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# Cadmium tolerance and its absorption ability in fibre flax and linseed varieties

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**Abstract:** Cadmium pollution now is one of the main environmental problems. Growing of plants hyper accumulators of Cd can help to remediate the polluted soils. Species *Linum usitatissimum* L. is one of such crops. Six fibre flax and two linseed varieties were evaluated for their resistance to different cadmium sulfate 8/3-hydrate $3CdSO_4 \times 8H_2O$ concentrations at the seedlings stage in the Jacobsen apparatus. After this, two fibre flax and two linseed varieties were selected and tested for adult plants' cadmium resistance and absorption ability. Cadmium concentrations were analysed in the ashes of adult plants by x-ray fluorescence analyzer BRA – 18. The presence of pollutants did not influence flax germination ability, but tested genotypes showed different degrees of cadmium resistance. They also differed in dynamics of growth and development suppression affected by Cd contamination, which probably is caused by different mechanisms of resistance. Fibre flax varieties Svetoch and Priziv 81 appeared to be more susceptible to pollution and absorb less cadmium per mass unit than linseed varieties Voronezhskiy 1308 and Bakhmalskiy 1056. So, high cadmium absorption ability of the plant provides its relative resistance to contaminant toxic influence by immobilization of metal ions. Obtained results also show the possibility of selection of genotypes with high cadmium resistance and absorption ability.

**Key words:** Cadmium accumulation, cadmium tolerance, fibre flax, linseed, soil remediation

## 1. Introduction

Though heavy metals, including cadmium, appear in the upper soil layers by natural tectonic processes, nowadays industrial development of mankind resulted in the outstanding cadmium pollution of the environment. The degree of this pollution increased dramatically in the 20th century and this process is continuously escalating. Now cadmium is the main soil contaminant among heavy metals (Cadmium tolerance in plants, 2019).

Evaluation of plants' tolerance to heavy metals resulted in the discovery of two main mechanisms, based on opposite physiological strategies (Baker, 1981). One of them is based on the creation of a biological barrier, preventing metals absorption. Such species were called excluders. The opposite strategy is based on the process of absorption of heavy metals from the soil and their inactivation inside the plant. Such species were called accumulators. In most plants, the critical level of cadmium toxicity is 5–10 mg / kg, although there are species that exceed this indicator by more than an order of magnitude (Kramer, 2010). These plants could be used for soil remediation, but most of them produce low biomass and are ineffective. Therefore, it is more perspective to use for bioremediation plants with high biomass (Bhargava, 2012). For example, such industrial crops as sunflower, flax, etc., are capable of phytoextraction of heavy metals from the soil (Pokrovskaya, 1995).

Cadmium is an unnecessary element for plants and special mechanisms of its transport have not been developed by evolution. So, cadmium ions pass in plants using transporters involved in the uptake of other divalent cations, such as Ca, Mg, Fe, Zn, Mn, and Cu (Huang X, et al., 2020; Sterckeman T and Thomineb S, 2020). It is known that the toxicity of heavy metals and, in particular, Cd, is due to their affinity with the SH – groups of active domains of various enzymes (Seregin, 2009). By binding to SH-groups of proteins, cadmium is accumulated in the apoplast. An important factor is that apoplastic transport occurs without consuming the plant's energy. As Cd is transported by apoplast, particularly its volume plays the

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main role in its accumulation and isolation, thus providing Cd detoxication (Seregin and Kozhevnikova, 2008). On the other hand, cadmium affinity with the SH – groups plays important role in its detoxication by phytochelatins, which contain cysteine-bearing SH – group. The leading role of phytochelatins in cadmium detoxication and accumulation in the cytosol was shown also for flax (Najmanova J et al., 2012). Nevertheless, till now, particular mechanisms of cadmium transport in plants have not been clarified.

