

# Variability in Heavy Metal Tolerance between Saransk (Russian) *Taraxacum officinale* Populations

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## ABSTRACT

Dandelion (*Taraxacum officinale* Wigg.) plants growing wild in Saransk City and its precincts were the subject of this investigation. Initially the heavy metal (HM) concentration of 8-10 individuals from four ecological zones (equivalent to 10 ecotopes) was measured and an index of biological accumulation was determined. Growth responses of dandelion seedlings to various HMs at several concentrations were assessed in *in vitro* bioassays. Seeds (100-200) from plants in the exact same locations were collected and stimulated to germinate on modified Knop medium with different concentrations of four HMs (Ni<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup> or Cu<sup>2+</sup>). HM-tolerant dandelion ecotypes were identified from several of the HM-contaminated sites. These ecotypes differed in their magnitude of tolerance, which depended on the concentration of the heavy metal in the growth medium and could be grouped into two edaphotypes: those from industrial and habitation zones, the phytostabilizers (concentrate and keep HMs in roots) and those from suburban zones, the phytoextractors (concentrate HMs in aboveground organs). Dandelion was considered an Fe-hyperaccumulator in all ecotopes.

**Keywords:** dandelion, ecotopes, growth, root, seed germination, shoot

**Abbreviations:** HM, heavy metals; I<sub>a</sub>, index of biological accumulation; WTI, Wilkinson tolerance index

## INTRODUCTION

Interest in geochemical anomalies previously led to the biogeochemical exploration of trace elements and in more recent years, as a result of rapid development, industrialization and global technogenic pollution of the environment, elemental abnormalities with an industrial genesis have begun to re-attract attention (Antoniadis *et al.* 2006; Chandra Sekhar and Prasad 2006; Reddy *et al.* 2006). Now, in many regions of the world the environment has become more and more chemically "aggressive", and in the last two decades biogeochemistry has once again become the focus of researchers' attention (Bashmakov *et al.* 2006; Loganathan *et al.* 2006; Prasad *et al.* 2006).

By studying natural ecosystems in which plants have acclimatized to a high level of heavy metals (HMs), knowledge of their physiology is necessary to predict vegetation development patches in regions with increasing HM contamination, and conducting scientific research into their conservation is the next logic step. Natural HM-tolerant populations can be used at the initial stages of settling substrates which have been polluted with HMs (Prasad 2006; Prasad and Freitas 2006).

Exploring HM accumulation in plants is important to assess the state of the plant (suppressed vital signs, nature of acclimatization to high HM concentrations, appearance of ecotypes and populations resistant (tolerant) to HMs), and also has interest for humans who use them as indicators, monitors and remediators of the environment (Chandra Sekhar and Prasad 2006; Vandenhove 2006). Data on HM accumulation by plants in urban ecosystems is also necessary for studying the biogeochemical cycling of HMs within the urban environment.

The aim of this study was to identify HM-tolerant ecotypes of *Taraxacum officinale* Wigg. in Saransk and its neighborhoods in Russia. Three objectives were defined: a) to investigate the effects of different HM concentrations on

growth characteristics of different ecotypes; b) to define the tolerance of plants from various ecotopes with different contamination levels of HMs; c) to determine HM contents in soil and plant samples from diverse contaminated points in Saransk city and surroundings.

## MATERIALS AND METHODS

### Site description

Saransk City, the capital of the Mordovian Republic, Russia, occupies a wide area (254.3 km<sup>2</sup>; 54° N, 45° E) and was the site of this investigation. It is located at the junction of steppes and forests in the central part of the Russian flats (on high ground Privolzhskaia). The mean annual air temperature is +3.5 to +4.1°C; in July it is +19.2 to +20°C, with a maximum of +39°C, and in January it is -11.3 to -12.8 °C, with a minimum of -44°C. The vegetative season is 175 to 180 days. The warm season accounts for 70% of the annual precipitation. Chernozioms, gray wood and alluvial soils are present in the terrain of the city. About 30 heavy engineering, electrical engineering, chemical, and food-processing industries are located in Saransk City. The water salinity on sewage disposal sites is 900 to 1000 mg·l<sup>-1</sup> with a pH of 6.2 to 8.0. The sewage contains high doses of organics, salts and Cu, Zn, Pb, Hg, Ni, Cr, Mn, V, W, among others (Burenkov *et al.* 1993). In soils of the Mordovian Republic the microelement content varies widely, even within a single soil type (depending on mechanical composition). Ni content in clay soils is within the average abundance range (40-100 mg·kg<sup>-1</sup>), and in light ones (sandy soils) it is almost 10-fold above Clarke (the average maintenance in the earth's crust, %). The permissible level of pollution accounts for 36% of the territory, more than 46% are moderately dangerous sources of pollution, ~16% hazardous, and around 1% extremely hazardous (Burenkov *et al.* 1993).

The basic indicator of a technogenic influence intensity is the chemical element concentration ratio (K<sub>c</sub>), which is defined by the attitude of the real (abnormal) maintenance of a contaminant in a

concrete natural body to its background (global, regional) level, i.e.  $K_c = C_r / C_b$ . As technogenic anomalies have, as a rule, polyelement compounds the total pollution index ( $Z_c$ ), which is equal to the sum  $K_c$  of chemical elements, is used and characterizes the effect of influence of a bunch of elements:

$$Z_c = \sum_{i=1}^n K_c - (n-1)$$

where  $n$  is the number of considered elements.

