FACULTY OF BIOLOGY

DOCTORAL SCHOOL OF BIOLOGY

RESEARCH ON THE EFFECTS OF TOXICITY OF SOILS CONTAMINATED WITH HEAVY METALS ON SOME UPPER PLANT SPECIES. CASE STUDY *TRITICUM AESTIVUM* L.

DOCTORAL THESIS ABSTRACT

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IASI

2022

THANKS

Now, at the end of this stage of my life, I deeply want to address a few words of thanks to all those wonderful people who guided or supported me throughout doctoral studies and who broke their free time to offer me support and help.

First of all, I would like to express my sincere thanks the doctoral supervisor, Mrs. Professor univ. Dr. Maria-Magdalena Zamfirache, for her permanent and rigorous guidance, for her continuous support and encouragement throughout the period of doctoral preparation and thesis elaboration.

With great consideration, I would like to thank the members of the References Committee for their valuable time, for their kindness in analyzing this paper, for the valuable scientific advice, and for the competent and permanent guidance during the elaboration and realization of this doctoral thesis.

This doctoral thesis would not have been complete without the essential help of Mr. Professor Dr. Gabi Drochioiu, who directed my steps towards scientific research, by proposing the research topic of the paper and I want to thank him for all the moral and scientific support. granted during these years of study.

I would like to thank Dr. Zenovia Olteanu, Professor and Dr. Anișoara Stratu, Head of Works, for all the useful suggestions and valuable observations offered during the scientific reports, observations that contributed to the completion of this paper.

In this way, I would like to thank my colleagues Dr. Olga Pintilie and Associate Professor Dr. Andrei Lobiuc, for their help and useful advice, in terms of statistical analysis of data, as well as in conducting some of the laboratory experiments.

I thank my family for all the understanding and patience shown during the most difficult times, during these years and for the unconditional support during the elaboration of the doctoral thesis.

I thank all those mentioned, who helped me during the years of writing this thesis and I want to emphasize that without the help received from you, the completion of this paper could not have been possible.

THESIS CONTENTS

INTRODUCTION

The existence of heavy metals and metalloids such as arsenic in soil and water, which have occurred as a result of mining in Romania is a major problem for both human health and the entire forest food chain. Due to their high toxicity, storage capacity and contamination, these potentially harmful elements for all ecosystems pose a real danger to humans and animals in the contaminated area.

Pollution caused by mining and the presence of large amounts of heavy metals and arsenic in the environment induced by human activity is one of the most important tasks of current environmental problems. In Romania, contamination with these elements, especially in forest areas [79], where there were mining operations, is a real challenge for research, as is the Tarnița area, in Ostra commune, Suceava county. In the Tarniţa-Ostra forest mining area subject to the study within the doctoral thesis, the waste dumps and tailings ponds remained, which represent a real danger for the health of the food chain. Excessive amounts of heavy metals, such as iron, copper, zinc, lead, cadmium and metalloids such as arsenic, have been detected both in and around the dumps. Excessive increase in the amounts of these elements in the soil, mainly due to pollution, leads to metabolic disorders in plants also affecting the quality of food, which could be harmful to human health [14].

The motivation of the research carried out in the doctoral thesis is represented by the fact that contaminated soil, surface and groundwater, but also the air in the Tarnița area can be significant major sources for the absorption of heavy metals by plants, can accumulate through roots and leaves. . Heavy metals such as Cd, Hg, Pb, Fe and excess Cu are very toxic to humans and the environment, with plants being considered as potential biosorbents to remove traces of metals from the soil. Therefore, the research carried out aimed at decontamination of soils affected by metal pollution [88], but also of extraction waters, ie supernatants obtained from mining materials, before performing germination tests on the species *Triticum aestivum* L..

Doctoral thesis entitled " Research on the effects of toxicity of soils contaminated with heavy metals on higher plant species. Case study *Triticum aestivum* L. " had as main objective the evaluation of the unfavorable effects induced to the common wheat species by the presence of heavy metals and arsenic in the dumps and soils coming from the Tarnița mining area. Thus , in a first phase of the doctoral research, determinations of the concentration of heavy metals present in the samples of mining and soil waste collected from the Tarnița area and in the samples with mining residues (toxic supernatants) were performed, using ICP-OES spectrophotometric techniques. and AAS. Similar to research conducted in previous years in the area of the former barite mine by Ștumbea in 2013 and then by Chicoș and collaborators in 2016, were identified in the Tarnița forest area both in landfills, in the surrounding soils, and and in tailings ponds remaining after mining significant concentrations of heavy metals such as iron, copper, barium, zinc, lead and others [77, 11]. Also, a correlation was made between the concentrations of the main contaminants (iron, copper, arsenic) identified in the samples and the values of germination parameters obtained for the species *Triticum aestivum* L. after 3 and 7 days of treatment, respectively. The first visible symptoms related to the toxicity of heavy metals included a slowdown in the germination process of caryopsis, a decrease in seedling growth, a reduced growth of the root, but also changes in root morphology, the roots being more affected than the leaves, because they were the organs. which came into direct contact with the toxic element. Therefore, the use of cereal crops for the management of soil contaminated with heavy metals has become an ecological way of remediation strategy.

