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EFFECTIVENESS OF THE DENITE[®]CR FOR BIOLOGICAL RECULTIVATION OF HEAVY METALS CONTAMINATED SOIL

It is known that heavy metals have high biological activity, ability to bioaccumulate and move along food chains. In this regard, plants that directly absorb heavy metals from the soil play an important role in their accumulation and delivery to the human body, which poses a huge threat to human health.

At the Department of Genetics and Molecular Biology, Kazakh National University. Al-Farabi carried out comprehensive studies to study the effect of cadmium chloride (CdCl₂) and heavy metal immobiliser Denite[®]CR on two varieties of soft wheat: Kazakhstanskaya-19 and Samgau. The studies were carried out at the cellular, tissue and organism levels. The object was 14-day-old seedlings of two varieties. The work used cytogenetic, botanical, histological methods, morphometry, biochemistry and biostatistics.

The paper shows that Denite[®]CR: (i) reduces the toxic effect of cadmium on cell division of the root system, restores the values of the mitotic index, almost to control values; the binding of cadmium ions contributed to the adaptation of wheat plants to growth in soil contaminated with cadmium at a concentration five times higher than the Maximum allowed concentration; (ii) statistically significantly increases the thickness of the primary root coat up to 31.89% with neutralization of Cd (Maximum allowed concentration + Denite).

Key words: heavy metals, immobilisation, toxic effect, reclamation, disturbed lands.

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Ауыр металдармен ластанған топырақты биологиялық тазарту үшін Denite[®]CR иммобилизаторының тиімділігін зерттеу

Ауыр металдардың биологиялық белсенділігі жоғары, биожинақтау және қоректік тізбектер бойымен қозғалу қабілеті бар екені белгілі. Осыған байланысты ауыр металдарды топырақтан тікелей сіңіретін өсімдіктер олардың жиналып, адам ағзасына жеткізілуінде маңызды рөл атқарады, бұл адам денсаулығына үлкен қауіп төндіреді.

Әл-Фараби атындағы ҚазҰУ генетика және молекулалық биология кафедрасында жұмсақ бидайдың екі сортына: Казахстанская-19 және Самгау сорттарына кадмий хлориді тұзы (CdCl₂) және ауыр металл иммобилизаторы Denite[®]CR әсерін зерттеу бойынша кешенді зерттеулер жүргізді. Зерттеулер жасушалық, тіндік және ағзалық деңгейде жүргізілді. Нысан екі сорттың 14 күндік өскіндері болды. Жұмыста цитогенетикалық, ботаникалық, гистологиялық әдістер, морфометрия, биохимия және биостатистика қолданылды.

Denite[®]CR: (i) тамыр жүйесінің жасушаларының бөлінуіне кадмийдің уытты әсерін төмендеті отырып, өсімдік жасушаларының жалпы тіршілігінің негізгі көрсеткіші – митоздық индекс мәндерін қалпына келтіретіні жұмыста дәлелденді. дерлік мәндерді басқаруға; кадмий иондарының байланысуы бидай өсімдіктерінің максималды рұқсат етілген концентрациядан 5 есе жоғары концентрацияда кадмиймен ластанған топырақта өсуге бейімделуіне ықпал етті; (ii) Cd (максималды рұқсат етілген концентрация + Denite[®]CR) бейтараптандыру кезінде бастапқы түбір қабығының қалыңдығын статистикалық түрде 31,89%-ға дейін арттырады.

Түйін сөздер: ауыр металдар, иммобилизация, токсикалық әсер, мелиорация, бұзылған жерлер.

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Исследование эффективности иммобилизатора Denite®CR для биологической рекультивации загрязненной тяжелыми металлами почвы

Известно, что тяжелые металлы имеют высокую биологическую активность, способность к биоаккумуляции и перемещению по пищевым цепям. В связи с этим, важную роль в их накоплении и доставке в организм человека играют растения, непосредственно поглощающие тяжелые металлы из почвы, что представляет огромную угрозу для здоровья человека.

На Кафедре генетики и молекулярной биологии КазНУ им. аль-Фараби проведены комплексные исследования по изучению влияния соли хлористого кадмия (CdCl_2) и иммобилизатора тяжелых металлов Denite®CR на два сорта мягкой пшеницы: Казахстанская-19 и Самгау. Исследования проведены на клеточном, тканевом и организменном уровнях. Объектом служили 14-дневные проростки двух сортов. В работе применяли цитогенетические, ботанические, гистологические методы, морфометрии и биостатистики.