On the basis of empirical analysis the assessment scale of soil contamination is developed:  $Z_c < 16$  = admissible;  $16 < Z_c < 32$  = moderately hazardous;  $32 < Z_c < 128$  = hazardous;  $Z_c > 128$  = dangerous (extremely hazardous) pollution (Burenkov *et al.* 1993).

## Botanical description

Dandelion (blowball) (*Taraxacum officinale* Wigg., family Asteraceae, subfamily Liguliflorae) is a perennial plant with a well developed tap root containing inulin, and sometimes attaining 50 cm in length. The root collar is fuzzy. Leaves are numerous, level with the soil or upright. Flower stalks are 10-30 cm in height. Flowers are assembled in large anthodia. Sheath leaflets are grey-green, scurfous and numerous. All flowers are semi-florets, bisexual, bright- or light yellow. Fruits are light-brown or olive-brown achenes with a white floccus of 10 mm in length (<http://en.wikipedia.org/wiki/Dandelion>).

## Plant material, sampling and heavy metal determination

Plants (roots, stems, and leaves) of dandelion were harvested from 100-m<sup>2</sup> plots. The samples were harvested in the beginning (May 9-15, 1997), middle (June 30 – July 7, 1997), and end (September 22 – October 5, 1997) of the growing season in 10 regions of industrial and domestic waste (Table 1). Seeds were collected from these 10 different sites to determine the 1000-seed mass.

Plant samples (0.5 kg fresh weight) were collected from 8-10 individuals per sampling plot. These plants were washed with distilled water and dried in the shade at room temperature for a week. HM contents were determined with the help of X-ray spectrometric analysis. The method involves the deposition of metal ions from aqueous solution in a zirconium hydroxide residue at pH 6 to 7, with subsequent filtration and analysis of the residue on an X-ray spectrometer ("Spectroskan", St. Petersburg, 1994). The HM content in plant samples (mg per kg of air dry weight) was calculated by placing the values into the following equation ("Spectroskan", 1994):

$$C_p = \frac{(c - c_1) \times 100 \times 1000}{m \times 50},$$

where  $c$  and  $c_1$  are the cumulative masses of four HMs (Pb, Ni, Zn, Cu) in the analyzed sample or blank, respectively (in mg);  $m$  is the air-dry weight of the plant sample taken for analysis (in g). These four HMs in particular were studied since anthropogenic contamination with Cu and Pb is widespread; Ni and Zn exist by co-natural processes in city bedrocks.

For data interpretation we applied an index of biological accumulation for each heavy metal ( $I_a$ ):  $I_a = C_p / C_s$ , where  $C_p$  is the HM concentration in a plant or a plant part,  $C_s$  is the content of the HM in soil (Il'in 1991). A value of  $I_a$  from 1 up to 10 indicates intense accumulation of an element by a plant; from 0.1 up to 1 indi-

cates average accumulation; from 0.01 up to 0.1 the element is poorly absorbed; from 0.001 up to 0.01 biological accumulation of the element is absent.

## Experimental design

Dandelion seeds from the same localities (but from several plants) (Table 1), as used for HM analysis, were treated with KMnO<sub>4</sub> for 5 min and germinated in Petri dishes (about 150 seeds per dish) in Knop's medium (1865) (20 ml per dish) supplemented with sublethal (1 mM) or physiological (10 μM) levels of Ni<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup> or Cu<sup>2+</sup> under constant conditions (14°C, 10 h photoperiod, illumination about 80 μM photons·m<sup>-2</sup>·s<sup>-1</sup>) within 14 days. Knop's medium was prepared as follows: 1% Ca(NO<sub>3</sub>)<sub>2</sub> – 8 ml, 5% KH<sub>2</sub>PO<sub>4</sub> – 4 ml, 10% KNO<sub>3</sub> – 2 ml, 1% MgSO<sub>4</sub>·7H<sub>2</sub>O – 2 ml, 10% KCl – 1 ml, 0.8% Fe citrate – 5 ml per liter of distilled water. On the 7<sup>th</sup> and 14<sup>th</sup> day the length of axial organs (roots and shoots) and seed germination capacity (%) were measured.

To assess the tolerance of dandelion seedlings to HM we applied a Wilkinson tolerance index (WTI):  $I_t = (l_{me} / l_c) \times 100\%$ , where  $l_{me}$  indicates the increase in root growth in a metal ion solution and  $l_c$  is the increase in root growth in the control (Koorneeff *et al.* 1997).

All experiments were conducted in triplicate, and each experiment consisted of 100-200 seeds or seedlings for each experimental variant. For all measurements averages and standard errors were calculated by standard mathematical methods using Microsoft Excell 2000, Biostat, and Statistica v. 2.6. The differences between the means were assessed by Tukey's method at  $P = 0.05$ .