Also, the methods of decontamination and remediation of contaminated soils and toxic supernatants obtained by extraction, containing heavy metals and arsenic, have been studied and applied in the laboratory, in order to reduce their toxicity on caryopsis and seedlings of *Triticum aestivum* L. . The use of the test species *Triticum aestivum* L. can provide technical efficiency, first for measuring germination parameters in the presence of contaminants, and then for removing heavy metals from polluted soils [66, 71, 85]. The results obtained *in vitro* , under experimental laboratory conditions, will provide information on the potential risks of heavy metals and arsenic in areas contaminated by wheat, ie changes in the processes of caryopsis germination and plant growth, the effect on macromolecules or activity. antioxidant enzymes in monocotyledonous cultures. These will help to identify the problems that may occur when cultivating plants in contaminated areas, specifying whether the Putna variety is suitable and resistant to be grown in the Tarnița area.

Thus, an important objective of doctoral research was to investigate methods of decontamination of waste from landfills, soils and waters and remediation of toxic compounds, methods that can be applied in the mining area Tarniţa: physico-chemical decontamination by extracting contaminants with water; bioremediation of toxic supernatants / mining residues using active and inactivated yeast cultures Saccharomyces cerevisiae Meyen ex EC Hansen; chemical decontamination of toxic supernatants / mining residues with glutathione, as well as by hydroxide precipitation, chemical precipitation being one of the most common and extensive remedial techniques for removing heavy metals from industrial effluents containing these toxic metals [6].

Due to the high content of contaminants in the metalliferous mining waste from the Tarniţa mining area, another objective of the doctoral research aimed to investigate the individual relationship of iron, copper and arsenic ions (in the form of arsenite and arsenate ions) with the species. *Triticum aestivum* L. in germination experiments performed *in vitro* , in laboratory conditions. In this sense, the germination of caryopsis and the growth of wheat seedlings in the presence of said ions were determined, at low concentrations [63, 72]. The absorption and distribution of trace elements and metals in crop plants have been studied very carefully, due to their importance [81].

The research carried out within the same chapter of the doctoral thesis also focused on the toxicity of the arsenic forms (arsenite and arsenate), which are found in the Tarniţa mining area. The studies were performed on the species *Triticum aestivum* L. Putna variety in the presence of arsenite and arsenate solutions, in order to highlight their toxicity by germination tests performed in laboratory conditions and to evaluate the possibility of plant protection in arsenite ion poisoning , using glutathione.

The test species, common wheat (*Triticum aestivum* L.) is a plant of nutritional importance, which can be an important means of transmitting heavy metals to higher trophic levels. At the same time, plant residues obtained from wheat plants after soil decontamination could be used as lignocellulosic biomass for energy production by biochemical or thermochemical degradation, namely for the production of bioethanol, as a partial substitute for fossil fuels [32]. is a renewable biomass rich in sugars such as starch and lignocellulose, being the main raw material for the production of bioethanol [38].

PURPOSE AND OBJECTIVES OF THE THESIS

The purpose of the research was to identify and investigate the effects of toxicity of soils contaminated with heavy metals and arsenic in the mining area Tarnita, Ostra commune, Suceava county (decommissioned barite mine), on common wheat test plants - *Triticum aestivum* L., Putna variety - by experimental tests performed *in vitro* , in laboratory conditions, on the germination process of caryopsis and the growth process of the resulting seedlings , expressed by specific morphological parameters (average mass, average height).

