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# Accumulating capacity of different varieties of rapeseed under conditions of anthropogenic pollution of soils by heavy metals

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**Abstract.** The results of a study of the content of heavy metals in the soil, underground and elevated parts of three varieties of rapeseed grown on soil samples from reference and anthropogenically disturbed sites are presented. Under the conditions of increasing chemical pollution, a decrease in the level of accumulation and transition of heavy metals has been established, which indicates the presence of protective mechanisms that begin to work in the area with high content of toxicants in the soil. According to a set of indicators characterizing the phytoremediation potential (resistance to loads, accumulating abilities), the Highlight variety is preferable for phytostabilization of sites contaminated with heavy metals.

## 1. Introduction

Anthropogenic activities such as mining, burning fossil fuels, the use of fertilizers, metal processing lead to the accumulation of heavy metals (HM) in the environment [1].

Heavy metals are not biodegradable. An increase in their content in soil and water poses a danger to the environment and human health, as they are concentrated in the tissues of living organisms and exhibit phytotoxic, cytogenetic, and biochemical effects [2].

Various engineering methods are used to restore contaminated soils: sequestration (removal of the most contaminated surface layer and its placement in landfills), dilution (plowing for mixing with less contaminated deeper layers of the soil), earth mulching (covering with clean soil), chemoextraction (washing the soil with special reagents), electrokinetic remediation (effect of direct electric current on the contaminated layer) [3,4].

However, the use of these non-biological methods destroys the biotic components of the soil, which is technically difficult and expensive. An alternative technology is phytoremediation, which uses the ability of plants to absorb, translocate, bioaccumulate, store, and degrade HM [5-8].

Plants with high phytoremediation potential should have good ability to accumulate HM intended for extraction, rapid growth and large biomass; a suitable plant phenotype for easy harvesting; tolerance to growing conditions and the possibility of further utilization of the grown biomass [9].



Based on these circumstances, rapeseed - *Brassica napus* L., which belongs to the genus *Brassica* L., the family Brassicaceae, like most hyperaccumulators known at present, was chosen as the object of research in this work [10].

This study is devoted to the study of the accumulation of heavy metals by different varieties of rapeseed to assess their phytoremediation potential.

## 2. Materials and methods

The objects of the study were rapeseed raw materials (*Brassica napus*) of three varieties (table 1) grown in 2018 on soils selected in the zone of influence of the following enterprises: Karabashmed CJSC (1.5 km from the enterprise), Satkinskiy Chugunoplavil'nyy Zavod JSC (2 km from the enterprise) and Uchaly Mining and Metallurgical Combine OJSC (1.2 km from the enterprise). The reference sample was rapeseed samples grown in the Blagovarsky district, taken outside the scope of local anthropogenic impact. Mineral fertilizers in the form of ANP fertilizer in an amount of N120P120K120 were introduced into all variants of the experiment (including reference ones).

Given the high concentrations of heavy metals in the selected soil samples, to reduce its toxicity, rapeseed was grown in the soil prepared by mixing 70% of the soil taken in the zone of influence of enterprises with 30% of the soil in the reference site. The content of gross forms of heavy metals (Mn, Fe, Cu, Zn) in soil and plants was determined by the atomic absorption method after acid decomposition.

**Table 1.** Description of rapeseed varieties.

Name	Description	Hybrid/Variety
Builder	Mid-season ripening hybrid of spring rapeseed of a new generation, providing a stable crop in unstable climatic conditions.	00-spring rapeseed hybrid
Brander	Mid-early ripening hybrid of spring rapeseed of intensive type with high yield potential.	00-spring rapeseed hybrid
Highlight	A linear spring rapeseed variety that forms small, compact plants with very early ripening	00-spring rapeseed variety

To detect polysaccharides in the shoots of rapeseed, qualitative reactions with aqueous extraction were carried out. Water extraction was prepared according to the method of quantitative determination of polysaccharides: 30 ml of alcohol 96% was added to 10 ml of solution A and mixed. Flocs were observed which precipitated while standing. This precipitate indicates the presence of polysaccharides in the material.

Quantitative determination of polysaccharides was carried out by the gravimetric method followed by weighing the precipitate. Ethyl alcohol 70% was used as precipitant.