В работе установлено, что Denite®CR: (i) снижая токсическое влияние кадмия на деление клеток корневой системы, восстанавливает значения митотического индекса, практически до контрольных значений; связывание ионов кадмия способствовало приспособлению растений пшеницы к росту в почве, загрязненной кадмием в концентрации, в пять раз превышающей предельно допустимую концентрацию; (ii) статистически достоверно увеличивает толщину первичной оболочки корней до 31,89% при нейтрализации Cd (предельно допустимую концентрацию + Denite®CR).

Ключевые слова: тяжелые металлы, иммобилизация, токсическое действие, рекультивация, нарушенные земли.

Introduction

Heavy metals have a negative impact on human life and health. Which is aggravated by their ability to accumulate in body tissues due to the cumulative effect [1,2]. One of these metals is cadmium and its salts, which have no physiological functions in the human body. The toxicity of cadmium is manifested, among other things, in promoting the development of cancer, osteoporosis, liver disease, kidney disease, etc [3–5].

This is especially important for some areas of Kazakhstan, where high levels of cadmium are noted, usually around industrial cities, metallurgical, chemical and other enterprises [6].

As a result, the legislation of different countries has taken measures to monitor, regulate, and reduce emissions of cadmium and its salts into the environment [7]. Despite the fact that there is positive experience in reducing the concentration of heavy metals as a result of the introduction of such regulations as, for example, the maximum permissible content of cadmium in food products [8], more and more of these chemical compounds continue to be emitted by various industrial enterprises, cars and other sources of emissions into the environment [9,10].

In this regard, there is a need to reduce the concentration of cadmium and its salts in the environment. The main methods for achieving this goal are, firstly, the reduction of cadmium emissions, and secondly, the neutralization of cadmium already accumulated in the environment. The latter includes immobilization of cadmium in soil by means of special chemicals, for example, Denite®CR [11].

The purpose of this study was to determine the efficiency of binding of cadmium salt (CdCl_2) by Denite®CR through the use of indicators of plant seedlings sensitive to pollution (bread wheat), which are a clear indicator of environmental pollution [12], provided that the level of pollution does not exceed the maximum extreme points (according to the corresponding indicator of tolerance to cadmium).

Materials and Methods

Study design

In this study, cadmium chloride salt (CdCl_2) was used as a cadmium compound simulating soil cadmium contamination. This choice is justified by the increased toxicity of this compound compared to pure cadmium [13].

To fulfill the purpose of the study, the experiment was divided into one control (C) and four experimental variants:

(C) Control – blank soil (no treatment).

Adding of 0.5 mg/kg of CdCl₂ (equivalent of one Maximum allowed concentration (MAC) to soil [14].

Adding of 2.5 mg/kg CdCl₂ (equivalent of five MAC) to soil.

Adding of 0.5 mg/kg of CdCl₂ and 30 g/kg of Denite®CR to soil.

Adding of 2.5 mg/kg of CdCl₂ and 30 g/kg of Denite®CR to soil.

This division into five variants of three replicates is necessary to establish the effect of CdCl₂ on wheat seedlings (compared to the control), and to determine whether Denite®CR is able to immobilize CdCl₂ by binding it.

The objects of study of the effects of these chemical treatments were seedlings of two Kazakh wheat varieties (*Triticum aestivum* L.) – Samgau and Kazakhstanskaya-19. Adding of CdCl₂ into soil was carried out prior to sowing wheat seeds. The study of the influence of the treatments was carried out on the 14th day after seed germination.

Mitotic index

To study the effect of CdCl₂ on wheat seedlings, their indicators were used. The first indicator of the condition of the seedlings was cell division of the root meristem. It was studied by determining the mitotic index (MI). The MI is determined by the number of dividing cells at all stages of mitosis – in prophase, metaphase, anaphase and telophase:

$$M = \frac{P + M + A + T}{I + P + M + A + T} = 100 \%$$

where:

M – mitotic index

P – prophase

M – metaphase

A – anaphase

T – telophase

I – interphase

Anatomical structure of plants

In addition to MI, morphometric indicators of the internal anatomical structure of wheat seedlings leaves and roots served as indicators in this study. The study of morphometric indicators

of leaves was carried out according to the following indicators:

- the thickness of the lower and upper epidermis of the leaves

- the thickness of the leaf blade itself

- general area of conductive vessels

- area of conducting xylem vessels.

