

## Surface Pollution of Meadow Plants during the Period of Reduction of Atmospheric Emissions from a Copper Smelter

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Heavy metals (HMs) sorbed on dust particles are a major component of atmospheric emissions from the metallurgical industries. There are two main routes of HM entry into plant tissues: from polluted soil through the roots and from dust deposited on the surface of aboveground plant parts [1]. To separate their contributions, it has been proposed to compare metal concentrations in untreated plant samples and samples washed from metal-containing dust. This approach makes it possible to estimate the contribution of metal intake from the soil and correctly compare it with their intake from the surface [2]. As a rule, washing significantly reduces the concentration of most metals (Al, Cd, Cr, Cu, Fe, Ni, Pb, Ti, V) and metalloids (As, Sb) on the plant surface of plants, but does not affect the concentrations of macroelements (N, P, K, Ca, Mg, Cl, S) [2].

Atmospheric emissions (including those containing heavy metals) in many industrial areas are being reduced due to the closure or renovation of industries [3]. Under these conditions, the polluted soil becomes the main and, in the long term, the only source of metal intake for plants. Theoretically, the cessation of atmospheric pollution of plant surface should lead to the absence of a difference between untreated and washed samples, but, on the other hand, secondary contamination of plants with soil particles is possible. We are not aware of the attempts to empirically test this assumption.

Analysis of the content of metal-containing dust on the surface of plants is important in terms of its strong negative effect on all components of terrestrial ecosystems. Dust-associated heavy metals can enter plant tissues through the cuticle and stomata and accumulate there [4]. The dust deposited on the leaf surface blocks the stomata in the open position, which leads to loss of moisture, general deterioration of plant life state, and decrease in drought and frost tolerance [5]. In addition, dust on the surface of plants can be a

source of heavy metals for phytophages. The greatest toxic effect can be expected in the case of invertebrates with gnawing mouthparts, which ingest dusty surface tissues of plants. This was indirectly confirmed in our study on meadow communities during the period of high emissions from a copper smelter: the abundance of gnawing phytophages (as opposed to sucking) was greatly reduced in the herbaceous layer grass stand near the polluter [6]. In general, reduction in the amount of dust should lead to a decrease in toxic impact on a wide range of biota components.

In this study, we analyzed metal contents in herbaceous plants of meadow ecosystems after a strong decrease in atmospheric pollution by emissions from the Middle Ural Copper Smelter (MUCS). Its purpose was to check the assumption that the contents of metals (Cu, Zn, Cd and Pb) in untreated and washed plants would not differ under these conditions.

The study was carried out in the vicinity of the MUCS, which is located in the outskirts Revda (Sverdlovsk Oblast). Annual emissions (SO<sub>2</sub> and heavy metals associated with dust particles) from the smelter amounted to 225 thousand tons in 1980, 148 thousand tons in 1990, and 63 thousand tons in 2000. In 2010, after an overhaul of the MUCS, emissions practically ceased, decreasing to only 3000 t/year. Between 1980 and 2012, the total annual amount of emissions decreased 75-fold, with a 116-fold decrease in SO<sub>2</sub> (from 201 to 1.7 thousand tons) and 44-fold decrease in dust particles (from 21 to 0.5 thousand tons). Emissions of Cu decreased 5500-fold (from 4400 to 0.8 t/year); of As, 1571-fold (from 900 to 0.6 t/year); of Pb, 16-fold (from 1000 to 70 t/year); and of Zn, 15-fold (from 1800 t in 1989 to 100 t in 2012). More detailed data are available on the composition and dynamics of emissions from the MUCS [7] and on technogenic transformation of ecosystems in a pollution gradient [8].

Key plots were located west of the MUCS (against the prevailing wind direction) in the impact (1 km from the smelter), buffer (4 km) and background (30 km) zones, in slightly depressed areas of secondary upland meadows in forest glades about 5000 m<sup>2</sup> in size formed as a result of felling operations about 60 years ago. The floristic composition of meadow vegetation varies greatly between zones with different pollution levels, which is due to the disappearance of sensitive forb species and their replacement by grasses near the smelter. There are forb meadows in the background zone, forb–grass meadows in the buffer zone, and grass meadows with absolute dominance of *Agrostis capillaris* L. in the impact zone. Characteristics of the herbaceous layer were described in detail previously [6]. No livestock grazing or haymaking occurred in the plots during the study period.