To analyze the obtained data, the rank correlation coefficients (Spearman's coefficient) were calculated. Spearman's rank correlation coefficient is a nonparametric method that is used to statistically study the relationship between phenomena [11]. In this case, the actual degree of parallelism between the two quantitative series of the studied characteristics is determined, and the tightness of the established relationship is estimated using a quantitatively expressed coefficient [12]. The calculations of the rank correlation coefficient (Spearman's coefficient) were carried out according to the formula:

$$P = 1 - \frac{6 \sum (R_x - R_y)^2}{n(n^2 - 1)} \times 100\% \quad (1)$$

where  $P$  — Spearman's coefficient;  $R_x$  — ranks of values of the content of heavy metals in soil samples;  $R_y$  — ranks of values of the content of heavy metals (or the content of polysaccharides) in the elevated part of rapeseed;  $n$  — number of observations.

If  $P = \pm 1$ , then the connection between the parameters is functional;  $P$  from  $-0.3$  to  $0$  or from  $0$  to  $0.3$  - indicators of weak connection tightness;  $P$  from  $-0.6$  to  $-0.3$  or from  $0.3$  to  $0.6$  - indicators of moderate connection tightness;  $P$  from  $-1$  to  $-0.6$  or  $0.6$  to  $1$  - indicators of high connection tightness;  $P = 0$  - no connection.

To assess the total soil pollution and to determine the level of toxic load on plants, the integral indicator was used:

$$S_i = \frac{1}{n} \sum_i \frac{C_i}{C_f}, \quad (2)$$

where  $C_i$  – the concentration of elements that are considered as priority pollutants in technologically disturbed areas,  $C_f$  – the content of corresponding metals in the objects of the reference zone,  $n$  – number of elements included in the analysis [13].

### 3. Results and discussion

#### 3.1. The content of heavy metals in the soil

The study found that the average content of heavy metals in the reference soil sample and the soil of anthropogenically disturbed sites on average varies from 13750 to 24469 mg/kg for iron, from 34 to 755 mg/kg for zinc, from 15.4 to 1469 mg/kg for copper, and from 618 to 894 mg/kg for manganese (table 2).

**Table 2.** Gross content of heavy metals in the soil (mg/kg) and the level of total toxic load  $S_i$ .

Sampling place	Fe	Zn	Cu	Mn	Si
Karabash	24469	755	1469	894	27.65
Satka	24275	43	23	649	1.25
Uchaly	23938	34	116	805	2.78
Reference	13750	63	15,4	618	1.00
APC	25000*	55	33	1500	-

Notes: \* - percentage abundance in the crust; the excess of the APC is highlighted in the table.

From table 2 it can be seen that the zinc content exceeds the approximate permissible concentration (APC) in the reference sample and in the soil of Karabash. In copper, the excess of APC is in the soils of Karabash and Uchalov. The content of iron and manganese does not exceed the standards in all soils.

As an integral indicator of soil pollution, the level of total toxic load calculated by formula 2 was used, which in the studied sites varied from 1 to 27.7 rel. units (table 2). Based on this criterion, the studied territories were assigned to the reference ( $S_i = 1.0$ ; 1.04 and 1.25 rel. units), buffer ( $S_i = 2.78$ , 7.37 rel. units) and impact ( $S_i = 26.43$  and 27.65 rel. units) pollution zones.

#### 3.2. The content of heavy metals in rapeseed

The gross content of heavy metals in rapeseed samples of three varieties is presented in tables 3 - 5.

**Table 3.** Gross content of heavy metals in Highlight rapeseed, mg/kg.

Toxic load rel. units	Plant part	Fe	Zn	Cu	Mn
27.65	Shoots	344	177	60	48
	Roots	450	90	54	31
26.43	Shoots	483	107	45	47
	Roots	1977	123	118	111
7.37	Shoots	1475	10.69	1.91	48.75
	Roots	2109	36	6.6	67
2.78	Shoots	3156	72	13	238
	Roots	3736	65	9.6	97
1.25	Shoots	1616	46	4.7	443
	Roots	1085	18	3	26
1.04	Shoots	468	20.1	3.16	28.13
	Roots	2322	16	5.4	83
1	Shoots	376	77	12	34
	Roots	1973	103	86	88

**Table 4.** Gross content of heavy metals in Builder rapeseed, mg/kg.

Toxic load rel. units	Plant part	Fe	Zn	Cu	Mn
27.65	Shoots	205	144	27	30
	Roots	3007	272	388	216
26.43	Shoots	232	140	24	29
	Roots	2924	165	12	137
7.37	Shoots	1565	55	5.6	54
	Roots	2230	55	9.4	86
2.78	Shoots	669	149	4.3	117
	Roots	718	61	6.2	33
1.25	Shoots	1031	35	3.5	77
	Roots	8150	30	6.6	217
1	Shoots	831	21	3.8	53
	Roots	6859	38	13	275