When studying the effect of cadmium ions on the anatomical structure of the roots the following indicators were considered:

- the thickness of the primary root membrane

- thickness of the endoderm (inner cortex)

- diameter of the central cylinder

- area of xylem.

Results and Discussion

Effect of CdCl₂ and CdCl₂ + Denite®CR on cell division activity (MI) of plant root meristem

In the Control of Samgau variety, the MI index was 73.54% (Table 1). Introduction of CdCl₂ at a concentration of one MAC reduced MI to 58.34%, and at five MAC – to 55.24%. Therefore, CdCl₂ reduces the cell division activity of the wheat root system by 20.67% at concentration of one MAC and by 24.89% at concentration of five MAC.

Introduction of CdCl₂ caused severe decrease of MI in wheat seedlings cells. However, the application of Denite®CR neutralized this negative effect. Denite®CR increased MI to 71.26% at a soil cadmium concentration of one MAC and to 70.85% at a cadmium concentration of five MAC.

Mitotic index of control seedlings of wheat variety Kazakhstanskaya-19 was 80.12% (Table 2), being slightly higher than that of Samgau variety. Mitotic index of control seedlings of wheat variety Kazakhstanskaya-19 was 80.12% (Table 2), being slightly higher than that of Samgau variety. The concentration of CdCl₂ at one MAC reduced MI to 65.32%, and the concentration of CdCl₂ at five MAC to 56.94%.

The introduction of CdCl₂ into the soil had a negative effect on the MI of plant roots, cell division decreased by 18.48% at a cadmium concentration of one MAC and to 28.94% at five MAC of cadmium.

Similar to the Samgau variety the treatment of cadmium-contaminated soil with the Denite®CR immobilizer led to an increase in MI almost to the control values: at one MAC mitotic index was 79.10%, at five MAC – 77.46%.

Table 1 – Effect of Cadmium Chloride and immobiliser Denite®CR on the Mitotic Index of Samgau wheat

Experiment	The number of cells in the stages of division (mitosis)				Interphase	Mitotic Index, %
	prophase	metaphase	anaphase	telophase		
Blank (Control)	370	375	369	372	534	73.54
Cd 1 MAC	255	249	256	255	724	58.34
Cd 5 MAC	165	145	164	163	516	55.24
Cd 1 MAC + Denite®CR	285	279	283	284	456	71.26
Cd 5 MAC + Denite®CR	261	275	269	267	441	70.85

Table 2 – Effect of Cadmium Chloride and immobiliser Denite®CR on the Mitotic Index of Kazakhstanskaya-19 wheat

Experiment	The number of cells in the stages of division (mitosis)				Interphase	Mitotic Index, %
	prophase	metaphase	anaphase	telophase		
Blank (Control)	361	365	363	362	360	80.12
Cd 1 MAC	224	211	212	218	459	65.32
Cd 5 MAC	158	127	157	155	451	56.94
Cd 1 MAC + Denite®CR	260	265	269	268	280	79.10
Cd 5 MAC + Denite®CR	276	268	271	269	315	77.46

Effect of CdCl₂ and CdCl₂ + Denite®CR on the anatomical structure of leaves and roots of wheat Samgau

Cadmium Chloride at a concentration of one MAC and five MAC had a statistically significant effect on the thickness of the lower and upper ep-

idermis, and are of xylem vessels of Samgau variety (Table 3). Thus, the experimental variants with CdCl₂ at one MAC + Denite®CR and at five MAC + Denite®CR statistically significantly increased the area of xylem vessels by 25.0% and 10.0%, relative to the Control.

Table 3 – Effect of Cadmium Chloride and Denite®CR on the Morphometric Indices of the Internal Anatomical Structure of Samgau wheat leaves

Experiment	Leaf epidermis thickness, µm		The thickness of the leaf blade, µm	Area of conductive vessels, x10 ⁻³ mm ²	Area of xylem vessels, x10 ⁻³ mm ²
	upper	lower			
Blank (Control)	24.19±0.37	21.52±0.06	217.22±4.36	44.02±2.59	2.25±0.11
Cd 1 MAC	26.21±1.28***	22.59±0.14	211.6±5.39	44.36±1.32	2.65±0.08
Cd 5 MAC	25.22±0.29***	22.53±0.14	214.85±21.69	43.57±8.67	2.70±0.065
Cd 1 MAC + Denite®CR	22.85±0.67	20.87±0.18***	187.52±4.52	46.48±4.40	2.82±0.04*
Cd 5 MAC + Denite®CR	24.29±0.31	20.08±0.48***	221.71±4.97	45.20±4.69	2.48±0.07