## RESULTS AND DISCUSSION

### Dandelion seed mass depends on ecotope

HMs are strong stress factors and one of the main toxins to plants and have a major impact on the environment. Some plants are capable of adapting to increased soil HM contents because of natural geochemical anomalies (Schat and Vooijs 1997) and these adaptations can be genetically fixed (Briat and Lebrun 1999). However, there is still no evidence of any species generating HM-tolerant populations in conditions of technogenic abnormalities. Moreover, HM toxicity has not been studied in many weeds (ruderal species, i.e. growing in waste places) at early ontogenic stages. We investigated the response patterns in *T. officinale* seedlings with Pb, Cu, Zn and Ni. Seeds were collected from differently contaminated areas of Saransk (Table 1).

The mass of 1000 seeds is a parameter that characterizes seed quality. The mass of 1000 dandelion seeds, collected from 10 different sites, is shown in Fig. 1. There is a trend for seed mass to increase from country-side regions to industrial zones. Seeds from the northern industrial zone had the greatest nutrient reserve. 1000-seed mass from this territory was  $873 \pm 41$  mg, but from the control area (sampling point 1) it was only  $373 \pm 50$  mg. The 1000-seed mass from the suburban area was comparable with that of the control. However, the samples from southwest forest territory (sample location 4) exceeded the control ( $453 \pm 22$  mg). The average values of the parameters are shown in Fig.

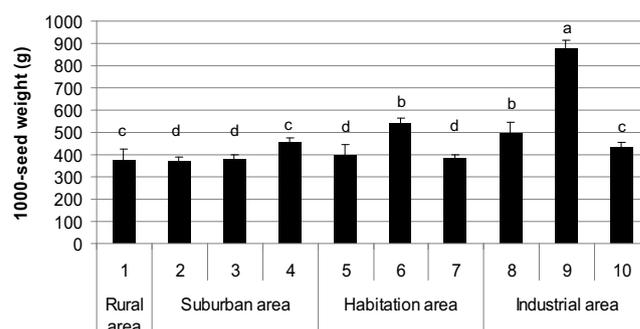


Fig. 1 1000-seed weight of dandelion depends on the ecotope.

Table 1 Sample points in different ecotypes of Saransk and surroundings.

Ecological zones	Ecotypes
Rural area	1 Country forest (15 km to the northeast from Saransk)
Suburban zone	2 Forest near municipal "Zarechnyj" borough
	3 Forest in municipal "Svetotehstroj" borough
	4 Forest near municipal "Southwest" borough
	5 "Southwest", a habitable area
Habitation zone	6 "Zarechnyj", a habitable area
	7 The Insar river floodplain, a storage zone
	8 Central industrial zone
Industrial zone	9 Northern industrial zone
	10 Urban treatment facilities

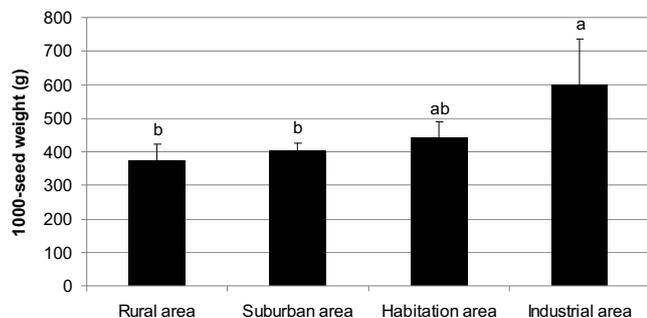


Fig. 2 The average values of 1000-seed weight parameter in different anthropogenic loading areas.

2. Although no suitable explanation could be found in the literature, perhaps this is a survival mechanism to increase the sink in seeds before the plant dies through contamination. The greater the nutrient reserve, the larger is the volume of the seed endosperm, and thus the plants will have a better chance of germination. As the parameter of 1000-seed mass is genetically determined (Brubaker 1966), it is reasonable to assume that the dandelion genotypes growing in polluted and clean areas are different from each other.

Probably, there was a generation of edaphic ecotypes of these species that are resistant to anthropogenic effects. To verify this assumption the weed seeds, collected from different sites of Saransk City with diverse HM levels, were germinated at 14°C and a 10-hr photoperiod on water or 1

mM or 10 µM HM solutions. Germinating capacity of the seeds as well as the length of roots and hypocotyls was determined.

### Germinating capacity of seeds in the presence of HM ions

Toxicity HM for wild-growing species is not studied practically, especially at early stages of an ontogenesis. Germination is the first and most important stage of development of a plant.

Dandelion seeds collected from different contaminated areas had variable germination capacity both in water and in HM solution (Table 2).

*T. officinale* seeds from contaminated areas (sampling points 8, 9 and 10) possessed higher germination capacity than non-contaminated sites. Thus in variants with low (physiological) Pb<sup>2+</sup> and Ni<sup>2+</sup> concentrations germinability was significantly higher than the control but at a sublethal level (1 mM) it remained at or slightly exceeded the level of the control. The difference to the control treatment (i.e. water) was significant only for the smallest Pb and Zn concentrations. Thus exposure to 10 µM Zn<sup>2+</sup> resulted in a 67% germinating capacity and was, at 10 µM Pb<sup>2+</sup>, 60%. Germinating capacity of suburban zone seeds exceeded several times the control treatment when exposed to Pb<sup>2+</sup> ions at the same concentration (sampling point 4 = 1.5-fold; sampling point 3 = 2.9-fold; sampling point 2 = 1.7-fold).

