

EFFECTS OF HEAVY METALS, CONTAINED IN FLOTATION TAILINGS, ON PLANTS USED IN REVEGETATION

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Abstract: During the processing of precious and base metal ores from the Baia Mare mining area, which contained Au, Ag, Cu, Pb and Zn, large amounts of flotation tailings resulted, which were deposited in the Bozânta tailings management facility, located between Săsar and Bozânta Mare villages, 4 km west of Baia Mare. Due to the presence of minerals containing metal sulphides (mainly pyrite), the acid rock drainage (ARD) has become very active, catalysed by iron- and sulphur-oxidizing bacteria, which naturally occur at these sites. Decreasing the surface of the pond as a result of evaporation, combined with the increase of the beach surface and the absence of any protection at the embankment ridge, led to the entrainment of the tailings by wind at considerable distances. They often end up in neighbouring settlements, especially in the two mentioned villages, as a result of prevailing winds from the NE-SW direction. The purpose of the research was to test the resistance of some plant species (*Sinapis alba* L. – white mustard; *Trifolium pratense* L. – red clover and *Lolium perenne* L. – ray grass) to the conditions generated by the tailings on the surface of the depot, in order to create a vegetation cover with a role in diminishing tailings discharges in the adjacent areas. There were tested the effects of the industrial eluate (acid mine water, pH 4.3), obtained from the tailings pond, over seed germination parameters: germination capacity (GC); germinative energy (GE) and relative seed germination (RG). The results of the tests pointed out that the least affected was white mustard: GC – 95.3%; the differences in the values obtained in the control samples and the GE test – 5.3%; the results for RG – 99.3%, confirm that the white mustard seeds were the least influenced by the industrial eluate, followed by ray grass and red clover. In the tests for plant growth on the tailings pond, 5 experimental variants were used: a) soil (S); b) flotation tailings (T); c) mixture of flotation tailings and soil (T+S); d) mixture of flotation tailings and organo-zeolitic material (T+OZ); e) mixture of flotation tailings, organo-zeolitic material and soil (T+OZ+S). It is recommended to use the T+OZ+S mixture on the pond surface and sowing with *Sinapis alba* and *Trifolium pratense*. For both species, double seed sowing is recommended.

Keywords: tailings pond, flotation tailings, ARD, heavy metals, revegetation

1. INTRODUCTION

The mining activity carried out in the Baia Mare area has produced several negative effects on the environment: changes in the landscape and relief; the use of large areas of land for the mining storage of waste rock, and for industrial installations; landslides, slopes and tailings ponds causing serious environmental accidents; pollution of surface waters and groundwater; chemical pollution of the air and soil (Jelea & Jelea, 2007; Mihali et al., 2013).

As a result of the processing of precious and base metal ores from Baia Mare mining area, containing non-ferrous minerals as Au, Ag, Cu, Pb,

Zn, large amounts of flotation tailings resulted, which were deposited on Bozânta tailings depot (Figure 1).

Due to the presence of sulphide minerals (mainly pyrite) the acid rock drainage (ARD) has become very active, catalysed by iron and sulphur-oxidizing bacteria, which naturally occur at this site. Bacterial oxidation of these minerals leads to the occurrence of acid rock drainage, which contaminates the environment with heavy metals (Jelea, 2015; Jelea et al., 2007; Jelea et al., 2015; Schippers et al., 2000).

The Bozânta tailings management facility (120 ha with annexes) functioned between 1976 and 2007 and it is located between Săsar and Bozânta Mare villages, 4 km west of Baia Mare.



Figure 1. Tailings beach and the pond.

The tailings create a hostile environment that disrupts the ecosystem of the area (Figure 2). The polluting effects are manifested by the entrainment of the fine mineral particles into the atmosphere and their deposition on the soil in the surrounding areas, with their heavy metals content (Mihali et al., 2013; Roba et al., 2015).



Figure 2. Dry vegetation on the tailings depot.

Heavy metals cannot be degraded naturally, consequently they have a long remanence time in the environment, and they can accumulate in the food chain (Singh & Kalamdhad, 2011; Big et al., 2012; Mihali et al., 2013; Roba et al., 2016; Suman et al., 2018; Adnan et al., 2022). Through the food chain, heavy metals can reach the human body where they accumulate by bioamplification, thus posing a major threat to human health (Jelea & Jelea, 2007; Yan et al., 2020; Gurzău et al., 2012; Adnan et al., 2022).

Phytoremediation has become one of the major methods of remediation of soils polluted with heavy metals because it has several advantages related to: ease of operation, relatively low cost, and improvement of soil without secondary pollution

(Jelea et al., 2015; Cui et al., 2018, Yan et al., 2020).

The plants used for the phytoremediation process are selected based on their properties of rapid growth, biomass development, pollutant tolerance and detoxification potential (Pandey & Singh, 2020). Studies conducted by Gerencsér et al., (2010), Gratiano et al., (2022) and Pandey & Singh (2022) highlighted the importance of *Sinapis alba*, *Trifolium pratense* and *Lolium perenne* species in phytostabilizing contaminated sites.

Revegetation can be a permanent and relatively inexpensive solution. An effective revegetation technique is that of sowing with balanced grass / legume mixtures combined with various amendments (Tordoff et al., 2000).

A combined effect of the use of a plant species and a natural amendment in the soil could provide the necessary conditions for the phytoremediation of these soils.

Numerous studies (Damian et al., 2007; 2013; 2018; 2019) have been conducted on the importance of zeolites in the recovery of soils polluted with heavy metals. Data from the literature claims that organo-zeolites retain metals in soil (Shi et al., 2009; Lee et al., 2019), increase soil pH (Prisa, 2020), improve soil quality (Lee et al., 2019), increase the cation exchange capacity for NH_4^+ , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} (Lee et al., 2019; Langella et al., 2000).

The purpose of the current research was to test the resistance of some plant species to the conditions offered by the flotation tailings on the surface of the depot, to create a vegetation cover with a role in diminishing tailings discharges in the adjacent areas.

The following types of activities were carried out within the research.

1. Testing the effects of industrial eluate (acid mine water) obtained from the tailings pond, over seed germination parameters: germination capacity, germinative energy and relative seed germination.

2. Testing the effects of industrial eluate on the growth of vegetative organs of plant (root and shoot – stem + leaf).

3. Testing plant growth on flotation tailings and tailings mixed with various additives in order to improve plant nutrition conditions, namely soil and organo-zeolitic material.