In order to fulfill the Purpose thus stated, the following **specific objectives have been formulated** :

- identification of areas contaminated with heavy metals in the research area, determination of the composition of metalliferous mining waste collected from the landfill and surrounding soils and correlation of the characteristics of samples taken from the work area (heavy metal content, with emphasis on excess concentrations of iron, copper and arsenic) with certain functional processes of test seedlings resulting from wheat caryopsis germinated under experimental laboratory conditions, on environments contaminated with heavy metals, expressed by the intensity of the determined germination process (energy and germination capacity), as well as by the value of specific morphological parameters (average mass, average height);
- identification of methods for decontamination of soils and waters contaminated with heavy metals and arsenic from the mining area observed under experimental laboratory conditions, in order to reduce their toxicity before performing germination and seedling growth tests common wheat test - *Triticum aestivum* L., Putna variety, methods that can be applied within the Tarniţa area:
    - physico-chemical methods, such as soil extraction with water;
    - microbiological methods of bioremediation of toxic supernatants / mining residues, using yeast cultures *Saccharomyces cerevisiae* Meyen ex EC Hansen, active and inactivated;
    - chemical methods of chemical decontamination of toxic supernatants / mining residues with glutathione, as well as by hydroxide precipitation.
- investigation of the effects of iron, copper and arsenic ions (in the form of arsenite and arsenate), at lower concentrations, compared to those of metalliferous mining waste in the Tarnița area, applying the specific technique of germination of caryopsis of *Triticum aestivum* L., Putna variety in *in vitro experimental conditions* .
- dissemination of results at scientific events and their publication in national and / or international journals.

In accordance with the objectives pursued, *in vitro* experiments were carried out in the germination of wheat caryopsis and in the growth of test seedlings in the presence of toxicity of contaminated soils from metal mining waste dumps and experiments to remedy the quantities of heavy metals contained in in order to inhibit the toxicity of mining residues / toxic supernatants before performing germination tests.

The doctoral thesis is structured in two parts:

Part I - LITERATURE STUDY - comprises a single chapter, in which they are presented: general considerations about heavy metals and metalloids such as arsenic, as well as soil pollution with these chemical elements, the influence of heavy metals and metalloids on plants, with an emphasis on iron, copper and arsenic, the effect of heavy metals on test plant species such as *Triticum aestivum* L.; physico-chemical methods decontamination of soils contaminated with heavy metals by water extraction of contaminants, methods of microbiological remediation of mining residues with yeast cultures *Saccharomyces cerevisiae* Meyen ex EC Hansen and chemical methods of remediation of toxicity of mining residues with glutathione and subsequently by precipitation with hydroxides.

Part II - OWN RESEARCH - consisting of four chapters: one chapter comprising *Research Materials and Methods* and two chapters of personal results, each including the *Research Objective*, *the Working Method*, the *Results obtained* and *the Preliminary Conclusions.*

The paper ends with some *Conclusions general*, *Dissemination of the obtained results* and *Bibliography* .

PART I - LITERATURE STUDY

Chapter 1. Current state of research

1.1. General considerations

Heavy metals have been identified as a major research priority, in order to remedy the polluted forest areas in Romania. Pollution of the soil with heavy metals and metalloids is a great danger for the environment , therefore an important concern is its protection against the toxic effects of these elements, as well as their accumulation along the food chain, which leads to environmental problems and serious health. Some heavy metals in low concentrations are essential for plants, but in high concentrations can cause metabolic disorders and inhibit the growth of most plant species. Heavy metals can accumulate in the food chain, posing a significant danger to the environment and human health [29].

Compared to iron and copper, arsenic is not essential for plants and other organisms. Plants accumulate arsenic in the root and transport it to the stem, through active or passive mechanisms. Exposure of plants, even at low concentrations in arsenic, can cause changes in their morphology, physiology, but also their biochemistry [49].

1.2. The effect of heavy metals on the test species *Triticum aestivum* L.

Gang and collaborators studied in 2013 the effect of different concentrations of heavy metals, such as copper, cobalt and chromium on the germination of caryopsis and the growth of common wheat seedlings (*Triticum aestivum* L.) [25]. The results obtained by them showed that at high concentrations of heavy metals there was a decrease in the percentage of germinated caryopsis compared to the control, but the differences were not significant up to concentrations of heavy metals of 100 ppm (Cu, Co and Cr). However, all three heavy metals significantly reduced the seed germination capacity, compared to the control, at 200-500 ppm concentrations ($P \leq 0.01$).

Mahmood and colleagues studied in 2007 the effect of copper at concentrations of 1-10 ppm on the common wheat species (*Triticum aestivum* L.) and found that at 10 ppm copper (10 μM) there is a reduction of more than 35% in germination. cariposelor [45].

Singh et al. (2007) studied the effect of copper at concentrations of 5-100 ppm (mg / L) on common wheat caryopsis (*Triticum aestivum* L.) and found that at 100 ppm copper, the germination capacity of the seeds is approximately 65% [72].