Germinating capacity of seeds in most cases was Cu < Zn < Ni < Pb (excluding seeds from sampling point 6). In

Table 2 Effect of HM treatment on germination of *Taraxacum officinale* seeds, collected from different ecological zones of Saransk City.

Sampling points	Germinating capacity (%)								
	Water	Cu		Zn		Ni		Pb	
		10 µM	1 mM	10 µM	1 mM	10 µM	1 mM	10 µM	1 mM
1	40 ± 2 a	30 ± 4 bc	14 ± 3 c	67 ± 5 a	20 ± 3 b	37 ± 4 b	37 ± 4 ab	30 ± 2 d	60 ± 5 a
2	18 ± 2 d	15 ± 3 d	13 ± 2 c	26 ± 5 cd	17 ± 3 b	27 ± 4 c	26 ± 3 c	18 ± 2 e	30 ± 4 c
3	22 ± 2 d	18 ± 4 cd	22 ± 3 ab	40 ± 5 bc	29 ± 3 a	22 ± 4 c	21 ± 3 cd	40 ± 5 bcd	64 ± 6 a
4	35 ± 1 b	25 ± 4 bc	12 ± 2 c	13 ± 3 e	27 ± 3 a	35 ± 4 bc	34 ± 3 bc	41 ± 2 c	54 ± 5 a
5	35 ± 3 abc	25 ± 3 c	24 ± 4 ab	32 ± 3 c	15 ± 2 b	26 ± 4 c	18 ± 3 d	50 ± 6 b	41 ± 4 b
6	37 ± 3 abc	34 ± 5 b	15 ± 3 c	39 ± 5 bc	31 ± 4 a	32 ± 4 bc	27 ± 3 c	22 ± 2 e	21 ± 3 d
7	33 ± 4 bc	29 ± 4 bc	22 ± 3 ab	24 ± 4 cd	18 ± 3 b	28 ± 4 c	21 ± 3 cd	58 ± 5 ab	22 ± 3 d
8	20 ± 2 d	24 ± 4 c	22 ± 3 ab	24 ± 3 d	20 ± 2 b	30 ± 3 bc	16 ± 2 d	31 ± 4 d	29 ± 4 cd
9	30 ± 3 c	52 ± 5 a	28 ± 3 a	48 ± 4 b	29 ± 3 a	55 ± 5 a	43 ± 5 a	34 ± 3 d	35 ± 4 bc
10	22 ± 3 d	23 ± 3 c	21 ± 2 b	22 ± 4 d	21 ± 3 b	31 ± 5 bc	29 ± 4 bc	69 ± 6 a	54 ± 5 a

Mean ± SE. n = 25

Note: different letters indicate significant differences at P ≤ 0.05 separately for each variant of the experiment, i.e. within a column.

Table 3 HM effects on 7-day length of dandelion axial organs.

Sampling point	Length (mm)								
	Water	Cu		Zn		Ni		Pb	
		10 µM	1 mM	10 µM	1 mM	10 µM	1 mM	10 µM	1 mM
1	35 ± 5 ab	30 ± 2 ab	9 ± 2 b	32 ± 3 bc	20 ± 5 ab	21 ± 4 d	4 ± 2 bc	38 ± 3 b	28 ± 5 abc
2	31 ± 4 b	27 ± 3 ab	4 ± 1 b	37 ± 4 a	11 ± 3 ab	38 ± 5 a	4 ± 1 bc	32 ± 3 bc	20 ± 2 ab
3	19 ± 1 d	11 ± 1 d	7 ± 1 b	20 ± 1 e	7 ± 3 c	13 ± 2 e	5 ± 2 bc	38 ± 1 b	14 ± 2 d
4	15 ± 1 d	7 ± 2 d	6 ± 2 ab	22 ± 4 bc	15 ± 3 a	30 ± 4 bc	7 ± 1 ab	30 ± 2 bc	22 ± 3 a
5	17 ± 2 d	13 ± 4 cd	4 ± 3 b	21 ± 1 e	6 ± 1 c	18 ± 2 d	3 ± 2 bc	22 ± 1 d	14 ± 1 d
6	18 ± 3 d	10 ± 2 cd	5 ± 2 ab	21 ± 4 bc	13 ± 3 ab	19 ± 3 c	5 ± 2 abc	40 ± 5 ab	21 ± 3 ab
7	29 ± 2 bc	26 ± 3 b	6 ± 2 b	13 ± 1 f	10 ± 4 bc	20 ± 5 cde	5 ± 1 bc	28 ± 4 c	13 ± 1 d
8	22 ± 4 cd	26 ± 4 ab	5 ± 2 ab	21 ± 3 bc	5 ± 1 c	42 ± 5 a	1 ± 1 c	25 ± 3 cd	15 ± 3 bc
9	24 ± 1 c	18 ± 3 c	15 ± 4 ab	21 ± 2 e	18 ± 3 ab	28 ± 1 b	3 ± 1 c	29 ± 2 c	27 ± 1 bc
10	30 ± 5 bc	21 ± 3 b	3 ± 1 b	22 ± 4 bc	5 ± 2 bc	24 ± 4 bc	4 ± 2 bc	26 ± 3 bcd	6 ± 2 d
1	30 ± 4 bc	27 ± 2 b	10 ± 3 b	31 ± 2 c	15 ± 1 b	30 ± 2 b	4 ± 3 bc	39 ± 4 b	24 ± 2 c
2	18 ± 3 d	13 ± 2 c	4 ± 2 b	18 ± 4 c	6 ± 2 bc	22 ± 5 c	3 ± 1 bc	23 ± 3 d	5 ± 1 d
3	26 ± 3 c	20 ± 5 bc	9 ± 3 b	26 ± 2 d	19 ± 1 a	23 ± 1 cd	6 ± 1 b	35 ± 1 b	26 ± 4 bc
4	24 ± 2 c	22 ± 2 b	9 ± 2 a	25 ± 3 bc	5 ± 1 c	36 ± 3 ab	6 ± 2 ab	49 ± 4 a	14 ± 2 c
5	36 ± 3 ab	32 ± 3 ab	16 ± 2 a	44 ± 3 a	23 ± 3 a	40 ± 5 a	8 ± 1 ab	49 ± 4 a	32 ± 1 a
6	40 ± 1 a	30 ± 3 a	4 ± 2 b	25 ± 3 bc	11 ± 2 ab	30 ± 2 b	5 ± 1 b	45 ± 5 a	10 ± 3 cd
7	38 ± 2 a	34 ± 4 a	18 ± 3 a	40 ± 2 a	19 ± 1 a	43 ± 3 a	10 ± 2 a	46 ± 3 ab	29 ± 1 b
8	35 ± 2 b	33 ± 4 a	4 ± 1 b	26 ± 2 b	9 ± 2 bc	28 ± 4 bc	9 ± 2 a	48 ± 5 a	10 ± 3 cd
9	31 ± 4 bc	28 ± 3 ab	19 ± 2 a	35 ± 1 b	21 ± 2 a	39 ± 4 a	7 ± 1 ab	48 ± 5 ab	33 ± 2 a
10	22 ± 1 cd	22 ± 2 b	2 ± 1 b	23 ± 3 bc	8 ± 3 bc	20 ± 2 c	2 ± 1 c	23 ± 4 cd	6 ± 2 d