*Linum usitatissimum* L. belongs to the group of accumulators and is also promising in this regard because it combines resistance to cadmium with the ability to accumulate it (Shi and Cai, 2009). It is considered cost-effective to utilize flax biomass, as well as the biomass of other similarly used plants, in order to obtain biofuels (Syc et al., 2011). Cadmium is accumulated mostly in the flax roots, and consistently lower concentrations are found in the stems, bolls, seeds (Bjelkova et al., 2011). At the same time, it should be taken into account that flax seeds can accumulate cadmium in quantities exceeding the maximum permitted concentration, which, for example, is 0.1 mg / kg in the Russian Federation (Melnikov et al., 2013). Therefore, flax seeds grown on land contaminated with cadmium should not be used for food. On the other hand, screening of 74 flax genotypes showed significant differences in the content of Cd in seeds from 0.14 to 1.37 mg / kg, which indicates the prospects of selection to reduce this level (Li et al., 1997).

Varieties of fibre flax and linseed differ in their ability to absorb cadmium from the soil. Ferther more they differ in the ability of cadmium transport and accumulation in stems, which is important for soil remediation. So the selection of the most effective genotypes is perspective (Bjelkova et al., 2011). It is interesting to note that the ability to accumulate cadmium is characteristic only for cultivated flax and is not peculiar to its wild species (Gontcharuke, 2000).

The current experiment is devoted to the evaluation of fiber flax and linseed tolerance to cadmium contamination, the vaous ways to accumulate cadmium removed from the soil, and the possibilities of the specific initial material selection for breeding of super cadmium accumulating varieties.

## 2. Material and methods

Firstly, eight standard varieties of fibre flax and linseed (Table 1) from the All-Russian Institute of Plant Genetic Resources flax collection were used for the evaluation of their seedlings' resistance to different concentrations of cadmium. The reaction of genotypes was analysed by the detection of the growth intensity of seedlings in the Jacobsen apparatus. For flax germination, a temperature of 20 °C was set. Seeds germination energy was determined on the third day after sowing; growth intensity was determined on the fifth day. For the control, distilled water was poured into the apparatus, in the experimental variants a solution of cadmium sulfate 8/3-hydrate $3CdSO_4 \times 8H_2O$ (molar mass 769.536; Cd content in 1 mole – 112.41) at different concentrations in distilled water: $CdSO_4$ $1 \times 10^{-4}$ M; $CdSO_4$ $1 \times 10^{-3}$ M; $CdSO_4$ $2 \times 10^{-3}$ M; $CdSO_4$ $5 \times 10^{-3}$ M were used. In each variant, the length of the two main parts of seedlings (root and hypocotyl) were measured for 40 seedlings.

On the next stage of experiment, four contrasting cadmium resistance varieties: Svetoch and Priziv 81 – as weakly resistant and Voronezhskiy 1308 and Bakhmalskiy 1056 – as highly resistant ones, were tested for their cadmium tolerance in vegetative trials in a greenhouse. Seeds were sown on February, 28 in 5-liter pots – 15 seeds per pot. Control pots contained no cadmium, experimental pots contained $2 \times 10^{-4}$ M of cadmium, concentration, which was chosen on the basis of preliminary experiments (data are not shown). For each variant, two pots were used. Seeds were germinated in 3 – 4 days after sowing. Plants'

**Table 1.** Varieties from the VIR flax collections, evaluated for cadmium pollution resistance.

| VIR catalog number | Name | Origin | *Linum* type |
|---|---|---|---|
| 5333 | Svetoch | Russia | Fibre |
| 6815 | K-6 | Russia | Fibre |
| 6807 | Orshanskiy 2 | Belorussia | Fibre |
| 7472 | Priziv 81 | Belorussia | Fibre |
| 7697 | Dashkovskiy | Belorussia | Fibre |
| 7804 | Mogilevskiy 2 | Belorussia | Fibre |
| 5579 | Voronezhskiy 1308 | Russia | Linseed |
| 6056 | Bakhmalskiy 1056 | Uzbekistan | Linseed |