The material was collected in July–August 2012 in the central parts of the meadow areas, at a distance of 10–15 m from the forest edge. Permanent sampling plots (SPs) used for this purpose were established in 2006 to study the structure of invertebrate communities in the herbaceous layer [6]. Three 50 × 50-m plot in each zone were located at distances of 100–300 m from each other.

Meadow plants were harvested by hand; the whole aboveground parts of plants (i.e., stems and leaf blades) were selected for analysis. Due to the severe degradation of meadow vegetation, only three species were represented in the impact zone: *A. capillaris*, *Deschampsia cespitosa* Beauv. and *Lychnis flos-cuculi* L. These species were sampled in all three zones. In addition to them, samples of ten dominant species were collected in the background and buffer zones (Table 1). Three plants of each species were taken from each SP. Thus, the material from each zone included 3 ind. × 3 SPs = 9 ind. of each species, and a total of 522 samples were collected.

Each plant was divided into two parts: one was thoroughly washed in a 0.5% solution of Fairy washing-up liquid [9], and the other remained unwashed. The plants were dried in a drying cabinet at 80°C for 24 h and then wet-ashed in 70% nitric acid in Teflon glasses in a Speedwave MWS-2 microwave digestion system (Berghof, Germany). The contents of heavy metals (Cu, Zn, Cd and Pb) were determined by atomic absorption spectrometry using an AAS 6 instrument (Analytik Jena, Germany). Chemical analyzes were carried out in the Laboratory of Ecotoxicology of Populations and Communities (Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences) certified for technical competence (certificate ROSS.RU0001.515630).

Standard descriptive statistics were calculated (mean ± standard error); an individual plant was considered an accounting unit. Differences between the untreated and washed parts were evaluated using one-way ANOVA with repeated measures (separately for

each species in each of the contaminated zones,  $n = 9$ ). The expected false discovery rate (FDR) was controlled using the Benjamini–Yekutieli procedure. The influence of species identity and pollution zone was assessed by two-way ANOVA according to two complementary schemes that took into account (1) three species found in all pollution zones ( $n = 81$ ) or (2) ten species found only in the background or buffer zone ( $n = 180$ ). Before analysis, the concentrations of metals were converted to logarithmic form ( $y = \ln(x)$ ).

The metal contents in plants significantly differed between the pollution zones: scheme 1,  $F_{(2;72)} = 34.3–209.6$  ( $p < 0.001$  in all cases); scheme 2,  $F_{(1;160)} = 95.3–419.6$ ,  $p < 0.001$  (except for Pb:  $F_{(1;160)} = 2.8$ ,  $p = 0.098$ ). Such differences are quite expectable and have been repeatedly recorded near other industries, which indicates that the intake of metals from the soil is strongly increased near the emission source. Differences in metal contents between plant species are also significant: scheme 1,  $F_{(2;72)} = 17.4–595.5$ ,  $p < 0.001$ ; scheme 2,  $F_{(9;160)} = 6.2–340.6$ ,  $p < 0.001$ . This is apparently explained by specific features of their physiology, anatomy, and morphology. It is well known that the accumulation of metals by plants depends on their species-specific features such as surface structure, total surface area, the presence of wax coating, etc. However, this aspect requires special analysis and is beyond the scope of this study. The diversity of “strategies” of metal accumulation by plants accounts for the universality of our results as regards the surface pollution in question.

A comparison of untreated and washed plants demonstrates the absence of statistically significant differences for all species in all pollution zones: in all cases,  $p > 0.05$  (see Table 1). This means that the contents of heavy metals in untreated plants correspond to their intake from the soil, which may be explained by the absence of plant surface pollution with metal-containing dust particles.

The most correct way to assess changes in surface metal intake in plants is to compare relevant data over the periods of high and low emissions. Unfortunately, there is no available data on the period of high emissions from the MUCS, but heavy pollution of the plant surface with metal-containing dust has been described near other active pollution sources of a similar type. Thus, in plants near a Pb–Zn smelter in England, the surface intake of Cd accounted for up to 28% of the total metal content; of Zn, up to 45%; and of Pb, up to 85% [10]. On the surface of hazel leaves in the most polluted areas near the mining and smelting complex in Serbia, the content of Cu was up to 57% of the total; of Zn, up to 56%; and of Pb, up to 73% [11]. In the impact zone of the Severonikel Ni–Cu smelter, up to 90% of the total Cu content in the aboveground parts of birch seedlings was received from the plant surface [9]. The amount of metal-containing dust emissions from the Severonikel works is comparable to that from