Note: upper value for each sampling point is average shoot length, while the lower value is average root length (Mean ± SE. n = 25).

Different letters indicate significant differences at P ≤ 0.05 (separately for each variant of the experiment – metal or water – and for shoots and roots).

all variants, germinating capacity was only significantly ( $P < 0.05$ ) repressed when exposed to HM sublethal (1 mM) concentrations. The most toxic effect on germinability was in the  $\text{Cu}^{2+}$  ion treatment. The seedcoat is able to selectively transport ions in medium. Possibly, such an effect (i.e. significant difference in seed germinating capacity to the control treatment) is related to the role of the seedcoat barrier (Obrucheva and Antipova 1997).

These findings create the perception that dandelion seed populations from contaminated zones of Saransk are adapted to increased soil HM content.

### Growth of dandelion axial organs in HM solutions

In the literature there is no data about the effects of HMs on the growth of wild plants. However, even within existent studies within a given species the influence of HM depends not only on the species, population and age of plants, but also on the concentration used, exposure time, and other factors beyond the discussion of this study. Since this is the first such study using wild plants, we decided that a study that compared the effect of Cu, Pb, Ni and Zn ions on dandelion axial organ growth (Table 3) would be very informative.

High HM concentrations (1 mM) inhibited *T. officinale* root and shoot growth. The greatest toxic effect was when  $\text{Cu}^{2+}$  ions were applied. In all variants when young plants were exposed to  $\text{Cu}^{2+}$  ions, the axial organs were shorter than the control.  $\text{Pb}^{2+}$  ions stimulated seedling growth (except roots from sample point 5). And in variants where the concentration of Pb was at physiological (10 mM) or sublethal (1 mM) concentrations the roots and shoots were significantly longer than the control. In most cases root growth was more resistant to HMs than shoots indicating how the roots serve as a physical barrier.

Physiological HM concentrations (10  $\mu\text{M}$ ) had stimulating effects on shoot growth of dandelion plants sampled from industrial zones. For example, the shoot length of seedlings from the central industrial zone (sampling point 8), when exposed to  $\text{Ni}^{2+}$  ions, was 40 mm, to  $\text{Zn}^{2+}$  ions, 44 mm, and to  $\text{Pb}^{2+}$  ions, 49 mm, while in the control it was only 36 mm. The same concentrations inhibited seedling growth from relatively unpolluted areas. The length of axial organs of seedlings from suburban zones were insignificantly different or below that of control plants (in most cases).

### Wilkinson tolerance index for dandelion

The WTI was calculated (Table 4) to assess the toxic effect of HM concentration on root growth.

Plants collected from industrial zones developed more tolerance at physiological concentrations of all HMs investigated (i.e. higher WTI). Plants exposed to lethal concentrations of Pb and Ni had the highest tolerance index. For example, the WTI of plants from the central industrial zone (sampling point 8) in  $\text{Pb}^{2+}$  solution was 64%, in an  $\text{Ni}^{2+}$  ion

solution, 53%.