A number of studies have reported that wheat seedlings have responded rapidly to a higher concentration of metals in terrestrial ecosystems by changing germination parameters and disrupting root growth compared to that of the stem [30]. The change in root growth is probably due to the consequences of direct exposure to heavy metal toxicity and the preferential accumulation of these elements in the root, followed by slow mobility to other vegetative organs of the plant [27]. Such an effect can be explained by the fact that the affected roots can cause a slower movement of metals to the other vegetative organs of the plant [21].

At concentrations of 10-100 µM, iron affects seedling growth and leaf development [42], by producing excess reactive oxygen species [53]. It has been observed that iron toxicity may decrease superoxide dismutase activity in wheat and rice plants [76].

Iron, in the ferrous and rarely ferric state, binds to the tetrapyrrole ring, forming existing cytochromes in chloroplasts, which catalyze various reactions underlying photosynthesis. At the same time, iron plays an important role in the respiration process [27].

For common wheat, the accessibility of metals is strongly influenced by soil moisture and reaction. Thus, with the increase of humidity to about 60% of the water capacity of the soil, the solubility of copper has a tendency to increase, and after this value begins to decrease substantially. As for iron, as the soil moisture approaches 100% of the water capacity, the solubility increases 20 times. Regarding the reaction of the soil, the more acidic the pH, the solubility of heavy metals increases, especially iron.

1.3. Methods for decontamination and remediation of soil toxicity and mining residues in the Tarniţa mining area

1. 3 .1. Physico-chemical methods for decontamination of soils polluted with heavy metals

The methods of remediation and depollution of some mining areas are dependent on the types of soil, on the properties of the chemicals found in the respective areas, on the depth of contamination, as well as on the natural processes that can manifest at the contaminated place [51] .

The extraction of heavy metals from the soil and metalliferous residues is a practical process of washing with water or various solutions, in order to purify the soils ex-situ, in order to remove impurities. By this process soil contaminants can be removed in two ways: either by dissolving or retaining them in the washing solution, which is subsequently treated by conventional wastewater treatment methods, or by concentrating them in a smaller volume of soil and then gravitational separation of particles by washing, a method similar to the techniques used for sand and gravel [62].

1.3.2. Microbiological remediation of mining residues with yeast cultures Saccharomyces cerevisiae Meyen ex EC Hansen active and inactivated

Among the yeasts of the genus *Saccharomyces* , *S. cerevisiae* Meyen ex EC Hansen is of special interest as a biosorbent [22, 82]. It is a harmless yeast, widely used in the food industry. *S. cerevisiae* Meyen ex EC Hansen has the ability to remove heavy metals from aqueous solutions at low concentrations, as well as to tolerate changes in pH and temperature during various processes [44, 84]. Biomass of *S. cerevisiae* Meyen ex EC Hansen can remove heavy metals, whether it is metabolically active (alive) or inactivated (dead) [23]. The advantages of using inactivated microorganisms include the elimination of culture media and special nutrients, thus reducing the cost of decontamination. In addition, it is possible to regenerate and reuse biomass, with the option to immobilize it and facilitate the mathematical modeling of the phenomena involved in the yeast-metal union [15].

1. 3 . 3 . Chemical methods for remediation of toxicity of mining residues

1.3.3.1. Determining the protective role of glutathione during the growth of plants exposed to excessive levels of heavy metals and arsenic

Glutathione is considered the most powerful natural antioxidant, the best tool for cell protection, the guarantor of the body's health [73].

In 2006, Ciobanu and collaborators showed that the germination parameters decreased significantly when the cariposes of the species *Triticum aestivum* L. were treated with Cu $^{2+}$ ions at a concentration of 10 $^{-2}$ M, and these parameters were recovered upon addition to the solution. treatment of glutathione [13] .

GSH acts as a precursor of the synthesis of phytochelatins, peptides synthesized posttranslationally, which play an important role in regulating the intracellular concentrations of heavy metals and not only [31, 61, 68]. Phytochelatins retain the metal and form a complex which is then transported in vacuoles [26, 65].

It was found that during the germination of common wheat caryopsis, glutathione at a concentration of 10 mM shows a protective role against the toxicity of sodium arsenite, protecting the germination process and stimulating seedling growth [8].

1. 3.3.2. Remediation of heavy metal toxicity from mining residues by precipitation with hydroxides

The most widely used chemical precipitation technique is hydroxide precipitation due to its relative simplicity, low cost and pH control [34]. The solubilities of the different metal hydroxides are reduced to a minimum, in the pH range 8.0 - 11.0. Metal hydroxides can be removed by flocculation and sedimentation.

