements studied varied from 0.68 to 0.89 ($F_{2, 22}$ = 26.15–93.65, $p \ll 0.0001$ in all cases); in the impact zone, R^2 = 0.45–0.89 ($F_{2, 21}$ = 10.32–91.25, $p < 0.001$ (at least)). This may explain the fact that acidity in combination with the concentrations of movable forms of metals or acidity alone were the main phytotoxicity determinants in the impact zone. Cadmium and zinc were the main determinants in the variants with a more concentrated and a more diluted suspensions,

Table 4. Pearson coefficients of linear correlation between mean root length and the concentrations of elements in different zones of toxic contamination and under different conditions of biological testing

Element	Litter-to-water ratio					
	1 : 10			1: 50		
	background	buffer	impact	background	buffer	impact
pH	0.12	−0.08	0.87*** (0.70***)	−0.30	0.20	0.78*** (0.34)
	Movable forms					
Cu	0.01	−0.41*	0.02 (0.35)	0.20	−0.65***	−0.12 (0.01)
Cd	0.19	−0.47*	0.59** (0.49*)	0.18	−0.46*	0.45* (0.07)
Pb	0.16	−0.19	−0.42* (−0.06)	0.21	−0.54**	−0.55** (−0.44*)
Zn	0.24	−0.45*	0.86*** (0.60**)	−0.06	−0.47*	0.76*** (0.15)
Ind	0.18	−0.41*	0.04 (0.34)	0.17	−0.64***	−0.11 (-0.04)
	Exchangeable forms					
Cu	−0.19	−0.38	−0.61** (−0.30)	0.13	−0.75***	−0.59** (−0.25)
Cd	−0.05	−0.58**	0.29 (0.31)	0.36	−0.75***	0.18 (0.03)
Pb	−0.21	−0.16	−0.45* (−0.31)	0.29	−0.59**	−0.43* (−0.26)
Zn	0.11	−0.57**	0.54** (0.35)	0.23	−0.77***	0.45* (0.08)
Ind	−0.22	−0.36	−0.57** (−0.29)	0.32	−0.75***	−0.55** (−0.26)

Note: The correlation coefficients for the impact zone calculated after the exclusion of the dropping-out point (see the text) are shown in parentheses. Ind is the toxic contamination index. The significance of differences of the correlation coefficients from zero: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 5. The parameters of significant ($p < 0.05$) multiple linear regression equations describing the relationship between mean root length and the contents of elements in the litter in different zones of toxic contamination

Parameter	Litter-to-water ratio			
	1 : 10		1 : 50	
	buffer	impact	buffer	impact
Explanatory variables	Cd-2 (−0.52)	pH (0.63)	Zn-2 (−0.57)	Pb-2 (−0.30)
			Pb-1 (−0.38)	pH (0.27)
R^2	0.27	0.41	0.58	0.19
F ratio	$F_{1, 23} = 9.78$	$F_{1, 21} = 16.49$	$F_{2, 22} = 17.54$	$F_{2, 20} = 3.50$
p	0.00473	0.00056	0.00003	0.04975

Note: In the background zone, multiple regression was nonsignificant ($p > 0.05$). For the impact zone, the equations were calculated after the exclusion of the dropping-out point (see the text). The figures at the symbols of elements indicate (*1*) movable forms and (*2*) exchangeable forms. The β coefficients at the explanatory variables in the regression equations are shown in parentheses; R^2 is the corrected multiple determination coefficient.

respectively. This "interchangeability" was accounted for by the fact that the concentrations of the exchangeable forms of these elements in the buffer zone exhibited an almost functional relationship (the correlation coefficient was 0.97, $p \ll 0.0001$). In general, the observed relationships agreed with the modern views on the relationship of the mobility, bioavailability, and toxicity of heavy metals with the acidity of the medium (Brun *et al.*, 1998; 2001; Salam and Helmke, 1998; Gray *et al.*, 1999; Alva *et al.*, 2000; Tyler and Olsson, 2001a, 2001b).