**Table 5.** Gross content of heavy metals in Brander rapeseed, mg/kg

Toxic load rel. units	Plant part	Fe	Zn	Cu	Mn
27.65	Shoots	106	48	8	21
	Roots	3455	183	233	202
26.43	Shoots	141	89	2.3	8.4
	Roots	1212	96	81	62
7.37	Shoots	1225	23	4.5	51

	Roots	2060	45	7.8	75
2.78	Shoots	1622	56	7.4	118
	Roots	7906	93	17	229
1.25	Shoots	663	16.4	2.3	7.9
	Roots	1806	32	4.4	79
1	Shoots	628	19	3.9	46
	Roots	2692	33	5.4	103

Analysis of the values given in tables 3-5 showed that all the studied varieties of rapeseed mainly accumulate heavy metals in the roots. The limited ability of plants to accumulate heavy metal ions in contaminated areas indicates the presence of protective mechanisms that begin to work in the area with high content of toxicants in the soil [14]. Perhaps, in rapeseed, regardless of the level of soil contamination, “similar” mechanisms work, regulating the intake of heavy metals into the plant organism and promoting their detoxification, which reduces the transport of ions into the shoots.

Let us assess the close relationship between the content of heavy metals in the soil and parts of plants. The values of the rank correlation coefficients calculated according to formula 1 are shown in table 6.

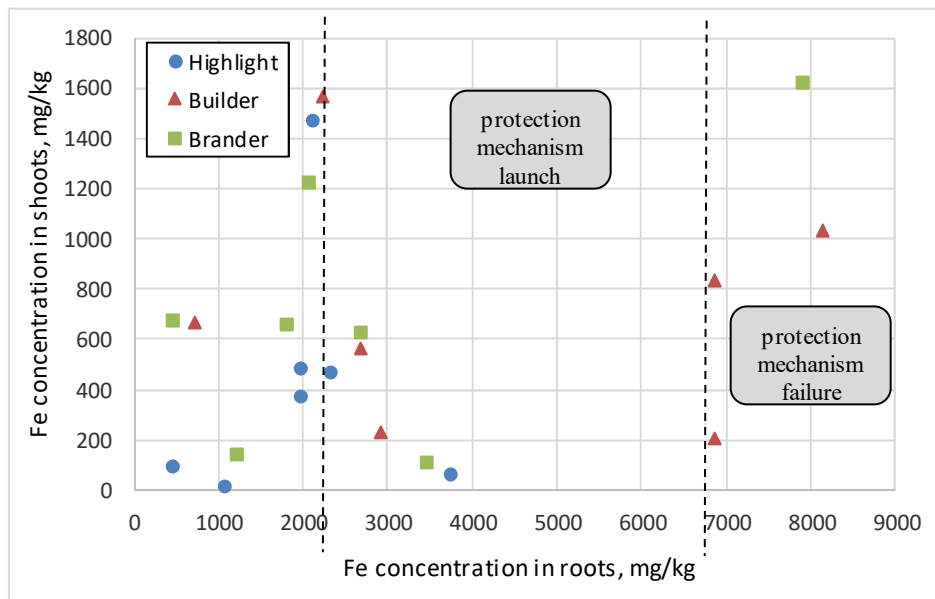
**Table 6.** Rank correlation coefficients (Spearman's coefficients) of the content of heavy metals in rapeseed shoots and soil.

Grade	Fe	Zn	Cu	Mn
Shoots-Soil				
Highlight	0.40	1.00	0.20	0.60
Builder	-0.80	0.40	0.80	0.00
Brander	-0.40	0.40	0.10	0.00
Roots-Soil				
Highlight	-0.20	0.80	0.40	0.20
Builder	-0.20	0.40	-0.20	-0.40
Brander	-1.00	0.40	0.80	-1.00

Let us determine the “threshold” concentrations of heavy metals in rapeseed roots, i.e. concentrations at which an increase in the content of heavy metals in underground parts does not lead to an increase in their content in elevated parts, i.e. plant defense mechanisms are triggered (Table 7). However, a further increase in metal ions in the underground parts can lead to a violation of the protective system and again to an increase in their concentrations in the elevated parts (Figure 2).