The process of precipitating hydroxide using calcium and sodium hydroxides to remove copper ions from wastewater was evaluated by Mirbagheri and Hosseini in 2005 [50]. The optimum pH for maximum precipitation of copper ions was approximately 12.0 for both calcium hydroxide and sodium hydroxide, and the copper concentration was reduced from 48.51 mg / L to 0.694 mg / L. .

Hydroxide treatment increased the particle size of the precipitate and significantly improved the efficiency of heavy metal removal. The concentrations of chromium, copper, lead and zinc in the effluents can be reduced from the initial concentration of 100.0 mg / L to 0.08; 0.14; 0.03 and 0.45 mg / L, respectively [10, 41].

Precipitation is a process that brings a substance from a solution to an insoluble form. This process alters the solubility of metal ions by reacting with specific chemicals, causing them to precipitate in solution. This approach can be adopted in the case of soils contaminated with heavy metals, in order to convert excess metals into insoluble forms, reduce their mobility in plants and the negative effects of their toxicity on the environment.

PART II - OWN RESEARCH

CHAPTER 2. Research materials and methods

In an effort to solve the research purpose of this thesis, presented at the beginning of this thesis, through the research we aimed to investigate the physiological responses of test plants (common wheat - *Triticum aerstivum* L., Putna variety) both to excess heavy metals from collected mining waste from the dump and from the soils located in the surroundings of the former Tarnița barite mine, Ostra commune, Suceava county, as well as to the supernatants obtained from the substrate in the respective area and decontaminated by microbiological and, respectively, chemical methods.

In accordance with the objectives pursued, in this doctoral thesis the concentrations of heavy metals and arsenic were determined from the samples collected from the perimeter of the experiment by the ICP-OES method (inductively coupled plasma optical emission spectroscopy) and the AAS (atomic absorption spectrometry) method , then performed experiments to germinate common wheat caryopsis and grow test seedlings under the influence of toxicity from mining waste from landfills, contaminated soils and toxic supernatants, as well as experiments to remedy quantities of heavy metals present in them by biological decontamination (treatments with yeast *Saccharomyces cerevisiae* Meyen ex EC Hansen *)* and chemical (treatments with glutathione and hydroxide precipitation) , in order to inhibit the toxicity of mining residues before performing germination tests on plant species test. At the same time, the effect of iron, copper and arsenic ions on the common wheat test variety was observed, as well as the protective effect that glutathione may have on this variety in case of toxicity caused by copper ions in excessive concentrations and, respectively, the presence of arsenate / arsenite ions. Germination and seedling cultivation tests were performed by *in vitro* experiments, in the research spaces of the Laboratory CERNESIM - Faculty of Biology and Biochemistry Laboratory - Faculty of Chemistry, within the "Alexandru Ioan Cuza" University of Iasi.

2.1. Materials

2.1.1. Biological species test

Spring wheat caryopsis (*Triticum aestivum* L.), Putna variety approved at the Agricultural Development Research Station Suceava, Romania, by Gaspar and collaborators were selected according to their size for uniformity and used in germination experiments in the presence of mining waste and some soil samples collected from the perimeter of the mining operation in Tarniţa, Suceava County. These common wheat caryopsis were treated with aqueous extracts obtained from metalliferous mining waste, as well as from soil samples collected around the mined area in question. Some aqueous extracts of these materials were also used after their microbiological decontamination with yeast or chemically with glutathione and, by precipitation with hydroxides, respectively. Separately, some chemical solutions of some heavy metal salts ($FeSO_4$, $CuSO_4$ and $CuCl_2$) and arsenic ions in the form of NaH_2AsO_3 and NaH_2AsO_4 were applied, to highlight the action of each type of ion.

The yeast mass *Saccharomyces cerevisiae* Meyen ex EC Hansen biologically active was purchased from a commercial company, SC ROMPAK SRL, Paşcani, Romania and maintained at 4 °C before use, being used in decontamination experiments due to the fact that the literature highlights, through numerous researches, the effectiveness of yeast in decontamination of heavy metal soils and waters [33, 43, 83].

2.2. Sampling

2.2.1. Sampling area and preparation of mining and soil waste samples

The copper and barite mine from Tarnița, closed and decommissioned since 2006, represents a great danger for the environment. The area contaminated with heavy metal salts and metalloids, such as arsenic compounds, as well as other elements, such as aluminum and heavy metal compounds, is located in the commune of Ostra, on the banks of the rivers Brăteasa and Tărnicioara, the latter being a right tributary of of the Moldova River.