* – P<0.05; ** – P<0.01; *** – P<0.001 vs. blank

Variants of the experiment with the introduction of Denite®CR into the soil statistically significantly reduced the thickness of the lower epidermis. Thus, cadmium at a concentration of one MAC reduced the thickness of the lower epidermis to $20.87 \pm 0.18 \mu\text{m}$, a concentration of five MAC to $20.08 \pm 0.48 \mu\text{m}$.

Introducing of cadmium slightly affected the thickness of the leaf blade. However, the variant with CdCl_2 at one MAC + Denite®CR significantly thinned it, i.e., $187.52 \pm 4.52 \mu\text{m}$ vs $217.22 \pm 4.36 \mu\text{m}$ in Control.

The thickness of the primary root coat and endoderm significantly increases by 31.89% and 6% under the treatment of one MAC (Table 4). The

effect of different concentrations of cadmium on Samgau variety, in general, manifested in an increase in the diameter of the central cylinder, and decrease of xylem vessels' area. However, for at one MAC + Denite®CR the xylem vessels' area statistically significantly increased to $2.42 \pm 0.01 \times 10^{-3} \text{mm}^2$.

Besides the xylem vessels, this experimental variant resulted with increase of all parameters, especially the primary root coat (to $134.81 \pm 18.58 \mu\text{m}$). While CdCl_2 at five MAC + Denite®CR variant resulted with significant decrease of all parameters. For example, the thickness of the endoderm is statistically significantly reduced by 22.85%.

Table 4 – Effect of Cadmium Chloride and Denite®CR on the Morphometric Indices of the Internal Anatomical Structure of Samgau wheat root

Experiment	Thickness of the primary root coat, μm	Thickness of the endoderm, μm	Thickness of the central cylinder, μm	Area of xylem vessels, $\times 10^{-3} \text{mm}^2$
Blank (Control)	102.21 ± 3.46	18.29 ± 0.11	182.16 ± 9.77	2.17 ± 0.02
Cd 1 MAC	$118.71 \pm 4.35^*$	$19.44 \pm 0.35^{**}$	194.28 ± 2.01	1.88 ± 0.01
Cd 5 MAC	102.82 ± 2.93	18.67 ± 0.18	186.87 ± 1.12	$1.70 \pm 0.01^*$
Cd 1 MAC + enite®CR	$134.81 \pm 18.58^*$	19.97 ± 0.22	192.46 ± 7.39	$2.42 \pm 0.01^*$
Cd 5 MAC + enite®CR	$80.81 \pm 5.14^*$	$14.11 \pm 5.01^{**}$	$128.10 \pm 8.65^*$	1.01 ± 0.11

* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ vs. blank

Kazakhstanskaya-19

Just like Samgau variety, with an increase in MAC, Kazakhstanskaya-19 variety observed a gradual thickening of the lower and upper leaves epidermis (Table 5). For example, $22.31 \pm 0.15 \mu\text{m}$

for upper epidermis in control and $30.82 \pm 0.61 \mu\text{m}$ at five MAC. However, both variants of Cadmium Chloride + Denite®CR demonstrated a statistically reliable reduction in the upper epidermis of the leaf, bringing it to Control parameters.

Table 5 – Effect of Cadmium Chloride and Denite®CR on the Morphometric Indices of the Internal Anatomical Structure of Kazakhstanskaya-19 wheat leaves

Experiment	Leaf epidermis thickness, μm		The thickness of the leaf blade, μm	Area of conductive vessels, $\times 10^{-3} \text{mm}^2$	Area of xylem vessels, $\times 10^{-3} \text{mm}^2$
	upper	lower			
Blank (Control)	22.31 ± 0.15	19.14 ± 0.23	164.58 ± 0.94	48.22 ± 1.26	2.29 ± 0.06
Cd 1 MAC	22.1 ± 1.02	$20.23 \pm 0.26^{**}$	147.34 ± 6.82	44.70 ± 1.22	2.25 ± 0.07
Cd 5 MAC	30.82 ± 0.61	29.13 ± 0.28	217.32 ± 3.80	$62.64 \pm 10.06^{**}$	$3.56 \pm 0.13^*$
Cd 1 MAC + Denite®CR	$21.44 \pm 0.91^*$	$19.99 \pm 0.31^{**}$	193.31 ± 1.74	45.33 ± 1.91	$2.58 \pm 0.06^*$
Cd 5 MAC + Denite®CR	22.36 ± 0.04	20.98 ± 0.27	196.43 ± 1.52	$53.39 \pm 4.03^*$	2.42 ± 0.12

* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ vs. blank

It should be noted that the area of conductive vessels increases, at both five MAC variants were significantly higher than control, i.e., $62.64 \pm 10.06 \mu\text{m}$ at five MAC and $53.39 \pm 4.03 \mu\text{m}$ at five MAC + Denite®CR versus $48.22 \pm 1.26 \mu\text{m}$ in Control. While, at both one MAC variants these parameters were lower than the in Control.

In Kazakhstanskaya-19 experimental variants, as the cadmium content in the soil increased, an increase in the thickness of the primary root coat was observed, e.g., CdCl_2 at one MAC

$-144.73 \pm 10.54 \mu\text{m}$ versus $135.31 \pm 9.7 \mu\text{m}$ for Control (Table 6).

As for the Samgau variety, CdCl_2 at five MAC + Denite®CR variant led to a significant decrease of all parameters, except xylem vessels' area which resulted in increased to 1.99 ± 0.09 versus $1.59 \pm 0.04 \times 10^{-3} \text{mm}^2$ in Control. While variant of one MAC + Denite®CR resulted in increase of all parameters, especially the primary root coat ($151.42 \pm 3.74 \mu\text{m}$), central cylinder ($213.64 \pm 7.72 \mu\text{m}$) and area of xylem vessels ($1.88 \pm 0.03 \times 10^{-3} \text{mm}^2$).

Table 6 – Effect of Cadmium Chloride and Denite®CR on the morphometric indices of the internal anatomical structure of Kazakhstanskaya-19 wheat root

Experiment	Thickness of the primary root coat, μm	Thickness of the endoderm, μm	Thickness of the central cylinder, μm	Area of xylem vessels, $\times 10^{-3} \text{mm}^2$
Blank (Control)	135.31 ± 9.7	19.09 ± 0.22	174.21 ± 5.91	1.59 ± 0.04
Cd 1 MAC	144.73 ± 10.54	18.74 ± 0.13	194.84 ± 3.24	1.77 ± 0.01
Cd 5 MAC	146.45 ± 1.72	18.65 ± 0.81	$196.02 \pm 2.06^*$	1.79 ± 0.02
Cd 1 MAC + Denite®CR	151.42 ± 3.74	19.71 ± 0.47	213.64 ± 7.72	1.88 ± 0.03
Cd 5 MAC + Denite®CR	134.97 ± 5.18	$17.73 \pm 0.44^{**}$	162.18 ± 5.67	1.99 ± 0.09
* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ vs. blank				

Contamination of soil with cadmium and its salts negatively affects the growth and development of plants. Thus, since the mitotic index is an important predictive indicator of the overall survival of living organisms and the activity of their cell division under the influence of negative environmental factors [15,16], its decrease in wheat seedlings of both varieties in experimental variants with the introduction of cadmium compared to the control (Table 1 and 2) allows us to conclude that soil contamination with cadmium inhibits the growth and development of plants at the cellular level. The most likely explanation for the increase in MI of plant seedlings growing in soil with CdCl_2 and Denite®CR is that CdCl_2 was immobilized by Denite®CR as stated by the manufacturer [11,17].

It is also figured out that Kazakhstanskaya-19 variety is more resistant to the cadmium contamination, compared with the Samgau variety, e.g., MI = 65.32% versus MI = 58.34% at one MAC (Tables 1 and 2). However, it might be explained by the generously higher MI of Kazakhstanskaya-19 variety in control, i.e., MI = 80.12% versus MI = 73.54% (ibid.).

Another proof of the negative impact of cadmium and its salts was the reaction of plants, expressed in changes in several of their anatomical and morphological parameters in variants of the experiment with soil contaminated with CdCl_2 . Thus, thickening of the lower and upper epidermis of the leaves of both varieties was observed (Table 3 and 5). The increase in the thickness of plant integumentary tissues apparently is a protective reaction of plants to the abiotic stress caused by the toxic effect of cadmium CdCl_2 , since these organs perform a protective function for plants [18]. This protective reaction is a typical response of plants to soil contamination with heavy metals. [19]. However, in all of the above variants of the experiment with the addition of cadmium CdCl_2 + Denite®CR, these parameters returned to the Control values. This, as well as the increase in MI, can be explained by the immobilization of CdCl_2 by Denite®CR.