2. MATERIALS AND METHODS

2.1. Materials used in the experiment

The following materials were used to perform the tests:

- The plant species selected correspond to the indications in Annex 2 (List of species historically

used in plant testing) of the 208–OECD Guidelines for the Testing of chemical (2006) and the protocol ISO (11269–2: 2005). We used seeds of: *Sinapis alba* L. – white mustard, Brassicaceae (Cruciferae) family; *Trifolium pratense* L. – red clover, Fabaceae (Leguminosae) family and *Lolium perenne* L. – perennial ryegrass, Poaceae (Gramineae) family.

- Flotation tailings, average sample 0–20 cm deep, taken from the tailings beach (Figure 3), with granulometry below 500 μm (Table 1) and 6.5% humidity.



Figure 3. Section in the beach of the pond, 140 cm deep.

Table 1. Flotation tailings granulometry.

Class	Sand					Dust
	Sieve mesh sizes (μm)	500-250	250-125	125-63	63-45	
Weight (%)	3.7	23.7	38.7	15.7	13.9	4.3

- Industrial eluate (acid mine water) 1:5, tailings pond: water, with low pH – 4.3, with the following composition (ppm): Cu – 1.986; Pb – 0.116; Zn – 64.2; Ni – 0.736; Cr – 0.118; Cd – 0.562; Mn – 156; Co – 0.734; Li – 0.034; Fe – 0.031; Ca – 832; Na – 860. Chemical analysis was performed by the AAS method. The oxidation reduction potential (ORP) of the solution was 289 mV, and the electrical conductivity (EC) was 3.02 mS/cm.

- Oligomineral water, with (ppm) Ca^{2+} – 44.9; Mg^{2+} – 14.3; Na^+ – 0.78; bicarbonates (HCO_3^-) – 199.5; nitrates (NO_3^-) – 1.85; total mineralization – 268.78; pH – 7.7.

- Soil, with organic substances 29–60%; mineral salts 0.35–0.75%; soluble potassium 52–85%; soluble phosphorus 32–55%; mineral nitrogen 25–50 ppm; Ca, Mg and microelements; pH 5.33; 43.2% humidity.

- The organo-zeolitic material with the following physicochemical features: C_{org} – 5.01%; humus – 8.64%; N_{total} – 0.989%; P – 4.968 $\text{mg}\cdot\text{kg}^{-1}$; K – 10046 $\text{mg}\cdot\text{kg}^{-1}$; C/N 5.9; pH 9.17; 5% humidity. The organo-zeolitic material is a mixture (2:1) of volcanic zeolitic tuff from Bârsana area (Maramures basin) SiO_2 – 67.86%; Al_2O_3 – 12.16%; FeO – 0.10%; Fe_2O_3 –

0.12%; MgO – 0.24%; CaO – 2.99%; Na_2O – 0.98%; K_2O – 2.51%; TiO_2 – 0.009%; Cr_2O_3 – 0.01%, fraction <2 mm, with waste from the poultry farm (Cochemé et al., 2003; Damian et al., 2013; Damian et al., 2019). Compost was obtained according to the Leggo method (2004).

2.2. Methods

2.2.1. Seed germination experiments

The seeds are produced by Agrosem Seed Centre Iași, Romania. The experiments were conducted in conditions of temperature between 20–22°C, average air humidity of 38–40% and a photoperiod of 10–11 hours.

Seed germination experiments for parameter analysis:

- Germination capacity – GC (synonyms: Germinative factor – GF; Germination percentage – GP), the number of seeds in a batch able to germinate;
- Germinative energy – GE (synonym: Germination rate – GR), the speed at which the germination process is triggered;
- Relative seed germination – RG (synonym: Germination index – GI) – can be used as an indicator of phytotoxicity in soils.

The seed germination experiments were made in Linhardt dishes. For each variant (control and test), we used 3 replicates, with 50 seeds each. The seeds from the control replicates were wetted with oligomineral water, and the seeds from the test replicates were wetted with industrial eluate.

The GC estimates the viability of a population of seeds. The ISTA (1999) formula was used to calculate GC. The formula (1) to calculate germination percentage is:

$$GC(\%) = SG/TS \times 100 \quad (1)$$

(where: SG – number of seeds germinated; TS – total number of seeds).

The number of germination days required to establish GC was 6 days for *Sinapis alba* and *Trifolium pratense* and 14 days for *Lolium perenne*.

GE provides a measure of the time course of seed germination. GE is determined by calculating the germination percentage at different time intervals after planting and then plotting these data: 3 days for *Sinapis alba* and *Trifolium pratense* and 7 days for *Lolium perenne* (Cupcea et al., 1965; Boldor et al., 1983).

The percentages of relative seed germination – RG can be used as an indicator of phytotoxicity in soils. The formula to calculate relative seed germination (2) after Fuentes et al. (2004), is:

$$RG(\%) = G_t/G_w \times 100 \quad (2)$$

(where: G_t – total seeds germinated in test; G_w - total seeds germinated in control).

2.2.2. Growth of vegetative organs

The plant growth is more sensitive to toxic substances than seed germination (Barral & Paradelo, 2011).

Measurements of the lengths of the vegetative organs (root and shoot) were made 7 and 14 days after germination experiments started (ISO 11269-1: 1993).

The tolerance index (TI%) for roots and shoots was calculated according to Wilkins' formula (3) (Wilkins, 1957):

$$TI = \frac{\text{mean length test}}{\text{mean length control}} \times 100 \quad (3)$$

The percentages of relative root growth (RRG) and relative shoot growth (RSG) was calculated according to the Fuentes et al. (2004) formula (4):

$$RRG_t / RRG_w \times 100 \quad (4)$$

(where: RRG_t – mean length in test; RRG_w - mean length in control).

Then root growth inhibition (GI%) was calculated following the Adamcová et al. (2016) equation (5):

$$GI(\%) = \frac{ML_w - ML_t}{ML_w} \times 100 \quad (5)$$

(where: ML – mean length of roots, t – in test or w – in control).

2.2.3. Experiments in pots

Experiments in pots on the growth of plants on the flotation tailings material and on the mixtures with soil and organo-zeolitic material (Figure 4) were performed according to the OECD test (2006).

In the tests for plant growth on the tailings pond, 5 experimental variants were used: (1) soil 100% (S); (2) flotation tailings 100% (T); (3) mixture of flotation tailings 85% + soil 15% (T+S); (4) mixture of flotation tailings 85% + organo-zeolitic material 15% (T+OZ); (5) mixture of flotation tailings 70% + organo-zeolitic material 15% + soil 15% (T+OZ+S) (Table 2).