Young plants exposed to  $\text{Pb}^{2+}$  ions were observed to have the highest WTI among seedlings from all sampling points. For example, the WTI for plants of the country forest at physiological concentrations of  $\text{Pb}^{2+}$  was 141%, of  $\text{Ni}^{2+}$ , 125%, of  $\text{Zn}^{2+}$ , 9%, and of  $\text{Cu}^{2+}$  ions, 87%; and at sublethal concentrations of  $\text{Pb}^{2+}$  ions it was only 38%, of  $\text{Ni}^{2+}$ , 24%, of  $\text{Zn}^{2+}$ , 16%, and of  $\text{Cu}^{2+}$ , 18% (Table 4).  $\text{Pb}^{2+}$  ions appeared to be least toxic to dandelion plants.

Cu was most toxic to plants, as the WTI of dandelion seedlings growing in  $\text{Cu}^{2+}$  ions was low: at 10  $\mu\text{M}$   $\text{Cu}^{2+}$  it was 146% for plants from the central industrial zone, and at 1 mM it was only 38% whereas at 10  $\mu\text{M}$  of  $\text{Ni}^{2+}$  and of  $\text{Zn}^{2+}$  the WTI averaged 170 and 160%, and at 1 mM, 47 and 53%, respectively.

So, the toxicity of the studied HMs, based on physiological and sublethal concentrations, showed a decrease in ranking:  $\text{Cu} > \text{Zn} > \text{Ni} > \text{Pb}$ .

It is possible to suggest that the greatest tolerance were of plants from the central and northern industrial zones. Plants from suburban areas (sampling points 2, 3 and 4) were least adapted to the presence of HMs in the environment. WTI calculations are well correlated with our data on total soil pollution of Saransk functional zones (Bashmakov and Lukatkin 2002). The concentrations of HM researched (Pb, Ni, Zn, Cu) in country and suburban soils quite often exceeds the concentrations in industrial areas. This proves that the predominance path of contaminant migration is aerogenic (Fig. 3). As a rule, the waterway of pollutant migration assumes that their concentration decreases with increasing distance from the source of pollution. In aerogenic contamination the maximum quantity of the pollution component is carry by wind and deposited at a distance from 5 to 40 relative heights of the pollution source. For example, if a factory chimney height is 200 m the contamination maximum will take place at a distance from 1 to 8 km from it. In our case this is equivalent to the suburban or country areas (Zones 1-4).

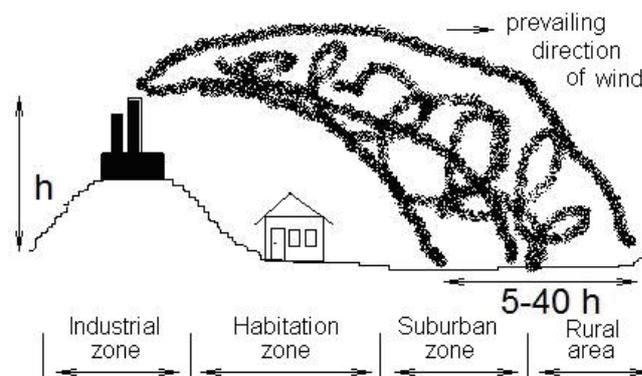


Fig. 3 Relative heights of the pollution source effects on pollutants distribution in aerogenic contamination pathway. h = relative heights of the pollution source.

Table 4 Wilkinson index for the dandelion plants which were grown from seeds, collected from various ecotypes of Saransk.

Sampling point	Index of tolerance (%)							
	Cu		Zn		Ni		Pb	
	10 $\mu\text{M}$	1 mM	10 $\mu\text{M}$	1 mM	10 $\mu\text{M}$	1 mM	10 $\mu\text{M}$	1 mM
1	87 ± 4 bcd	18 ± 1 c	90 ± 1 d	16 ± 1 e	125 ± 6 c	21 ± 1 e	141 ± 3 c	38 ± 2 d
2	58 ± 2 e	21 ± 3 bc	58 ± 6 e	23 ± 4 cd	77 ± 3 f	35 ± 6 c	50 ± 2 h	61 ± 3 ab
3	45 ± 5 f	19 ± 1 c	52 ± 2 e	20 ± 2 de	80 ± 1 f	40 ± 5 bc	99 ± 1 e	55 ± 4 b
4	59 ± 4 e	15 ± 5 cd	55 ± 3 e	17 ± 1 e	79 ± 2 f	23 ± 4 de	68 ± 2 f	21 ± 3 e
5	88 ± 3 b	13 ± 1 d	95 ± 2 cd	21 ± 4 cde	105 ± 4 d	21 ± 2 de	123 ± 2 d	23 ± 4 e
6	82 ± 4 cd	19 ± 4 bc	89 ± 3 d	26 ± 1 c	110 ± 6 d	29 ± 3 cd	140 ± 5 c	43 ± 2 c
7	81 ± 2 d	25 ± 2 b	84 ± 5 d	26 ± 2 c	95 ± 5 e	31 ± 1 c	98 ± 4 e	23 ± 1 e
8	146 ± 6 a	38 ± 4 a	160 ± 2 a	47 ± 4 a	170 ± 7 a	53 ± 3 a	180 ± 6 a	64 ± 1 a
9	83 ± 1 cd	21 ± 2 bc	130 ± 5 b	32 ± 1 b	145 ± 5 b	40 ± 2 bc	150 ± 5 b	54 ± 2 b
10	90 ± 2 b	18 ± 1 c	94 ± 4 cd	21 ± 2 d	123 ± 3 c	25 ± 1 d	150 ± 1 b	51 ± 1 b

Mean ± SE. n = 25

Note: different letters indicate significant differences at  $P \leq 0.05$  (separately for each variant of the experiment).