It is also interesting to see some parameters of the anatomical structure of leaves and roots. Thus, with the Kazakhstanskaya-19 variety, both variants of the experiment with and without a concentration of at five MAC + Denite®CR reliably increased the

area of conductive vessels in the leaves (Table 5). And also, there was an increase in the thickness of primary root coat of Kazakhstanskaya-19 variety (Table 6), and an increase in xylem vessels' area of Samgau roots' variety at one MAC + Denite®CR. As a consequence, the immobilizing abilities of Denite®CR may be questioned, or the immobilized CdCl₂ may still have toxic properties. Moreover, the decrease in all root parameters at one MAC + Denite®CR (ibid.) in both wheat varieties can be explained by the combined inhibitory effect of both Cadmium and Denite®CR, in which both varieties in the variant at low Cadmium concentration can still resist to the toxic effect of CdCl₂ and Denite®CR. With its increase they approach the upper limit of endurance and enter the pessimism zone. The mechanism of such a combined inhibitory effect remains unclear and requires further investigations.

Samgau and Kazakhstanskaya-19 varieties also demonstrated differences in anatomical structures. Thus, the differences between the area of conductive vessels in leaves in various variants of experiment at Samgau were insignificant (Table 3), while at Kazakhstanskaya-19, at five MAC and five MAC + Denite®CR variants conductive vessels were significantly higher than in Control (Table 5). This can be explained by the greater resistance of the Samgau variety, since an increase in the area of xylem vessels increases the plants' resilience to stress [20–22].

Cadmium and its compounds are easily absorbed by plants, and as a result, at their high concentrations on cultivated agricultural lands, they pose a particular danger to human health and well-being [23]. Determining the ability of Denite®CR to immobilize cadmium compounds in soils where one of the key crops that meet the energy needs of mankind grows [24, 25] is relevant.

In the future, the use of Denite®CR is possible for the immobilization of other heavy metals (for example, lead), given that the spread of cadmium pollution is associated with pollution by other heavy metals in Kazakhstan [6], which negatively affects the health of the population of these areas [26, 27].

Conclusion

Introduction of Denite®CR into the soil resulted in decrease of the toxic effect of cadmium on cell division of the root system.

Heavy metal immobilizer Denite®CR led to an increase in the synthesis of the antioxidant enzyme catalase and thus neutralized the toxic effect of CdCl₂ during the growth of wheat seedlings.

The obtained data on the study of the effect of cadmium chloride and Denite®CR immobilizer on Samgau and Kazakhstanskaya-19 varieties wheat seedlings led to the following conclusions:

It was found that Denite®CR, reducing the toxic effect of cadmium on cell division of the root meristem, restored the values of the main indicator of the overall survival of plant cells – the mitotic index, almost to Control values; the binding of cadmium ions contributed to the adaptation of wheat plants to growth in soil contaminated with CdCl₂ at a concentration up to five times higher than the maximum allowed concentration;

Introduction of Denite®CR into the Cadmium-contaminated soil statistically reliably increased the thickness of the upper and lower leaves epidermis of wheat sprouts of both varieties; showed a significant increase in the area of the conductive vessels and the diameter of the central cylinder of the roots of both varieties;

Introduction of Denite®CR into the soil contaminated by Cadmium salt at a concentration five times higher than the maximum allowed, resulted the inhibition of the development of seedling roots, without suppressing the anatomical and morphological characteristics of leaves and the mitotic index of both wheat varieties compared to the option without adding Denite®CR.

Thereby, despite the last conclusion the data obtained give grounds to believe that Denite®CR effectively immobilises cadmium ions, thereby neutralising its toxic effect on the growth and development of plants and can be recommended for use for land rehabilitation on lands contaminated with cadmium. However, the inhibitory effect of simultaneous introduction of cadmium chloride and Denite®CR into the soil requires further study.

We believe that studies of the effect of Denite®CR immobilizer on the growth and development of plants in the presence of other heavy metals, including mercury and lead, need to be continued. At the same time, the emphasis of research should be directed to the effect of the immobilizing ability of Denite®CR, on metabolism, elements of agricultural plant productivity.

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