The huge deposits of metalliferous mining waste left after the closure of the copper and barite mine in the Ostra-Tarnița area have severe negative effects on the environment, being deposited in four tailings ponds: Poarta Veche, Ostra, Tărnicioara and Valea Străjii, among all, the pond The tarmac containing the largest amount of mining waste.

In order to investigate the current state of the environment in the Tarniţa area, samples of metalliferous mining waste (DMM) were taken from the tailings dump and soil samples collected from the vicinity of the tailings dump (approximately 30 m away from it). As several tailings dumps were created as a result of barite mining, the sampling points are shown in Figure 2.1.

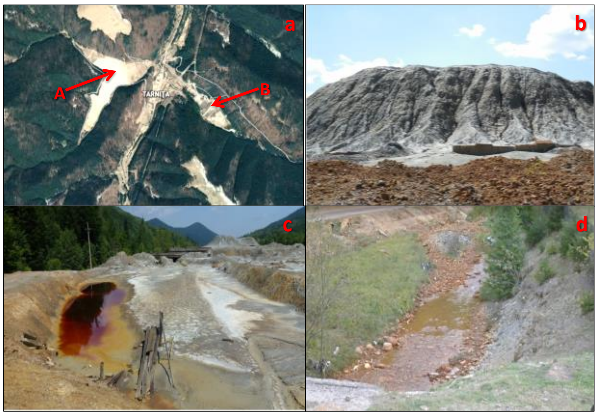

FIG. 2.1. Tarniţa mining complex, Suceava, Romania:
(a) Positioning of tailings dumps in the Tarniţa mining complex (A - Tărnicioara; B - Valea Straja; photo from Google Earth source); (b) Waste dump in the Straja Valley area; (c) Contaminated water around tailings dumps; (d) Tărnicioara contaminated river, located at approx. 30 m away from the landfill.

Samples collected from the metalliferous mining waste dump and in its vicinity (Figure 2.1, b) were taken from the surface horizons (20 - 30 cm depth), from bottom to top, in polyethylene bags. They were conditioned by drying in an oven at 40 °C, placed on a filter paper in a thin layer of about 2 cm, then screened through a 2 mm sieve and finally homogenized. All samples were stored in closed polyethylene vessels, stored at + 4 °C and analyzed by ICP-OES and AAS spectrometry.

In order to achieve the objectives of the doctoral thesis, the collected samples were used to test the toxicity of heavy metal ions and arsenic in these samples using common wheat caryopsis - *Triticum aestivum* L., Putna variety, and to identify methods for decontamination of soils polluted with significant amounts of heavy metals, soils taken from the copper mining area and barite Tarnița.

2.2.2. Germination tests on plants Triticum aestivum L. test, variety Putna

2.2.2.1. Disinfection of caryopsis

Disinfection of caryopsis was performed with 5% sodium hypochlorite for 5 minutes. Subsequently, caryopsis was washed several times with MilliQ ultrapure water, until the characteristic odor disappeared [4, 55].

2.2.2.2. Germination method of caryopsis

The experiments were performed in three repetitions, with batches of 50 caryopsis, which were placed in sufficiently large test tubes (180 x 18 mm) to allow their mixing with the treatment solutions , after which the treatment solution was added. corresponding to each sample. The mixtures in the tubes were incubated for one hour, with intermittent stirring, long enough to soak the seeds with the treatment solutions, according to the recommendations of ISTA (Seed Science and Technology, 1993) [48] .

After one hour, the caryopsis, together with the solution with which the treatment was performed, were evenly distributed in Petri dishes with a diameter of 9 cm, each with two overlapping layers of filter paper, according to ISTA recommendations (Seed Science and Technology, 1993) [48]. To preserve the sterile working environment, we worked under the hood of microbiological protection and PCR with vertical air flow . Petri dishes were kept throughout the germination period in the growth chamber, equipped with a temperature, humidity and light programming system. Experimental working parameters in the growth chamber (Snijders Scientific, Netherlands) temperature, humidity and light were selected so as to correspond to the living conditions specific to the species *Triticum aestivum* L. [58]. For germination, the following conditions were ensured in the growing chamber: temperature of 24 ºC (± 1) and a lighting regime of 16 hours light / 8 hours darkness. The whole germination process thus organized lasted for 7 days [20, 67].