1



2



3

Figure 4. Preparation of the mixtures: 1 - flotation tailings material; 2 - soil; 3 - organo-zeolitic material.

Table 2. Composition of experimental variants (g).

Experimental variants	Soil	Flotation tailings	Organo-zeolitic material
1. S	500*	-	-
2. T	-	500	-
3. T+S	75	425	-
4. T+OZ	-	425	75
5. T+OZ+S	75	350	75

*The mixtures were prepared taking into account the moisture content of the used materials.

The mixtures prepared for sowing, were placed 500 g in pots, moistened for one week with oligomineral water to uniformise the salts released by the constituent materials and to carry out the ion exchange. There have been conducted measurements of pH, oxidation-reduction potential (ORP) and air in the soil (Table 3).

The seeds were sown in three pots with 50 seeds for each work variant. The pots were placed under the same conditions as germination tests. Monitoring of seed growth was performed over 21 days (at 3, 6, 9, 12, 15, 18 and 21 days).

Table 3. Physico-chemical parameters of flotation tailings and mixtures with soil, and organo-zeolitic material.

Experimental variants	pH	ORP (mV)	Air in soil (%)
1. S	5.33	128	33
2. T	4.50	165	21
3. T+S	5.12	130	24
4. T+OZ	4.26	179	25
5. T+OZ+S	4.84	146	23

2.2.4. Data processing

The statistical estimation of the comparative growth differences between vegetative organs of control and test were achieved by applying the T-Student test. Elimination of aberrant values is based on the Chauvenet criterion (Snedecor & Cochran 1978; Weber, 1980). The value of p indicates: $p > 0.50$ insignificant; $p < 0.50$ – significant; $p < 0.001$ – very significant.

3. RESULTS AND DISCUSSIONS

3.1. Experiments of seeds germination

Seeds germination was very sensitive to environmental changes. The germination test is an important procedure that evaluates the toxic effects of heavy metals on plants (Bezini et al., 2019).

3.1.1. Seeds germination experiments of *Sinapis alba* L

In control (Figure 5) the total number of germinated seeds after 3 days was 143 (GE – 95.3%), compared to the test (Figure 6) where 135 seeds germinated (GE - 90%).

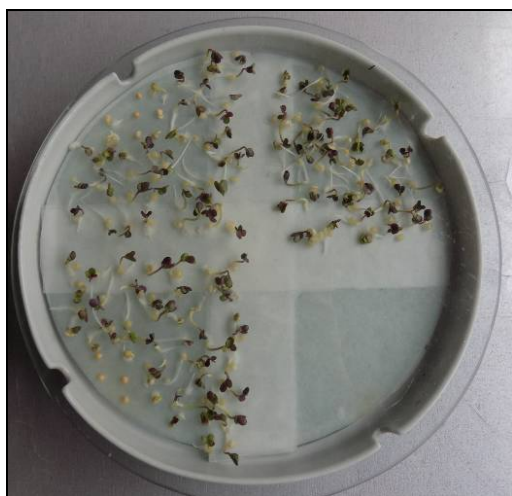


Figure 5. *Sinapis alba* - control at 3 days.



Figure 6. *Sinapis alba* - test at 3 days.

The influence of the substrate (water or industrial eluate) after 6 days can also be seen in the number of seedlings that had vegetative organs (a priori of the first leaves): 127 plantlets in the control (Figure 7) and 120 plantlets in the test (Figure 8).

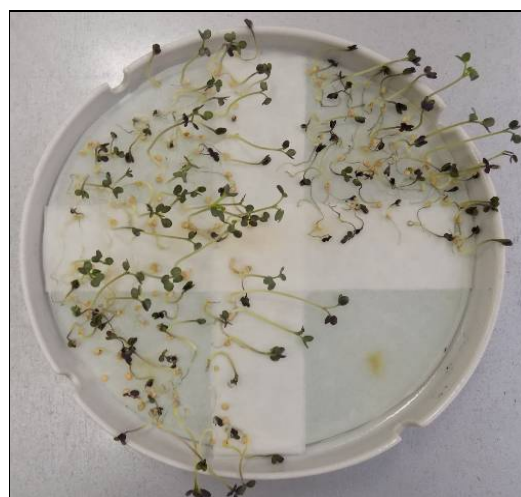


Figure 7. *Sinapis alba* - control at 6 days.

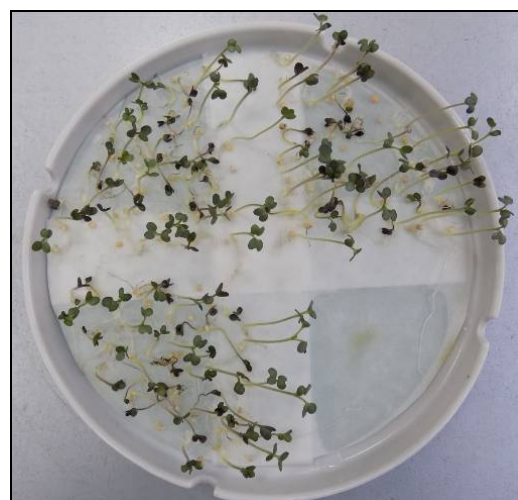


Figure 8. *Sinapis alba* - test at 6 days.

After 6 days, GC was 96% in the control (GC_w) and 95.3% in the test (GC_t). For the control, maximum GC was reached on day 7.98% while the test remained the same. After 7 days, 138 plantlets showed the development of leaves in the control and 127 plantlets in the test (Figure 9).

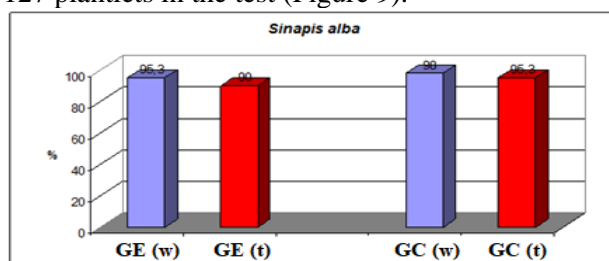


Figure 9. Germination energy (GE) and germination capacity (GC) at *Sinapis alba* (w – control; t – test).

In both variants most seeds germinated in the first 3 days, had a large GE, while the maximum GC was reached after 4 days and 7 days respectively (Figure 10).

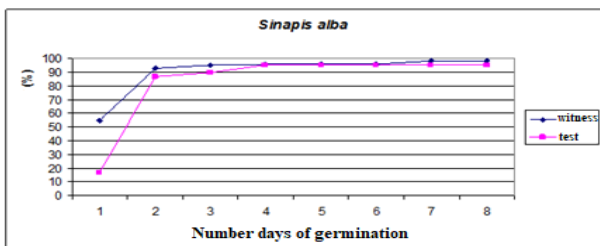


Figure 10. The dynamics of the germination process at *Sinapis alba*.