**Table 5** Contents of heavy metals in axial organs (shoots and roots) of *Taraxacum officinale* depending on ecotype.

Sampling point	HM concentration (mg/kg of dry biomass)					
	Pb	Ni	Cu	Zn	Mn	Fe
1	19.3 ± 6.1 e	33.9 ± 0.5 b	2.8 ± 0.2 f	351.5 ± 10.7 b	138.2 ± 7.9 c	5462.2 ± 111.5 a
	87.9 ± 6.5 a	6.5 ± 0 d	108.2 ± 11.2 d	422.2 ± 25.4 a	37.7 ± 5.5 de	3610.3 ± 115.7 a
2	3.9 ± 0.5 g	3.5 ± 0.4 cd	5.9 ± 0.5 e	8.5 ± 0.5 f	9.0 ± 2.5 h	1107.2 ± 31.1 g
	1.9 ± 0.8 e	6.0 ± 3.1 cde	15.5 ± 5.4 h	7.2 ± 3.1 f	3.8 ± 1.1 g	186.2 ± 19.3 f
3	140.5 ± 8.3 a	1.7 ± 0.1 e	92.9 ± 6.5 c	311.2 ± 22.4 c	102.6 ± 11.2 d	3600.5 ± 222.2 bc
	67.8 ± 9.7 b	4.4 ± 0.7 e	37.2 ± 7.3 g	124.8 ± 12.3 e	28.1 ± 8.2 e	2151.7 ± 23.0 c
4	1.5 ± 0.1 i	2.8 ± 0.4 d	7.8 ± 0.4 d	293.9 ± 19.6 cd	192.0 ± 13.5 b	1639.8 ± 40.1 e
	0.8 ± 0.5 e	6.5 ± 1.2 d	137.6 ± 16.6 c	368.9 ± 25.1 b	213.8 ± 14.5 a	2050.9 ± 85.1 c
5	1.9 ± 0.2 h	2.9 ± 0.2 d	134.5 ± 5.2 b	273.8 ± 9.6 d	29.3 ± 0.4 e	1662.8 ± 61.1 e
	1.3 ± 0.6 e	7.9 ± 2.1 cd	37.9 ± 9.7 fg	120.8 ± 6.4 e	10.2 ± 1.2 f	2260.0 ± 38.5 b
6	8.7 ± 0.3 f	3.7 ± 0.4 c	96.8 ± 3.0 c	179.5 ± 3.2 e	107.0 ± 12.0 d	920.0 ± 42.5 h
	5.9 ± 0.9 c	7.0 ± 3.1 cde	40.7 ± 7.5 fg	8.5 ± 2.3 f	32.3 ± 2.4 e	249.5 ± 12.3 e
7	59.6 ± 4.8 d	1.4 ± 0.3 e	132.3 ± 7.4 b	264.8 ± 18.1 d	17.6 ± 2.3 f	3815.4 ± 70.2 b
	85.3 ± 1.7 a	8.4 ± 0.5 c	54.0 ± 4.5 f	168.7 ± 12.1 d	130.1 ± 9.3 b	2355.4 ± 67.2 b
8	80.8 ± 5.1 c	56.7 ± 6.7 a	147.1 ± 14.5 b	414.1 ± 15.4 a	18.9 ± 1.6 f	1867.5 ± 40.7 d
	6.6 ± 0.5 c	47.8 ± 2.1 a	178.1 ± 8.1 b	254.6 ± 15.2 c	53.3 ± 6.1 c	3505.8 ± 45.5 a
9	7.4 ± 1.1 f	59.9 ± 4.6 a	148.5 ± 9.7 b	433.4 ± 12.8 a	374.5 ± 16.3 a	3462.2 ± 22.1 c
	0.9 ± 0.2 e	49.4 ± 3.4 a	291 ± 10.2 a	249.4 ± 13.1 c	42.2 ± 4.2 d	435.6 ± 9.4 d
10	104.9 ± 10.5 b	3.7 ± 0.2 c	194.6 ± 13.2 a	410.7 ± 20.3 a	5.3 ± 0.1 i	1531.0 ± 55.0 f
	3.4 ± 0.5 d	16.9 ± 2.1 b	76.9 ± 6.7 e	171.7 ± 11.6 d	39.0 ± 3.7 d	423.4 ± 10.5 d

Note: upper value for each sampling point is average shoot length, while the lower value is average root length (Mean ± SE.  $n = 25$ ). Different letters indicate significant differences at  $P \leq 0.05$  (separately for each variant of the experiment and for shoots and roots).

Although a fairly obvious observation, we conclude that the existence of different naturally occurring *T. officinale* edaphotypes on differentially contaminated ecotopes had different tolerance levels to HMs.