**Table 1.** Concentration of heavy metals ( $\mu\text{g/g}$ ) in the dominant species of herbaceous meadow plants in areas with different levels of pollution

Species	Pollution zone and treatment					
	Background		Buffer		Impact	
	untreated	washed	untreated	washed	untreated	washed
	Cu					
<i>Agrostis capillaris</i>	5.1 ± 0.4	4.5 ± 0.3	3.9 ± 0.2	3.4 ± 0.4	27.1 ± 4.5	16.5 ± 1.7
<i>Calamagrostis epigeios</i>	3.6 ± 0.5	3.4 ± 0.5	6.2 ± 0.9	5.6 ± 0.4	—	—
<i>Deschampsia cespitosa</i>	3.7 ± 0.4	3.8 ± 0.3	6.9 ± 0.3	5.4 ± 0.3	24.7 ± 7.8	14.1 ± 2.2
<i>Alchemilla</i> sp.	3.7 ± 0.3	3.4 ± 0.3	9.7 ± 1.2	11.7 ± 1.5	—	—
<i>Angelica sylvestris</i>	2.5 ± 0.2	3.0 ± 0.3	11.7 ± 2.1	12.8 ± 1.3	—	—
<i>Lychnis flos-cuculi</i>	3.3 ± 0.5	5.2 ± 1.9	29.3 ± 4.9	47.8 ± 13.0	93.9 ± 19.1	89.5 ± 9.4
<i>Equisetum sylvaticum</i>	2.0 ± 0.1	2.2 ± 0.2	5.8 ± 0.4	5.6 ± 0.5	—	—
<i>Filipendula ulmaria</i>	4.4 ± 0.2	4.2 ± 0.2	12.9 ± 1.3	13.5 ± 1.5	—	—
<i>Lathyrus pratensis</i>	5.2 ± 0.2	5.8 ± 0.1	7.9 ± 0.5	8.5 ± 0.5	—	—
<i>Potentilla erecta</i>	10.8 ± 1.3	10.7 ± 1.3	15.4 ± 1.0	13.2 ± 0.7	—	—
<i>Sanguisorba officinalis</i>	5.8 ± 0.3	5.3 ± 0.2	10.8 ± 1.0	9.9 ± 0.8	—	—
<i>Succisa pratensis</i>	3.7 ± 0.3	3.8 ± 0.3	5.4 ± 0.5	5.0 ± 0.7	—	—
<i>Veratrum lobelianum</i>	5.7 ± 0.2	5.7 ± 0.4	11.8 ± 1.3	11.2 ± 1.0	—	—
	Zn					
<i>Agrostis capillaris</i>	48.7 ± 3.3	47.5 ± 3.0	104.6 ± 8.7	96.9 ± 10.5	172.2 ± 15.0	157.9 ± 13.9
<i>Calamagrostis epigeios</i>	38.0 ± 6.1	39.9 ± 5.6	79.3 ± 4.6	88.4 ± 4.7	—	—
<i>Deschampsia cespitosa</i>	35.2 ± 3.7	39.8 ± 1.4	60.5 ± 6.2	67.5 ± 1.8	124.7 ± 19.7	121.9 ± 13.2
<i>Alchemilla</i> sp.	37.1 ± 3.1	45.3 ± 1.9	110.2 ± 24.2	177.2 ± 19.3	—	—
<i>Angelica sylvestris</i>	19.6 ± 0.8	24.5 ± 4.2	45.3 ± 4.9	53.4 ± 6.7	—	—
<i>Lychnis flos-cuculi</i>	150.9 ± 13.2	168.1 ± 19.3	916.2 ± 77.3	979.2 ± 66.1	803.2 ± 66.8	694.3 ± 58.7
<i>Equisetum sylvaticum</i>	27.9 ± 2.3	30.1 ± 3.1	111.2 ± 15.5	91.4 ± 8.1	—	—
<i>Filipendula ulmaria</i>	159.0 ± 9.0	162.7 ± 12.1	308.0 ± 51.5	339.5 ± 52.5	—	—
<i>Lathyrus pratensis</i>	84.3 ± 6.3	116.2 ± 10.0	207.8 ± 12.7	226.5 ± 15.2	—	—
<i>Potentilla erecta</i>	63.9 ± 6.3	72.0 ± 9.0	146.9 ± 17.5	157.7 ± 22.2	—	—
<i>Sanguisorba officinalis</i>	81.4 ± 9.9	91.1 ± 11.2	267.5 ± 25.4	295.4 ± 32.0	—	—