3.1.2. Experiments of seeds germination of *Trifolium pratense* L

The total number of seeds germinated after 3 days in the control was 93 out of 150 seeds (Figure 11), a GE of 62%, compared to the test, where GE was 46.6% as a result of germination of 70 seeds (Figure 12). Few seedlings showed the development of the first leaves after 3 days: 21 and 6 respectively.

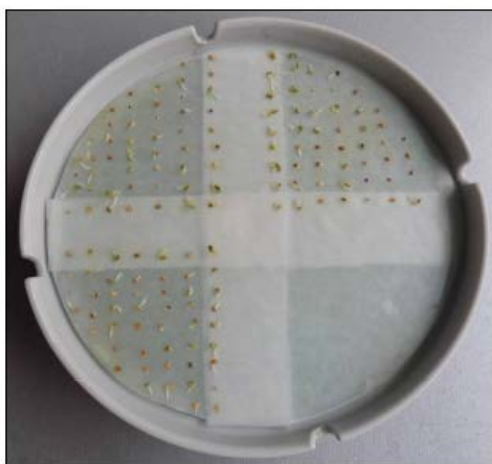


Figure 11. *Trifolium pratense* control at 3 days.

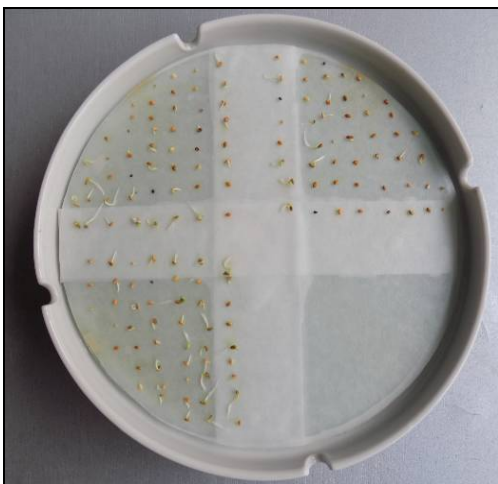


Figure 12. *Trifolium pratense* test at 3 days.

After 6 days, GC was 73% for the control and 60.6% for the test, as a result of the germination of 110 seeds (Figure 13) and 91 seeds respectively (Figure 14); 100 control plantlets have developed

leaves and only 64 plantlets in the test.

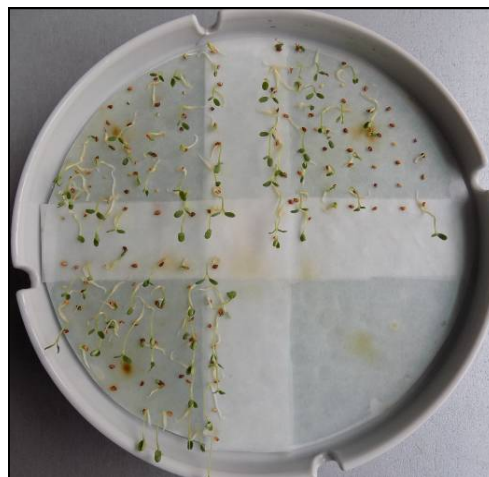


Figure 13. *Trifolium pratense* control at 6 days.



Figure 14. *Trifolium pratense* test at 6 days.

The maximum germination level GC was reached in both variants after 10 days: 78.7% (118 seeds germinated – 112 with leaves) in the control and 64.7% (97 germinated seeds – 78 with leaves) in the test (Figure 15).

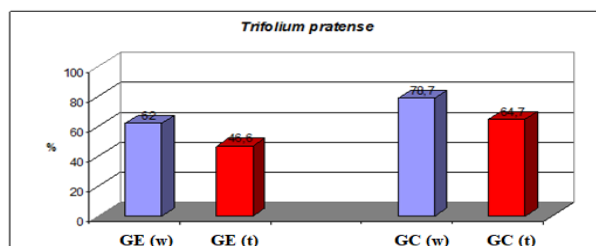


Figure 15. Germination energy (GE) and germination capacity (GC) at *Trifolium pratense* (w – control; t – test).

In control and test variants for *Trifolium pratense*, the dynamics of the germination process is similar over the course of 10 days but occurs at different levels due to different germinative factors under the conditions offered by the two variants

(Figure 16).

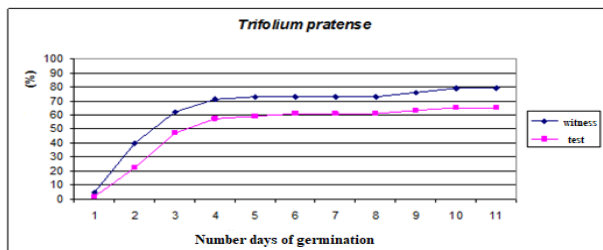


Figure 16. The dynamics of the germination process at *Trifolium pratense*.

3.1.3. Experiments of seeds germination of *Lolium perenne* L

Germinative energy, GE, of the ray grass seeds, calculated after 7 days of germination, was 40.7% (61 seeds germinated) under oligomineral water wetting conditions (Figures 17, 18) and 28.7% (43 seeds germinated) in the test industrial eluate (Figures 19, 20).

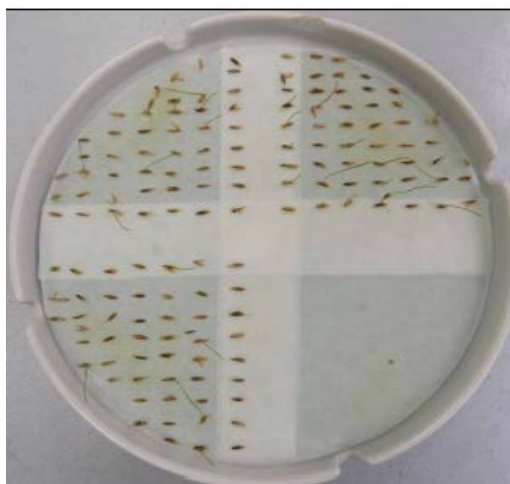


Figure 17. *Lolium perenne* – control at 3 days.