The experiments were performed in the Laboratory CERNESIM research of the Faculty of Biology and in the Biochemistry Laboratory within the Faculty of Chemistry, "Alexandru Ioan Cuza" University of Iaşi.

2.2.3. Repair of treatment solutions for germination tests

2.2.3.1. Preparation of toxic supernatant (ST)

In order to determine the effect of the mining residues from Tarniţa on the plant species subjected to germination and seedling growth in the biological tests, samples of 1 g of homogenized material were collected, in three repetitions, collected from the tailings in centrifuge tubes and subjected to extraction with 10 mL of ultrapure Mili-Q water on the ultrasonic bath for 15 minutes. For the extraction of heavy metals, the methods applied by Rauret and collaborators in 1989 and by Dœlsch were adapted. and collaborators in 2006, so the resulting suspensions were ultracentrifuged for 5 minutes at 5000 rpm [64, 16] . The soil and the original metallic material, as well as 1 g samples of the same solid materials from which the water-soluble residues were removed were uniformly deposited on the filter paper in the respective Petri dishes. The toxic supernatant was thus removed, but was used in germination experiments to verify the effect of the extracted heavy metals on some test plant species.

2.2.3.2. Method of decontamination of metalliferous mining waste (DMM) by water extraction

An amount of 1 g of DMM was taken and extracted with 10 mL of ultrapure Mili-Q water on the ultrasonic bath for 15 minutes. The toxic supernatant obtained after ultracentrifugation for 5 minutes at 5000 rpm was removed and the solid material from DMM was mixed with 5 mL of milli-Q ultrapure water and used in germination tests.

For the DMM sample extracted twice, after removing the supernatant obtained after the first extraction, 10 mL of Mili-Q ultrapure water was added to the DMM extract . The toxic supernatant obtained by ultracentrifugation for 5 minutes at 5000 rpm was removed and the solid extracted a second time was mixed with 5 mL of milli-Q ultrapure and also used in germination tests.

For the DMM sample extracted three times, after removal of the supernatant obtained after the second extraction, 10 mL of Mili-Q ultrapure was added to the DMM extract . The toxic supernatant obtained by ultracentrifugation for 5 minutes at 5000 rpm was removed and the DMM extract extracted for the third time was mixed with 5 mL of milli-Q ultrapure water and used in germination tests.

2.2.3.3. Method of removal of toxic compounds from the supernatant by hydroxide precipitation

To remedy the soil and the heavily contaminated area, it was proposed to remove the content of heavy metals and arsenic from the washing waters by the method of precipitation with hydroxide solutions (sodium, calcium, etc.), according to the method adapted and used by Jang and collaborators in 2007 [37]. Thus, a 4% NaOH solution to pH 5.0 was added to the toxic supernatant, and the mixture thus obtained was ultracentrifuged for 5 minutes at 5000 rpm. Alkalization was performed separately with the same alkaline solution to a pH of 12.0. The supernatant obtained was finally neutralized with a solution of 4% HCl and 4% NaOH to pH 6.9-7.0 so as not to affect the germination of the test plants. After centrifugation, to remove the precipitates obtained, all these hydroxide precipitation decontaminated solutions were also used in biological research. The heavy metal content of all solutions was measured by atomic absorption spectrometry (AAS).

2.2.3.4. Method of reducing the toxicity of supernatant with yeast cultures Saccharomyces cerevisiae Meyen ex EC Hansen

The active yeast sample used to prepare a suspension was obtained by mixing 5 g of yeast with 10 mL milli-Q ultrapure water for one hour at room temperature, stirring intermittently.

For the inactivated yeast sample, 5 g of yeast were treated with 10 mL of milli-Q ultrapure water at boiling point, a procedure which was carried out by intermittent stirring for one hour in a water bath at 100 °C. method adapted after Göksungur and collaborators, who inactivated yeast cultures by keeping them at 121 °C for 15 minutes [28].

Active yeast was used for bioremediation of the supernatant, i.e. 5 g of yeast were incubated for one hour with 10 mL of toxic solution (6 mL of toxic supernatant and 4 mL of ultra-pure milli-Q water).

In order to reduce the toxicity of the supernatant containing heavy metal ions, inactivated yeast was obtained, obtained from 5 g of yeast treated with 10 mL diluted supernatant solution (6 mL toxic supernatant and 4 mL milli-Q ultrapure water), which was at boiling point. ; the resulting mixture was further maintained for one hour, with intermittent stirring, on the water bath at 100 °C.