<i>Succisa pratensis</i>	13.5 ± 1.0	14.7 ± 0.9	104.9 ± 76.0	21.6 ± 3.5	—	—
<i>Veratrum lobelianum</i>	11.2 ± 0.4	12.9 ± 1.6	30.5 ± 7.4	25.8 ± 2.5	—	—
	Cd					
<i>Agrostis capillaris</i>	0.25 ± 0.05	0.23 ± 0.04	0.83 ± 0.17	0.76 ± 0.13	2.2 ± 0.3	2.2 ± 0.3
<i>Calamagrostis epigeios</i>	0.29 ± 0.04	0.23 ± 0.04	0.36 ± 0.06	0.22 ± 0.04	—	—
<i>Deschampsia cespitosa</i>	0.20 ± 0.05	0.13 ± 0.04	0.26 ± 0.03	0.24 ± 0.04	1.1 ± 0.5	0.9 ± 0.3
<i>Alchemilla</i> sp.	0.32 ± 0.06	0.39 ± 0.07	1.47 ± 0.41	2.43 ± 0.43	—	—
<i>Angelica sylvestris</i>	0.16 ± 0.07	0.28 ± 0.09	0.20 ± 0.03	0.18 ± 0.04	—	—
<i>Lychnis flos-cuculi</i>	1.28 ± 0.32	1.94 ± 0.57	5.33 ± 0.84	10.89 ± 4.15	20.2 ± 3.0	17.2 ± 1.4
<i>Equisetum sylvaticum</i>	0.21 ± 0.03	0.23 ± 0.04	0.82 ± 0.07	0.74 ± 0.11	—	—
<i>Filipendula ulmaria</i>	0.97 ± 0.17	0.97 ± 0.13	1.97 ± 0.47	2.06 ± 0.43	—	—
<i>Lathyrus pratensis</i>	0.13 ± 0.04	0.23 ± 0.05	0.25 ± 0.05	0.43 ± 0.13	—	—
<i>Potentilla erecta</i>	0.42 ± 0.07	0.44 ± 0.05	2.50 ± 0.26	2.74 ± 0.26	—	—
<i>Sanguisorba officinalis</i>	0.21 ± 0.04	0.16 ± 0.03	0.32 ± 0.07	0.42 ± 0.16	—	—
<i>Succisa pratensis</i>	0.16 ± 0.03	0.14 ± 0.04	0.78 ± 0.33	0.27 ± 0.04	—	—
<i>Veratrum lobelianum</i>	0.14 ± 0.01	0.15 ± 0.02	0.50 ± 0.09	0.54 ± 0.09	—	—
	Pb					
<i>Agrostis capillaris</i>	2.0 ± 0.2	2.5 ± 0.3	1.6 ± 0.2	1.9 ± 0.3	12.5 ± 2.8	9.0 ± 1.8
<i>Calamagrostis epigeios</i>	3.9 ± 1.0	1.7 ± 0.3	2.8 ± 0.5	2.5 ± 0.6	—	—
<i>Deschampsia cespitosa</i>	1.4 ± 0.3	1.5 ± 0.3	1.7 ± 0.5	1.5 ± 0.4	9.2 ± 4.0	4.7 ± 1.5
<i>Alchemilla</i> sp.	3.8 ± 0.8	2.3 ± 0.5	2.6 ± 0.5	3.5 ± 0.9	—	—
<i>Angelica sylvestris</i>	3.0 ± 1.0	2.1 ± 0.5	2.3 ± 0.4	2.1 ± 0.4	—	—
<i>Lychnis flos-cuculi</i>	3.8 ± 0.9	1.8 ± 0.6	7.5 ± 1.8	12.8 ± 5.0	25.1 ± 14.5	14.2 ± 3.6
<i>Equisetum sylvaticum</i>	1.3 ± 0.2	1.3 ± 0.2	2.3 ± 0.5	2.5 ± 0.6	—	—
<i>Filipendula ulmaria</i>	2.4 ± 0.3	2.3 ± 0.4	2.3 ± 0.3	2.0 ± 0.4	—	—
<i>Lathyrus pratensis</i>	1.5 ± 0.3	1.9 ± 0.3	1.5 ± 0.3	1.9 ± 0.4	—	—
<i>Potentilla erecta</i>	2.9 ± 0.4	3.5 ± 0.4	4.0 ± 0.7	4.3 ± 0.8	—	—
<i>Sanguisorba officinalis</i>	2.9 ± 0.4	2.5 ± 0.2	2.9 ± 0.5	2.7 ± 0.3	—	—
<i>Succisa pratensis</i>	2.0 ± 0.3	1.6 ± 0.3	2.3 ± 0.3	2.2 ± 0.4	—	—
<i>Veratrum lobelianum</i>	2.4 ± 0.4	2.4 ± 0.3	3.4 ± 0.2	3.2 ± 0.3	—	—