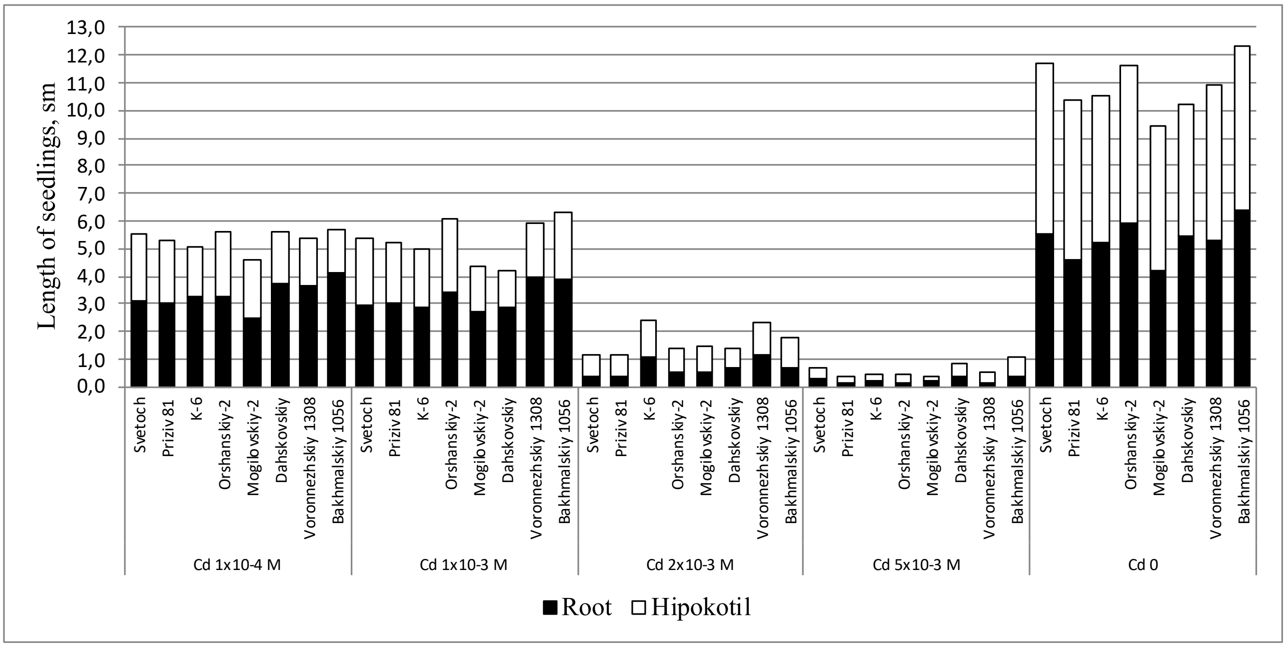
height was measured on the 17th, 29th, 39th, and 49th day after sawing. At this time, the vegetative experiment was stopped because of the plants' mass mortality.

For the measurement of cadmium concentration in the plants, they were pulled out of the soil separately from each pot and burned in order to increase the concentration of metal in the samples. Cadmium concentrations in the ashes were analysed by x-ray fluorescence analyzer BRA – 18. The relative amount of cadmium was estimated according to the fluorescence intensity.

## 3. Results and discussion

In the first stage of the experiment, the analysis of germination energy and general germination ability of seeds showed the absence of cadmium influence on them in all evaluated concentrations. In all variants of the experiment, cadmium contamination practically did not influence the germination ability of seeds. It varied in the range of 96%–100 %. Similar results were received by Najmanova J with colleagues (2012). The same team of scientists also showed that elongation of the seedling root was inhibited by Cd in a dose-dependent manner. In the control conditions of our experiment Svetoch, Orshanskiy 2 and Bakhmalskiy 1056 had the longest seedlings, due to the size of both the roots and hypocotyls (Figure 1). The variety Mogilevskiy had the shortest seedlings. The addition of cadmium, even in the lowest concentration ($1 \times 10^{-4}$ M), significantly reduced the size of all seedlings (Table 2). At the same time, the degree of growth suppression was different in different varieties. Linseed Bakhmalskiy 1056 and, to a less extent, the fibre flaxes (Priziv 81, Orshanskiy 2 and Dashkovskiy) preserved a substantial length of roots. All varieties radically reduced the hypocotyls.