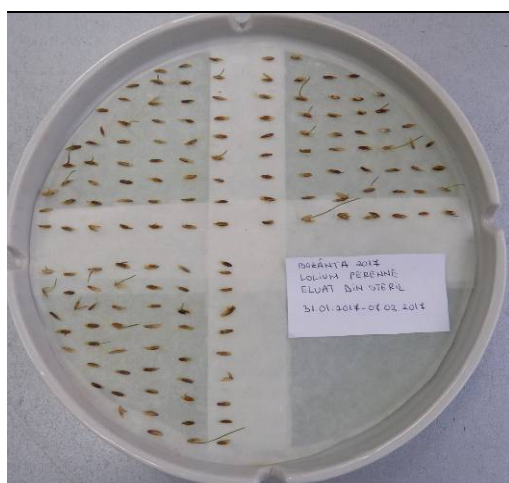


Figure 18. *Lolium perenne* – test at 3 days.

The germination capacity (GC) after 14 days of germination, was 72% (108 germinated seeds) for the

control, while the percentage for the test was 62% (with 93 germinated seeds) (Figure 21). For both variants, maximum GC was reached on day 13.



Figure 19. *Lolium perenne* - control at 6 days.



Figure 20. *Lolium perenne* – test at 6 days.

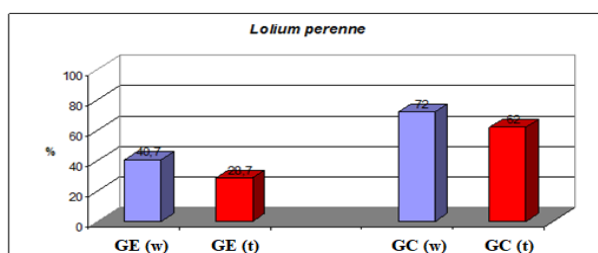


Figure 21. Germination energy (GE) and germination capacity (GC) at *Lolium perenne* (w – control; t – test).

Figure 22 shows that for both variants (control and test) the germination process start to be visible within 3 days, taking place in parallel during the 14 days, with a difference that remained constant.

By calculating the differences between the values obtained for each GE and GC parameter in the control and test and for each plant, one can see the extent to which the industrial eluate affected seeds germination of each species: for *Sinapis alba* the

differences between control and test for GE were 5.3% and 2.7% for GC; for *Trifolium pratense* the differences were 15.4% for GE and 11% for GC and for *Lolium perenne* the differences were 12% for GE and 10% for GC respectively.

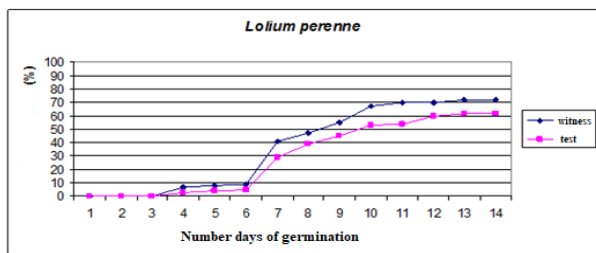


Figure 22. The dynamics of the germination process at *Lolium perenne*.

Considering that in the end the total number of germinated seeds is important, compared to the GC of the plants in the conditions of the industrial eluate, it is found that the smallest difference is 2.7%, so less affected were the white mustard seeds. For the ray grass and red clover seeds, the differences were 10% and 11%, which represents a greater effect that the industrial eluate had on the germination of the seeds of these plants.

The results obtained for the germination capacity give information about the quality of the seeds material. Based on these results it is possible to calculate the degree of cover of cultivated lands and the number of seeds required per hectare.

From the results one can observe that for seeds lots used for both *Trifolium pratense* and *Lolium perenne* under normal growing conditions it is necessary to supplement the seeds used for seeding with more than 20-30% above the recommended quantity, in order to obtain a vegetal carpet with optimal density. For *Sinapis alba*, the supplementation compared to the recommended quantity / ha is not required.

In the tests involving industrial eluate, the values of the monitored parameters were situated at the lowest levels compared to the results obtained under normal conditions.

The three seed lots used in the industrial elution test experiments had different GC: 95.3% *Sinapis alba*, 60.6% *Trifolium pratense* and 62% *Lolium perenne*, below those obtained in control, reflecting the effects negative of industrial eluate on seed germination.

In the context of the results obtained for the GC differences in the two variants and the GE, it is assumed that with the use of the three lots of seeds to revegetate the tailings depot, producing a vegetal carpet directly on the tailings, taking into account only the chemical parameters of the industrial eluate,

the *Sinapis alba* seeds will be most affected by germination, followed by *Lolium perenne* and *Trifolium repens*.

From the calculation of relative seed germination (RG), the following results were obtained: *Sinapis alba* – 99.3%; *Trifolium pratense* – 82.7% and *Lolium perenne* – 86.1%.

3.2. Highlighting the effects of industrial eluate on growth of vegetative organs (root and shoot)

Parameters for inhibiting plant growth are often used to evaluate the toxicity effects of heavy metals. Some authors (Cendón et al., 2005; Barral & Paradello, 2011) consider that plant growth is more sensitive to toxic substances than seed germination.

3.2.1. Effects of industrial eluate on growth of vegetative organs at *Sinapis alba* L

In case of the *Sinapis alba* plantlets, in the first 7 days, in two of the control variants (replicates 1 and 3) the mean values and statistical estimation for the roots were increased than those of the test variants. The percentage differences from the control were increased with 16.10% (replicate 1) and 8.09% (replicate 3), while in the case of the test – replicate 2, the percentage difference compared to the control decreased with 9.21% (Table 4).

Table 4. Influence of industrial eluate on the growth in length (mm) of *S. alba* roots (after 7 and 14 days)

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es*	X±Es*
7 days			
Control	12.42±0.85	15.97±0.99	14.95±0.81
Test	14.42±0.85	14.50±0.83	16.16±0.95
p	<0.25	<0.25	<0.5
D%	116.1	90.79	108.09
14 days			
Control	14.68±1.22	20.50±1.13	20.86±1.73
Test	13.67±1.19	19.40±1.51	18.82±1.83
p**	NS***	NS****	NS****
D%***	93.11	94.63	90.22

X±Es* – medium values ± statistical estimation; p** – statistical significance; D%*** - percentage differences from the control; NS**** - insignificant.

In other words, in two of the three test replicates, the average on the growth in length of roots were higher than in the case of the control replicates. The average on the growth in length of roots for the control was 14.4 mm and 15.0 mm for test, after 7 days. The percentage increase compared to the control was 4%.

After 14 days, the average on the growth in

length of roots for the control was 18.7 mm and 17.3 mm for test. It was observed a decrease of 7.45% on the growth in length of roots plantlets treated for 14 days with eluate. The visible effect of metal toxicity is the inhibition of root elongation. The root length can be used as most important tolerance index (Haque et al., 2020).