Comparison of the spatial distributions of the concentrations of movable forms of metals, acidity, and phytotoxicity (interpolation was performed via kriging in the framework of the spherical model using the Surfer 6.04 software) confirmed the conclusions from the results of correlation and regression analyses (Fig. 3). As seen from Fig. 3, the biological-test response to forest litter from the buffer zone is the result of the variously directed effects of metals accumulated in the litter and its acidity, with the spatial mosaics coinciding for whole areas of the plot studied, rather

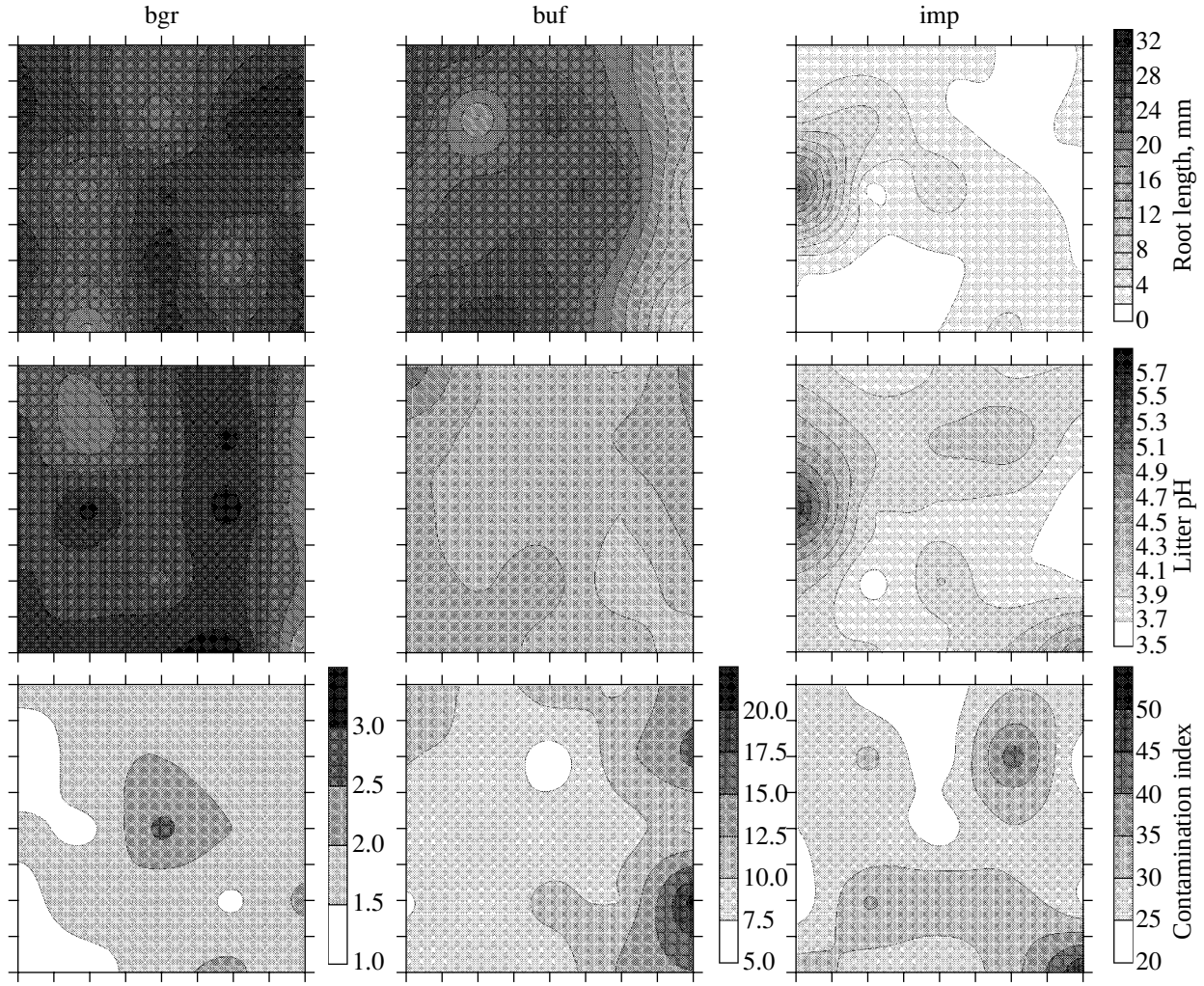

Fig. 3. Spatial variation of the phytotoxicity of forest litter (the substrate-to-water ratio is 1 : 50) estimated from seedling root lengths, pH of the litter, and index of substrate contamination with heavy metals (movable forms) within 100 × 100 m plots in the background (bgr), buffer (buf), and impact (imp) zones.

than for some individual points as we observed in the impact zone. In the background zone, the spatial mosaics of the parameters studied were independent.

The results obtained may be used when considering the general patterns of the biota responses to chemical pollution. The responses of many groups of organisms to toxic contamination, even estimated by integral indices, is distinctly nonlinear, which is reflected in the "classical" *S*-shaped dose–effect curves relating the inflow of pollutants into the ecosystem and the functioning of the biota (Vorobeichik *et al.*, 1994). The nonlinear relationship indicates that the diversity of the states of biota is drastically increased in the transition zone characterized by a narrow range of toxic contamination levels, so that both almost "background" and almost "impact" variants of the state of biota may be observed at the same level of contamination. In other words, the transition zone exhibits a trigger effect, i.e., a rapid "switching" of the biota from the normal (background) to the maximally deteriorated (impact) functional mode.

Undeniably, the root test that we used in this study is an oversimplified model of the biota response to toxic contamination. However, the observed dose–effect relationship shows a marked trigger effect: in the buffer zone, the phytotoxicity variation covers almost the entire range of possible values, whereas the transition from the background to the impact level uses only 15–19% of the total gradient of toxic contamination. Therefore, the spatial variation in the concentrations of pollutants in the buffer zone determines nonlinear responses of the biota even within relatively small areas, which may, to a certain degree, explain the trigger effect. Taking into account that we used a genetically homogeneous sample for biological testing, we may conclude that the considerable spatial variation in the concentrations of toxic forms of heavy metals (which, in turn, is determined by the superposition of