Further, a tenfold increase of the cadmium concentration to $1 \times 10^{-3}$ M did not lead to the radical changes in the seedlings. However, the fibre flax variety




| LSD | Root | Hypokotil |
|---|---|---|
| Cd 1×10-4 M | 0.4 | 0.2 |
| Cd 1×10-3 M | 0.4 | 0.3 |
| Cd 2×10-3 M | 0.2 | 0.2 |
| Cd 5×10-3 M | 0.1 | 0.1 |
| Cd 0 | 0.6 | 0.4 |

LSD - least significant difference

**Figure 1.** The average size of *Linum usitatissimum* seedlings at different concentrations of cadmium ($CdSO_4$).

**Table 2.** The degree of flax seedlings elongation depression caused by different cadmium concentrations in comparison with cadmium less condition.

| Cd concentration | Cd $1 \times 10^{-4}$ M | | Cd $1 \times 10^{-3}$ M | | Cd $2 \times 10^{-3}$ M | | Cd $5 \times 10^{-3}$ M | |
|---|---|---|---|---|---|---|---|---|
| Variety | Root | Hypocotyl | Root | Hypocotyl | Root | Hypocotyl | Root | Hypocotyl |
| Svetoch | 43% | 62% | 46% | 61% | 93% | 87% | 95% | 94% |
| Priziv 81 | 34% | 61% | 34% | 62% | 91% | 88% | 97% | 95% |
| Orshanskiy 2 | 37% | 55% | 45% | 60% | 79% | 76% | 96% | 95% |
| K-6 | 45% | 68% | 42% | 54% | 90% | 85% | 97% | 95% |
| Mogilevskiy 2 | 42% | 59% | 36% | 68% | 87% | 82% | 95% | 96% |
| Dashkovskiy | 32% | 60% | 48% | 71% | 87% | 84% | 92% | 91% |
| Voronezhskiy 1308 | 43% | 71% | 39% | 59% | 89% | 82% | 94% | 89% |
| Bakhmalskiy 1056 | 23% | 72% | 25% | 66% | 77% | 80% | 97% | 93% |
| LSD | 5.7 | 4.4 | 5.7 | 3.9 | 4.2 | 2.9 | 1.2 | 1.9 |

LSD - least significant difference

Orshanskiy-2, as well as linseed Bakhmalskiy 1056 and Voronezhskiy 1308 slightly increased the size of the seedlings mainly due to the elongation of the hypocotyls. In comparison with the previous concentration of cadmium, only the Dashkovskiy variety showed a significant depression of growth. Cadmium concentration of $2 \times 10^{-3}$ M caused an abrupt decrease in seedling size of all varieties. The most resistant to this amount of cadmium were varieties Orshanskiy 2 and Bakhmalskiy 1056. With an increase of the cadmium content in the solution to $5 \times 10^{-3}$ M, varieties Voronezhskiy 1308, Bakhmalskiy 1056, and Dashkovskiy showed the most intensive growth in comparison with the others.

The experiment showed that flax varieties demonstrated different responses to the impact of diverse contents of cadmium in the substrate and showed different degrees of resistance to the pollutant. As the concentration of metal ions increased, differences were detected in the dynamics of changes in the size of whole seedlings and their main components (roots and hypocotyls). This may indicate differences in the resistance mechanisms peculiar for different varieties. Selection and use of genotypes with different reactions to changes in cadmium concentration will help to discover the physiological mechanisms of its deactivation in the plant and the genes responsible for them.