Comparing the averages of the growth in length of shoots for the control (22.6 mm) and the average for the test (24.8 mm), there was an increase of 10.13% growth in length shoots of the plantlets grown on the industrial eluate after 7 days (Table 5).

Table 5. Influence of industrial eluate on the growth in length (mm) of the *S. alba* shoots (after 7 and 14 days).

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es	X±Es
7 days			
Control	17.00±1.03	25.44±1.45	25.33±1.38
Test	27.97±1.81	21.76±1.25	24.93±1.33
p	<0.001	<0.05	<0.5
D%	164.52	85.53	98.42
14 days			
Control	20.04±1.13	33.47±2.32	33.34±1.71
Test	36.97±2.69	30.97±2.12	34.67±2.11
P**	<0.001	NS****	NS
D%***	158.53	92.52	103.98

Explication at table 4.

It follows that after the first seven days of the germination of white mustard seeds, there was a further increase in the vegetative organs in the test, with industrial eluate, as compared to the control. The percentage differences from the control increased by 4% in the root and 10.13% in the case of the shoot.

The treatment for 14 days with industrial eluate at the *Sinapis alba* plants showed the decrease of growth in length of the roots slightly below those in the control. The percentage difference from the control ranged between -6.89% and -9.78%.

In replicate 1, after 14 days, the growth of shoots in the test is highly significant with a percentage difference from the control of 58.53% (p <0.001). In test replicates 2 and 3 there were decreases, respectively increases (-7.48% and +3.98%) compared to control. Taking into account the three replicates, the control obtained an average of 28.9 mm, and the test 34.2 mm, which corresponds to a difference of 18.13% in favour of the plants grown on the industrial eluate.

3.2.2. Effects of industrial eluate on growth of vegetative organs at *Trifolium pratense* L

In the case of *Trifolium pratense*, the results showed the significant increase in the roots length in the test variants (19.43% – replicate 1; 7.27% –

replicate 2; 12.82% – replicate 3) after 7 days.

After 14 days, the increase of the growth in length of roots was observed in two replicates. The increase compared to the control was 4.16% (replicate 1) and 47.87% (p<0.001; replicate 3).

The average growth length of the roots was 15.3 mm for the control and 15.7 mm for the test. The differences between the two lots are insignificant, the eluate having no visible effects on the length increase of the red clover root (Table 6).

Table 6. Influence of industrial eluate on the growth in length (mm) of *T. pratense* roots (after 7 and 14 days).

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es*	X±Es*
7 days			
Control	8.90±0.31	9.77±0.97	9.75±0.17
Test	10.63±0.61	10.48±0.5	11.00±0.55
p	<0.01	<0.25	<0.10
D%	119.43	107.27	112.82
14 days			
Control	12.96±1.16	20.42±1.40	12.47±1.23
Test	13.50±1.47	15.26±1.5	18.44±1.48
p**	NS****	<0.02	<0.001
D%*	104.16	74.73	147.87

Explication at table 4.

Table 7. Influence of industrial eluate on the growth in length (mm) of *T. pratense* shoots (after 7 and 14 days)

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es*	X±Es*
7 days			
Control	20.51±0,52	17.08±0,91	21.10±0,51
Test	18.36±1.24	17.88±0,45	17.32±0.64
p	<0.05	NS****	<0.001
D%	89.51	104.68	82.08
14 days			
Control	24.46±0.94	22.69±1.05	21.94±1.22
Test	19.47±0.83	20.59±0.77	20.57±0.77
p**	<0.001	<0.25	<0.50
D%***	79.59	90.78	93.75

Explication at table 4.

It is noted that in case of red clover plant shoots, the industrial eluate had a decrease in growth rate compared to the control (Table 7). Measurements of red clover shoots at 14 days after the experiment set up revealed the tendency for the first 7 days to slow the growth under the influence of industrial eluate against the control in all three batches. The percentage difference from the control was between - 20.41% (<0.001; replicate 1), - 9.22% (p<0.25; replicate 2) and -6.25% (<0.50; replicate 2). The average growth of the shoots was 23.0 mm in the control and 20.2 mm in the test, maintaining the differences observed in the measurements performed at 7 days.

3.2.3. Effects of industrial eluate on growth of vegetative organs at *Lolium perenne* L

After 7 days, the results indicate an increase of the roots in the test variants, the increase being 61.68% ($p < 0.05$; replication 1), 16.69% ($p < 0.10$; replication 2) and 18.73% (replication 3) compared to the control. The mean roots growth in the control was 5.9 mm, and 7.6 mm in the test (Table 8).

Table 8. Influence of industrial eluate on the growth in length (mm) of the *L. perenne* roots (after 7 and 14 days).

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es*	X±Es*
7 days			
Control	4.15±0.47	6.12±0.74	7.58±1.01
Test	6.71±1.1	7.20±1.37	9.00±1.12
p	<0.05	<0.10	NS****
D%	161.68	116.69	118.73
14 days			
Control	28.06±1.80	23.86±1.59	23.44±1.22
Test	29.09±1.49	33.07±2.18	34.70±2.45
p**	NS****	<0.001	<0.001
D%***	103.67	138.6	148.03

Explication at table 4.

As for the length of the shoots in the control variants, it had the values: 10.25±1.39 mm (replicate 1), 13.40±0.87 mm (replicate 2) and 8.62±1.44 mm (replicate 3) with an average of 10.8 mm.

In the test variants, the results obtained were 6.85±1.05 mm (replicate 1), 9.40±2.01 mm (replicate 2) and 9.63±2.11 mm (replicate 3) with an average of 8.6 mm (Table 9). There was a slowdown in the growth of *Lolium perenne* shoots in the test replicates relative to the control replicates.

Table 9. Influence of industrial eluate on the growth in length (mm) of the *L. perenne* shoots (after 7 and 14 days)

Variants	Replicate 1	Replicate 2	Replicate 3
	X±Es*	X±Es*	X±Es*
7 days			
Control	10.25±1.39	13.40±0.87	8.62±1.44
Test	6.85±1.05	9.40±2.01	9.63±2.11
p	<0.25	<0.05	NS****
D%	66.82	70.15	111.71
14 days			
Control	51.61±3.76	54.87±3.36	45.65±3.51
Test	53.50±1.19	60.07±3.75	65.03±2.88
p**	NS****	NS****	NS****
D%***	103.66	109.47	142.45

Explication at table 4.