### Heavy metal accumulation by dandelion

Many wild grasses are capable of accumulating HMs intensively without any morphological changes or physiological damage and are usually termed “metallophytes” or “hyperaccumulators”. “Pseudometallophytes” are plants capable of accumulating HMs, too although these are able to grow both on uncontaminated and on polluted soils. We estimated the accumulation of HMs in axial organs, namely shoots and roots, of dandelion plants from different ecological zones of Saransk (Table 5). *T. officinale* accumulated Zn, Cu, Ni, Mn more in contaminated areas (sampling points 8, 9 and 10) than Fe and Pb, which accumulated more in uncontaminated areas (sampling points 1 and 3). Fe and Zn were most accumulated, while Ni and Pb were least accumulated; the former are necessary, the latter are unnecessary elements.

According to Clemens (2001), plants turn into hyperaccumulators if they capable to accumulate in their tissues over 0.1% of HMs (on a dry mass basis). The exceptions to the rule are Zn (over 1%), and Cd (over 0.01% of dry weight). So dandelion was thus considered, according to this definition, an Fe-hyperaccumulator in all ecotopes.

To assess the bioconcentration intensity, a relative unit, the index of biological accumulation ( $I_a$ ), was used (Table 6). Cu and Zn were most intensively bioaccumulated; Fe and Mn were moderately bioconcentrated in the majority of ecotopes while Pb and Ni were in the low to moderate category. In some cases (when  $I_a > 1$ ) HM concentration in plant organs was significantly higher than the content in the soils in which they were growing. For example, Zn bioaccumulation in dandelion shoots in sampling points 1, 3, and 5-10; Cu accumulation in roots in ecotopes 1, 4, and 8-10.

Intensive Cu absorption by plants is connected to its relevant role and irreplaceability in plant metabolism (Doncheva 1998). The accumulation of Zn can be explained primarily by its considerable mobility in soils (Bansal *et al.* 1982). Most likely for Ni, the rise in  $I_a$  was connected with the acclimatization of plants to high natural HM contents in the region's soils. Fe accumulation by axial organs of plants was equally high in all ecotopes confirming the presence of strong Fe regulatory mechanisms in higher plants (Guerinot and Yi 1994; Grusak *et al.* 1995; Eide 1996). Pb is a trace

**Table 6** Index of HM bioaccumulation in dandelion axial organs (shoots and roots) from 10 different ecotopes.

Sampling point	Bioaccumulation index					
	Pb	Ni	Cu	Zn	Mn	Fe
1	0.10	0.20	0.10	2.76	0.06	0.09
	0.47	0.04	3.61	3.32	0.02	0.06
2	0.03	0.01	0.09	0.06	0.01	0.03
	0.01	0.02	0.23	0.05	0.02	0.05
3	1.30	0.01	0.74	2.53	0.07	0.09
	0.62	0.02	0.30	1.01	0.09	1.50
4	0.02	0.01	0.16	0.01	0.18	0.03
	0.01	0.03	2.87	4.55	0.20	0.04
5	0.04	0.10	3.13	1.52	0.04	0.03
	0.03	0.03	0.88	0.67	0.01	0.04
6	0.01	0.04	0.05	2.67	0.27	0.02
	0.02	0.02	0.05	0.13	0.08	0.01
7	0.34	0.10	1.27	2.55	0.04	0.11
	0.05	0.03	0.52	0.81	0.33	0.07
8	0.50	0.09	0.90	1.03	0.03	0.03
	0.04	0.08	1.09	0.63	0.07	0.06
9	0.03	0.10	1.25	2.33	0.60	0.07
	0.01	0.11	2.44	0.24	0.07	0.01
10	1.02	0.10	2.56	1.35	0.01	0.03
	0.03	0.06	1.02	0.52	0.06	0.01

Note: upper value for each sampling point is average shoot length, while the lower value is average root length (Mean.  $n = 25$ ).

element with an insignificant physiological role that enters roots without resistance: the intensity of Pb bioaccumulation increases with the total anthropogenic load of the soil (Reutse and Kyrstja 1986).

HM accumulation did not show a clear pattern in underground and aboveground organs of plants on polluted and uncontaminated ecotopes (Tables 5, 6). For example, in the northern industrial zone (sample point 8) *T. officinale* took up Zn much less into shoots ( $I_a = 0.24$ ) than roots ( $I_a = 2.33$ ). In most cases (Table 6) Pb was a weakly bioaccumulated element, but on treatment facilities (sample point 10)  $I_a$  (Pb) for roots was 1.02, but for shoots it was 0.03.

It is possible that under heavy soil contamination plants guard themselves from excessive soil HMs, storing them in roots. This was evident on technogenic sludge (industrial zones) where the majority of HMs accumulated in roots of plants.

*T. officinale* shoots concentrated Ni and Cu in suburban areas. Cu absorption in shoots was more intensive in the southwest forest ( $I_a = 2.87$ ) than on treatment facilities ( $I_a =$

1.02) and in the central industrial zone ( $I_a = 1.09$ ). Dandelion shoots from the country forest were accumulators of Cu ( $I_a = 3.61$ ), Zn ( $I_a = 3.22$ ) and Pb ( $I_a = 0.47$ ).

*T. officinale* accumulated the four HMs investigated under maximum anthropogenic load primarily in the roots. There are probably two groups of edaphotypes: those from industrial and habitation zones, the phytostabilizers (concentrate and keep HMs in roots) and those from suburban zones, the phytoextractors (concentrate HMs in aboveground organs).

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