On the next step of the experiment, four varieties with contrast reaction to the cadmium application (Svetoch, Priziv 81, Voronezhskiy 1308 and Bakhmalskiy 1056) were sown in the pots in a greenhouse. In the control conditions, two fibre flax varieties showed more intensive growth than the linseeds as it was expected. Also, about 60% of fibre flax plants started flowering before the experiment was stopped because of the mass mortality of the experimental plants. Linseed varieties did not start flowering, which is not surprising, because in field conditions, they flower later than fibre flax.

Like in the first experiment, linseed varieties showed higher resistance to cadmium than fibre flax ones (Tables 3 and 4, Figure 2). After germination 14 days Bakhmalskiy 1056 developed equally in the control condition and on cadmium polluted soil. Only 3% of plants died in the experimental conditions. During the same period, Voronezhskiy 1308 reduced plants height under pollution conditions only by 3% and lost no plants. Growth of both fibre flax varieties was suppressed by cadmium by 20%.

During the next period between 17 and 29 days of the experiment linseed varieties also showed higher resistance to cadmium. But this time Voronezhskiy 1308 showed superior resistance, then Bakhmalskiy 1056. Both fibre flax varieties had equal low resistance. But Svetoch lost another 10% of plants.

Between 29 and 39 days of the experiment the situation changed significantly. Svetoch lost more 23% of plants, but the rest appeared to be the most resistant ones. Priziv 81 also lost another 20% of plants, but the survived plants significantly increased their resistance. Though Voronezhskiy 1308 reduced its resistance, it still had all plants alive. Bakhmalskiy 1056 lost 30% of plants and the rest ones had the same level of resistance as during the previous period.

Between 39 and 49 days of the experiment when plants stopped the vegetative growth and started to form buds, survived Svetoch plants reduced their cadmium resistance. The same can be said about Bakhmalskiy 1056. Priziv 81 lost another 17% of plants, and resistance to cadmium

**Table 3.** The degree of flax stems elongation depression caused by Cd $2 \times 10^{-4}$ M concentration in comparison with cadmium less condition.

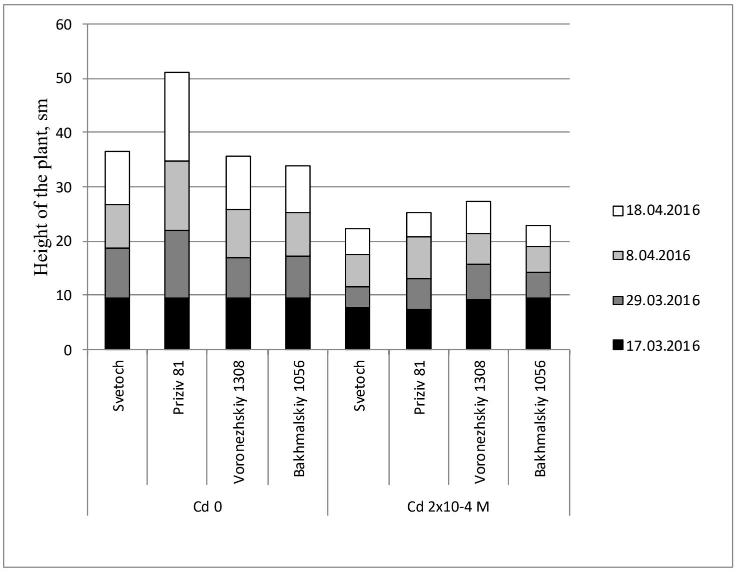
| | The degree of flax stems elongation depression caused by Cd | | | |
|---|---|---|---|---|
| Period of plants growth | Germination – 14 day | 15 – 26 day | 27 – 36 day | 37 – 46 day |
| Svetoch | 20% | 57% | 26% | 51% |
| Priziv 81 | 20% | 57% | 37% | 74% |
| Voronezhskiy 1308 | 3% | 15% | 35% | 78% |
| Bakhmalskiy 1056 | 0% | 38% | 41% | 57% |
| LSD | 11.4 | 20.7 | 6.5 | 15.5 |