After 14 days, measurements of the aerial parts revealed the growth of plant shoots in the test variants compared to the control variants. The increase was statistically insignificant, ranging between 3.66%

(replicate 1) and 42.45% (replicate 3).

For *Lolium perenne*, the metal concentrations in the eluate had positive effects: the mean for the control was 50.7 mm, and 59.5 mm for the test with industrial eluate.

3.2.4. Relative increase in vegetative organs

By comparing the results for the relative increase of the roots, the following features were observed (Table 10):

- after the first 7 days, there was a relative increase in plant roots length in the test – with industrial eluate for all plant species; the biggest difference was observed in *Lolium perenne*, followed by *Trifolium pratense* and *Sinapis alba* (very small);
- after 14 days, at the end of the experiment, the same difference was observed indicating a greater increase in length of the *Lolium perenne* roots in the test compared to the control; for *Trifolium pratense* the differences were very small, whereas for *Sinapis alba* the value of the relative dimensions of the roots in the test decreased compared to the control;
- the industrial eluate stimulated long-term growth of the *Lolium perenne* roots, it brought small changes of growth in the case of *Trifolium pratense*, while on the roots of *Sinapis alba* it had an inhibitory effect (Figure 23).

Table 10. Relative root growth and relative shoot growth

No. days	Species / Relative growth (%)		
	<i>S. alba</i>	<i>T. pratense</i>	<i>L. perenne</i>
Relative roots growth			
7	104	114	128
14	92	103	128
Relative shoot growth			
7	164	91	84
14	118	87	117

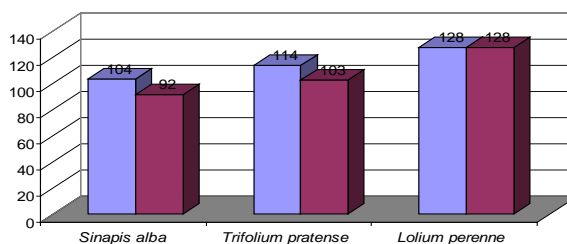


Figure 23. The relative roots growth (blue columns – 7 days; red columns – 14 days).

For the relative growth of shoots during the experiment, the following observations were made:

- after the first 7 days, there was a significant increase in the length of the *Sinapis alba* shoots in the variants exposed to the industrial eluate, whereas for *Trifolium pratense* and

Lolium perenne the industrial eluate had a slowing down effect on the growth of the shoots compared to the control (Figure 24);

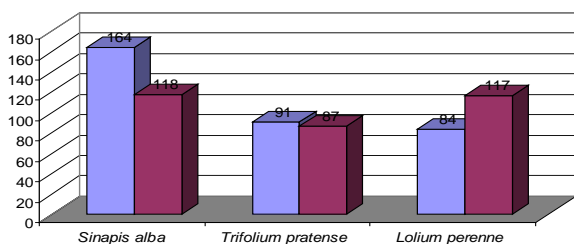


Figure 24. The relative shoots growth (blue columns – 7 days; red columns – 14 days)

- following the measurements made 14 days after the start of the experiment, for the shoots of *Sinapis alba*, dimensional differences remained in favour of the test, but at a difference less than 7 days;
- for *Trifolium pratense*, the growth rate of the shoots in the test variants further decreased compared to the control;
- in the test with *Lolium perenne*, a significant increase was observed (after stagnation in the first 7 days), the ray grass shoots in the test exceeded the size of the control.

3.2.5. Tolerance index

The effects of industrial eluate on the growth of vegetative organs were as follows:

- The roots length of *Sinapis alba* decreased (92%) slightly below the average of the results in the control, whereas the shoots were stimulated in the increase in length (118%);
- For *Trifolium pratense*, the differences obtained for the roots are also insignificant (102.94%), whereas the average of the plant shoots size in the test was lower than that of the control (87.75%);
- *Lolium perenne* showed an increase in the dimensions of the roots (115.1%) and shoots (117.39%) in the test compared to the control;
- Industrial eluate may have minimal effects on the germination for some plants but may have effects on the growth of vegetative organs of plants.

3.2.6. The percentage inhibition of roots

The percentage inhibition of roots (GI%) determination was based on the average values of the growth rate. $GI\% > 0$, denotes an inhibitory effect, while $GI\% < 0$, means growth stimulation (Vaverková et al., 2020).

For *Trifolium repens* and *Lolium perenne* the results obtained were negative (-2.94%; -15.24%) which indicates a stimulation of the roots growth. *Sinapis alba* belongs to sensitive species and is often

used to test toxicity. The GI% value for *Sinapis alba* was low (8.03%), indicating that the solution is less phytotoxic to *Sinapis alba*. The plants have ability to tolerate and adapt to stress (heavy metals in soil).

3.3. Experiments on plant growth on the flotation tailings and on mixtures with soil and organo-zeolitic material

Table 11 shows seeds germination results for the five variants. *Sinapis alba* seeds sown in soil, control variant V1, germinated at a rate of 94%. Of the test variants, V5 with T+OZ+S germinated and developed 44% of the seeds.

Table 11. Germinative capacity in experiments on plant growth on pots at 21 days

Species / Experimental variants	Germinative capacity (%)				
	V ₁ S	V ₂ T	V ₃ T+S	V ₄ T+OZ	V ₅ T+OZ+S
<i>S. alba</i>	94	0	18	10	44
<i>T. pratense</i>	88	16	56	46	56
<i>L. perenne</i>	40	0	4	4	4

In V3 (T+S) and V4 (T+OZ) variants, only 18% and 10% of the seeds germinated, while in V2, consisting only of flotation tailings, germination was totally inhibited.

In tests for *Trifolium pratense*, 88% of seeds sown in soil have germinated and developed. In test V₃ (T+S) and V₅ (T+OZ+S), 56% of seeds have germinated and 56% of seeds have developed, and in variant V₄ (T+OZ) with organo-zeolitic material, the percentage was 46%. The smallest percentage of germination, of 16%, was on flotation tailings substrate (V₂), but it is also the only one of the three plant species whose seeds germinated only on flotation tailings.

In the case of *Lolium perenne*, it is noted that in the control variant V1, with seeds sown on the fertile soil, germinated, and developed 40% of the seeds. In variants V3, V4 and V5, wherein the flotation tailings are mixed with soil and / or organo-zeolitic material, a small number of seeds has germinated, the tailings having a strong inhibiting effect on the germination of the grass seeds. Figure 25 shows the relative seed germination outcome in water, eluate and for the five experimental variants.