two spatial mosaics, namely, gross content and substrate acidity) is the key mechanism of the trigger effect of the biota response to toxic contamination.

Thus, the results of the analysis of the microscale spatial variation in the forest litter contamination with heavy metals and its phytotoxicity allow us to answer affirmatively the questions we asked above. In the case of moderate amounts of pollutants discharged from a point source, a wide variation in the toxicity of forest litter is observed even within a relatively small area. This variation is accounted for by considerable variations in both the concentrations of the metals and substrate acidity. The acidity may be considered the main factor modifying the toxicity of forest litter. Other conditions being equal, the proportion of the exchangeable fraction of metals, which is the most available for plants and, hence, is the main determinant of toxicity, directly depends on acidity in the pH range studied. The wide spatial variation in litter phytotoxicity results in a distinctly nonlinear dose–effect relationship: a relatively small change in toxic contamination is accompanied by the maximum variation in the responses of the biological test.

The possibility of the modification of pollutant effects by natural ecological factors was discovered long ago; however, most of these data were obtained in laboratory experiments, where fixed concentrations of pollutants were added to the medium, and all other parameters were strictly controlled. We used material from natural ecosystems to demonstrate that high and low acidity of the forest litter modified the toxicity of heavy metals. Therefore, the phenomena that have been observed in numerous laboratory experiments, such as the absence of growth suppression by heavy metals at high pH of the soil solution (Alva *et al.*, 2000), actually occur under natural conditions within the natural range of pH values. These phenomena should be taken into account when analyzing the response of the biota to toxic contamination.

ACKNOWLEDGMENTS

We are grateful to E.Kh. Akhunova for measuring the concentrations of heavy metals, E.V. Prokopovich for measuring acidity, and N.V. Kuznetsova and E.V. Ul'yanova for technical assistance in biological testing.

This study was supported by the Russian Foundation for Basic Research, project no. 01-05-65258.

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