LSD - least significant difference

**Table 4.** Portion of the dead plants under cadmium stress (Cd $2 \times 10^{-4}$ M).

| | % of the dead plants under cadmium pollution | | | |
|---|---|---|---|---|
| Day after germination | 14 | 26 | 36 | 46 |
| Svetoch | 7 | 10 | 33 | 33 |
| Priziv 81 | 0 | 0 | 20 | 37 |
| Voronezhskiy 1308 | 0 | 0 | 0 | 10 |
| Bakhmalskiy 1056 | 3 | 3 | 33 | 33 |
| LSD | 3.5 | 4.4 | 13.4 | 9.8 |

LSD - least significant difference




| LSD | 14 day after germination | 26 day after germination | 36 day after germination | 46 day after germination |
|---|---|---|---|---|
| Cd 0 | 0.1 | 2.3 | 1.9 | 2.8 |
| Cd 2×10-4 M | 1.1 | 1.0 | 1.2 | 0.8 |

LSD - least significant difference

**Figure 2.** The average growth of flax plants in the pots on the soil contaminated with cadmium ($CdSO_4$ in concentration of $2 \times 10^{-4}$ M) and without contamination.

of the rest reduced. Voronezhskiy 1308 for the first time in the experiment lost 10% of plants and showed high susceptibility to pollution.

The obtained results showed that different varieties have different degrees of cadmium resistance, which is consistent with the results of other researchers (Bjelkova et al., 2011). Also, they have different mechanisms of this resistance. In addition to that, some varieties can be heterogenic in this character. For example, Svetoch showed the "increase" of the resistance when susceptible plants died.

In general, Voronezhskiy 1308 showed the most stable and high resistance to cadmium pollution.

After 49 days of the experiment, plants were harvested separately from each pot, forming 2 recurrences of each variant and mineralized by burning to increase cadmium concentration in the samples. The content of cadmium in control samples (without its artificial addition to the soil) was lower than the resolution of the method used. The difference in the cadmium concentration in the experimental samples is shown in Figure 3. Priziv 81 from the second recurrence did not participate in the analysis. Mass death of the plants caused a lack of ash obtained after mineralization.

In mineralised samples of the same mass, the concentration of cadmium in the varieties Bakhmalskiy 1056 and Voronezhskiy 1308 was higher than that in Svetoch and Priziv 81. This means that the first two varieties absorb more metal per unit of plants' mass than the second two. At the same time, the varieties Voronezhskiy 1308 and Bakhmalskiy 1056 gave a large green mass. This effect was especially visible in the Voronezhskiy 1308 variety. Thus, Voronezhskiy 1308 not only absorbs more cadmium per mass unit but also, in general, has a larger size and height, as well as a larger number of surviving plants. Consequently, it is able to accumulate more cadmium in its tissues and can be used for soil remediation. These results are consistent with the data about the amassing and immobilization of cadmium by plants-accumulators. So, genotypes capable to accumulate (immobilize) Cd appear to be more resistant to its toxicity.

Previously, it was shown that fibre flaxes absorb more cadmium, then linseed ones (Bjelkova et al., 2011), but, in our experiment, we received just the opposite result. In both trials, only several genotypes were tested, so this discrepancy can appear because of the random selection of tested varieties. It means, that capacity of cadmium absorption depends on the particular genotype and is not a characteristic of linum type.

## 4. Conclusion

In frames of current experiments, it was confirmed that different flax varieties have different resistance to cadmium pollution and its absorption ability. It was found out that the high cadmium absorption ability of the plant provides its relative resistance to contaminant toxic influence. Obtained results show the possibility of selecting genotypes with high cadmium resistance and absorption ability. On the other hand, intraspecific diversity of cadmium resistance and absorption ability will help in the discovery of these features mechanisms and genetics.

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The authors declare no conflict of interests.

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