**Table 7.** Threshold concentrations of heavy metals in the roots of the studied plant species, mg/kg.

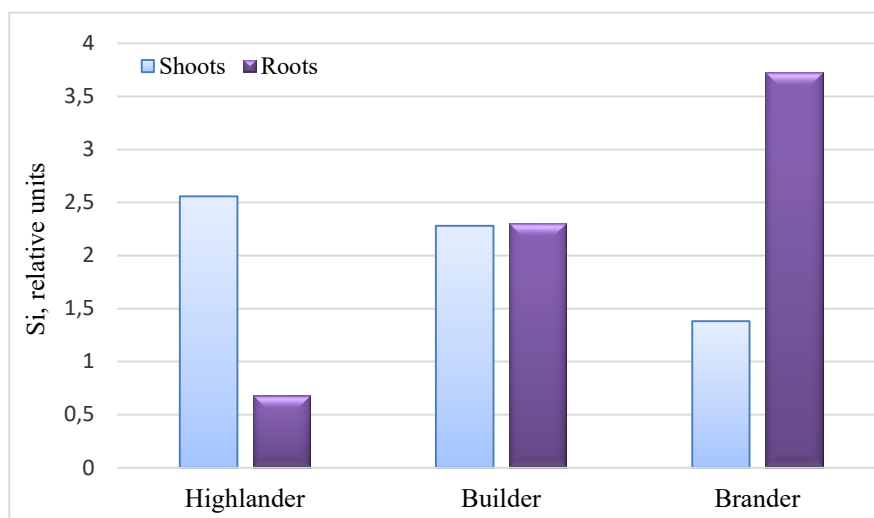
Variety	Fe	Zn	Cu
Highlight	2109	90	54
Builder	2230	61	12
Brander	2060	96	17



**Figure 1.** The relationship between the iron content in the roots and shoots of rapeseed.

The sequence of growth of threshold concentration values is consistent with the arrangement of elements in the phytotoxicity series of heavy metals:  $\text{Cu} > \text{Zn} > \text{Fe}$ . Maximum threshold concentrations are characteristic for iron; for copper and zinc, they are several orders of magnitude lower and relatively close. The earliest inclusion of protective mechanisms is characteristic of the Builder variety. High threshold concentrations were noted for Highlight, which indicates its greater metal resistance and accumulative abilities.

The reflection of the inclusion of protective mechanisms is the toxic load on the plants, the integral assessment of which was carried out using the indicator calculated by formula 2 (Figure 2).



**Figure 2.** Toxic load on the underground and elevated parts of plants.

The obtained values of the Sn coefficient show that the elevated part of the Highlight rapeseed and the underground part of the Brander rapeseed experience the maximum toxic effect. The Builder variety is characterized by an average level of toxic load of approximately the same magnitude in shoots and roots.

### 3.3. The content of polysaccharides in rapeseed

Let us assess the effect of concentrations of heavy metals in the soil on the change in the polysaccharide content in the studied varieties of rapeseed (table 8).

**Table 8.** Rank correlation coefficients (Spearman's coefficients) of the content of heavy metals and polysaccharides in rapeseed shoots.

Metal	Fe	Zn	Cu	Mn
Highlight	0.60	0.40	0.40	0.80
Builder	-0.20	1.00	1.00	0.40
Brander	-0.40	1.00	0.30	0.40

An analysis of the data in Table 8 shows that the relationship between the content of heavy metals and polysaccharides in the elevated part of rapeseed is positive for most elements and varieties. This suggests that heavy metals stimulate the synthesis of polysaccharides: the higher the concentration of metal elements in rapeseed shoots, the more polysaccharides are formed in them. A negative Spearman's coefficient is observed only for iron in the Builder and Brander varieties, which is explained by the high tolerance of most plant species to an excess of iron in the environment and its high biological need due to the need for the functioning of a number of vital enzymes.

Assessing the results obtained, it should be noted that an increase in the level of sugars in plant organs under stress is usually associated with adaptive responses in the form of activation of glycolysis. The established significant increase in the content of polysaccharides in rapeseed shoots in response to heavy metals is consistent with the well-known ideas about the degradation of pools of spare polysaccharides under stress or increased gluconeogenesis and glyoxylate cycle, which correlates well with the functional role of sugars as compatible osmolytes in case of water potential disturbances in plant cells [15].

## 4. Conclusions

Thus, the highest stability and ability to accumulate heavy metals from the soil is characteristic of Highlight rapeseed. Given the tendency for metals to accumulate mainly in the roots, it is more advisable to use this type of rapeseed for phytostabilization of soils contaminated with heavy metals. However, when cleaning, it is necessary to take into account the level of soil contamination, since the mechanism of the process of absorption of pollutants will depend on this indicator. At low concentrations of heavy metals, they can accumulate in the elevated part. The subsequent increase in the concentration of ions in the underground parts triggers protective mechanisms that impede their movement into the elevated part of the plant. The physiological meaning of this is to lower the concentrations of chemical elements in those areas where the processes of biosynthesis are most active. For example, delayed absorption of heavy metals by roots, synthesis of enzymes resistant to heavy metals [9]. A further increase in the content of metal ions in the underground parts can lead to the failure of this system, and their concentration in the shoots will increase.

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