Sinapis alba seeds

a) *S. alba* had the best germinative capacity (GC), germination energy (GE), both in the control variants with oligomineral water (98%) and soil (94%), but also in the germination test with industrial eluate (95%);

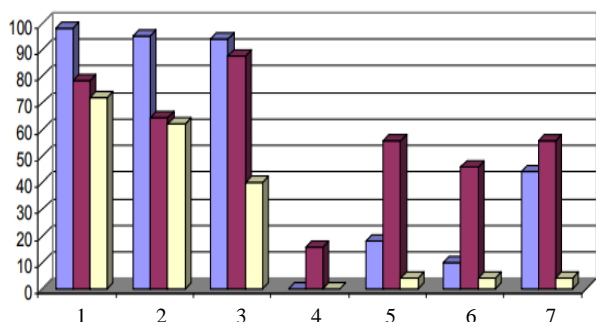


Figure 25. Relative seed germination in the variants with eluate and flotation tailings. 1. Water; 2. Eluate; 3. $V_1 - S$; 4. $V_2 - T$; 5. $V_3 - T+S$; 6. $V_4 - T+OZ$; 7. $V_5 - T+OZ+S$.

Blue columns – *Sinapis alba*, red columns – *Trifolium pratense*; yellow columns – *Lolium perenne*.

- b) most seeds germinated in the first 3 days;
- c) relative seed germination (RG) was over 99%, indicating that germination of mustard seeds was virtually unaffected by the chemical composition and pH of the industrial eluate;
- d) regarding the relative root growth (RRG) in the industrial eluate test, there is an increase and then an insignificant decrease – so the industrial eluate has not influenced the root growth;
- e) the relative shoot of growth (RSG) there was an accelerated increase in the industrial eluate in the first 7 days (64%), after which at the end of the 14 days period, the difference decreased to 18%, which can be considered a stimulating effect that the eluate had in the long-lasting growth of white mustard shoots in the first part of plant growth;
- f) tolerance index (TI) of 92% means an insignificant influence of the industrial eluate on roots growth of white mustard;
- g) in the tests performed on the flotation tailings, in the variant $V_5(T+OZ+S)$, the highest relative germination (44%) was obtained, this being the appropriate substrate for cultivation on the flotation tailings;
- h) given that the germination in $V_5(T+OZ+S)$, is 47% of that obtained in the $V_1(S)$, soil test, it is recommended to double the seeds norm at flotation tailings when using this type of mixture.

***Trifolium pratense* seeds**

- a) *T. pratense* had the largest germinative capacity (GC), 88%, in V_1-S (soil test); in the oligomineral water and industrial eluate test, the GC was much lower, 73% and 60.6%;
- b) most seeds germinate in the first 2 days with very good germinative energy (GE) of about (90%);
- c) relative seed germination (RG) was 83%, thus the industrial eluate influenced the germination of red clover seeds;
- d) the results for relative root growth (RRG), at the end of the 14 days did not indicate changes induced

- by industrial eluate in roots growth;
- e) regarding the relative shoot growth (RRS) there was a decrease of the height of the shoots in the industrial eluate with 13%, indicating a negative effect on growth;
- f) the tolerance index (TI) of 103% is the consequence of the industrial eluate and not of the changes in root;
- g) in the tests performed on the flotation tailings, in the vegetal soil variants $V_3(T+S)$ and $V_5(T+OZ+S)$, the highest germination index (56%) was obtained, followed by the variant with organo-zeolitic material $V_4(T+OZ)$; it is noteworthy that red clover seeds were the only ones that germinated in V_2 only with flotation tailings;
- h) in case of using this plant, it is also recommended to double the seeds norm.

***Lolium perenne* seeds**

- a) *L. perenne* had the lowest germination capacity (GC), both in oligomineral water (72%) and soil (40%) and in the industrial eluate (62%);
- b) most seeds germinated only after 9-10 days, having a low germinative energy (GE) at 7 days, of 41% in the control and 29% in the eluate;
- c) 86% relative seed germination (RG), indicating that ray grass seed germination was influenced by the chemical composition and pH of the industrial eluate;
- d) relative root growth (RRG), in the industrial eluate test, there was a significant increase of 28% over the 14 days; thus, the industrial eluate influenced the growth of the roots;
- e) regarding the relative shoot growth (RRS), there was a decrease in the case of industrial eluate in the first 7 days (84%), then the difference decreased, and in the end the shoots were 17% longer than in the control;
- f) the 115% tolerance index (IT) was given in particular to stimulate root growth under the influence of industrial eluate;
- g) in all tests performed with flotation tailings, the germination index was 4%;
- h) this species should not be used in the mixed variants tested for the Bozânta tailings depot.

4. CONCLUSIONS

The research aimed to test the resistance of the following plant species: *Sinapis alba* L (white mustard), *Trifolium pratense* (red clover) and *Lolium perenne* L (ray grass) to the conditions offered by the flotation tailings on the surface of the tailings depot, in order to achieve a vegetal carpet with a role in reducing tailings material mobilization by wind to the surrounding areas.

The results of the tests on the effects of

industrial eluate (with acid pH of 4.3 and heavy metal content) on the seed germination parameters show that the least affected was the germination of white mustard seeds, followed by the germination of ray grass seeds and then of red clover.

Analysing the results of experiments that highlighted the effects of industrial eluate on the growth of vegetative organs of plants, it was found that the eluate had an inhibitory effect on the growth of white mustard roots, did not influence the growth of red clover and stimulated the growth of ray grass.

Regarding the shoots, it was observed that industrial eluate stimulated growth of mustard and ray grass and inhibited the red clover. According to the toxicity index, red clover is the plant that had the largest deviations from the results obtained under standard conditions. It is recommended to prepare the mixture T+OZ+S on the surface of the depot and sow it with white mustard and red clover.

It is not recommended to use the *Lolium perenne* in the germination works in the mixed variants tested for the tailings from Bozânta depot, considering the inhibition of germination and growth of this species in all variants with flotation tailings.

Considering the results obtained in the germination and growth tests in the presence of the industrial eluate and of the germination and growth tests performed on the flotation tailings, the following can be stated:

- unlike the inhibitory factors represented by the physical and chemical parameters of the tested solutions (pH, Eh, heavy metal content), in the case of tailings, there are other stress factors with action on germination and growth of plants arising from the granulometry of stored materials (germination layer density, presence of air, capillary force, etc.);

- taking into account the complexity of the materials stored in the tailings depots and the effects they may have on plants, it is recommended that both types of tests would be performed to select the appropriate species for revegetation;

- a necessary step before the actual work is the testing at the pilot level, in the field, to make observations on the effects of environmental factors specific to the application area.

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