Data are presented as mean ± standard error,  $n = 9$ . A dash indicates the absence of a species. Differences between untreated and washed plants in each pollution zone lack statistical significance ( $p > 0.05$ ).

the MUCS during the period of high emissions: in the early 1990s, up to 15 thousand tons per year [9] and 16 thousand tons per year [7], respectively. This allows us to draw an analogy and suggest significant surface pollution of plants in the impact zone of MUCS.

Thus, the absence of differences between untreated and washed plants in the impact zone of MUCS after emission reduction must be due precisely to the absence of surface contamination. This conclusion is consistent with a significant decrease in the concentrations of heavy metals (by a factor of 2 to 42) in the untreated birch leaves in 2014 (after cessation of emissions) compared to 2008 [12]. It has been suggested that this decrease is due to a reduction in the content of airborne industrial dust, since the concentrations of metals in the soil remain high [7]. A similar situation was described for the Severonikel smelter: a reduction in atmospheric emissions (5- to 8-fold) resulted in a significant (2- to 16-fold) decrease in the content of heavy metals in the needles of spruce [13] and pine [13, 14], as well as in the leaves of several dwarf shrubs [14]. Given the absence of decrease in the contents of metals in the upper soil horizons [14], the authors attribute these changes to the decrease in atmospheric emissions of metal-containing dust.

Despite the absence of significant differences, the Cu content in the untreated plants of *A. capillaris* and *D. cespitosa* in the impact zone is somewhat higher than in the washed ones (by factors of 1.6 and 1.8, respectively) (Table 1). This is unlikely to be due to emissions from the MUCS, since the proportion of Cu in them in 2012 was extremely small (only 0.03%, 0.8 t/year). In the presence of airborne metal deposition on the surface of plants, one would have expected an increased content of Zn, whose proportion in emission is higher than that of Cu (3.9%, 118 t/year) [7]. On the other hand, the content of Cu in the litter and humus soil horizon in the impact zone exceeds significantly (by two orders of magnitude) the contents of other metals [7]. It is logical to assume that the cause of this may be the secondary surface contamination of grasses with soil particles due to wind transfer: the presence of open spaces of dust-forming soil [15] and a significant increase in wind speed [16] are typical of ecosystems that have become sparse under anthropogenic impact.

As an alternative explanation for the similarity of metal contents in untreated and washed plants, it can be assumed that the attempt to wash off the dust particles failed due to an incorrectly chosen method of washing. In most cases, bidistilled water is recommended for the treatment of plants as an effective and safe solvent [2]. Its use, however, may be limited by the characteristics of the plant species studied and the properties of pollutants, as shown for Cu, Zn, Cd and Pb [17, 18]. For the best cleaning of biological material from pollutants of any type, it is usually recommended to use weak solutions of surface-active substances (surfactants) [19], which allows removing 35–42% of

Cu [9, 20], 45 – 75% of Zn [10, 20], 28% of Cd, and 85% of Pb [10] settling on the surface of plants under intense atmospheric pollution. Contrary to some concerns [9], the use of surfactants does not lead to the leaching of elements from leaf tissues and is considered correct for estimating the proportion of external contamination [19, 21]. As a consequence, the similarity of metal concentrations in untreated and washed plants can only be explained by the absence of surface contamination.

Thus, after a drastic reduction of atmospheric emissions, despite the possibility of secondary pollution, the contents of metals on the surface of herbaceous plants has not increased. This result has been obtained for the first time for meadow ecosystems under conditions of reduced atmospheric emissions and is extremely important for assessing the contribution of plants to the movement of heavy metals along trophic chains and also as a basis for analyzing the processes of recovery in invertebrate communities of the herbaceous layer during the period of emission reduction. The cessation of surface pollution means a reduction in the total amount of metals supplied by plants to the trophic chains. In addition, it can be expected that the elimination of this component of pollution, despite the high concentrations of metals persisting in the soil and plants, will have a quick positive effect on invertebrate phytophages in polluted areas, primarily on those with gnawing mouthparts.

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#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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