All the cheeses obtained as above were centrifuged for 5 minutes at 5000 rpm, and 5 mL of the supernatant obtained was the sample of active / inactivated yeast and, respectively, active / inactivated yeast and toxic supernatant (ST). For the comparison of the samples with active yeast with those with inactivated yeast, control samples were performed in three repetitions, with 5 mL of ultrapure milli-Q water and 50 caryopsis of common wheat.

2.3. Research methods

2.3.1. Methods of analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES) of mining and soil waste samples taken from the Tarnița area

analyzes were performed in the Biochemistry laboratory at the Faculty of Chemistry, UAIC Iaşi and in the Analytical Chemistry laboratory, University of Chemical and Metallurgical Technology, Sofia, Bulgaria.

2.3.2. Methods of analysis by atomic absorption spectrometry (AAS) of the samples of mining and soil waste taken from the Tarnița area

Analyzes were performed in the Biochemistry laboratory at the Faculty of Chemistry, UAIC Iaşi and in the Laboratory of the ICAS Marian Dracea Research Station, Câmpulung Moldovenesc, Suceava.

2.3.3. Analysis of germination parameters

The process by which the embryo of a caryopsis passes from the dormant to the active state, as a result of which a seedling appears, is called germination. In the species *Triticum aestivum* L. germination is unipolar, ie the root and stem come out of the caryopsis through the same place [3].

In order to evaluate the germination parameters of caryopsis, the size and, respectively, the mass of the resulting seedlings, germination experiments were performed in the presence of treatment solutions, tests that lasted 3 to 7 days [20, 67].

After 3 days of treatment, the number of germinated seeds was determined (germination energy, Eg, expressed as a percentage).

Germination energy (E_g, %) , represents the ability of seeds to germinate (for *Triticum aestivum* L. - every 3 days).

It is determined as a ratio between the number of caryopsis germinated in the first third of the period (a) and the total number of caryopsis analyzed (n):

$$E_g = \frac{a}{n} \cdot 100 \tag{1}$$

Germination faculty (F_g,%) , represents the ability of the seeds to germinate until the end of the germination period (*Triticum aestivum* L. - 7 days).

It is determined as the ratio between the number of caryopsis germinated at the end of the period taken into account as germination days (b) and the total number of caryopsis analyzed (n):

$$F_g = \frac{b}{n} \cdot 100 \tag{2}$$

After 7 days of treatment, in each Petri dish was determined the number of seedlings formed, germinated caryopsis but from which no seedlings were formed (germination, F_g, expressed as a percentage) and non-germinated / dead caryopsis [70]. After Evers and Bechtel (1988) and Niță et al. (2004), respectively, the seedling represents the stage of ontogenetic development of the plant in which it has the main root with first-order secondary roots, the hypocotyl, cotyledon, first leaf and second, and the third is barely dissolving [19, 54]. According to Andrei et al. (2008) 2-3 days after germination, common wheat seedlings have a short primary root or root, which through growth penetrates coleorize [3].

For each seedling resulting from germinated wheat caryopsis, the coleoptile was harvested after 7 days of treatment, together with the first leaf, determining the average mass (m_p, mg) and the average height of all seedlings formed (cm) in each Petri dish.

To determine the germination capacity, the normally germinated caryopsis from each repetition were counted. It was considered that a caryopsis germinated when the root was about 2 mm long [1, 55]. Caryopsis found swollen, rotten, moldy at the end of the germination period were considered ungerminated.

2.3.4. Statistical data processing

To process data from germination experiments of test plants grown on environments with heavy metal-rich mining residues, the Tukey test was used [74]. A 95% confidence interval was used as the margin of error. For each treatment performed in three repetitions, the germination parameters were calculated, after which the standard deviation of the obtained values was calculated. The average of the three repetitions was compared with a calculated D value to see if the difference was significant. For the difference to be significant, the average of three repetitions must be greater than the calculated value D.

Chapter 3. Heavy metal and arsenic pollution from copper and barite mines Tarnița and decontamination methods

3.1. Heavy metals and arsenic in mining and soil waste samples from Tarnița copper and barite mines

3.1.1. The objective of the research

The copper and barite mines in the Tarnița mining area are currently closed, but the open-cast metalliferous ore deposits, as polluting waste, still contain, in significant quantities, toxic chemical elements. The deposits and their immediate vicinity represent the first area of contamination, as it contains large quantities of elements such as iron, lead, barium, aluminum, arsenic, copper, etc. These elements are in the form of minerals such as insoluble iron and copper sulfides, which continuously release, by oxidation and hydrolysis, significant amounts of metal ions into the environment.

In this context, the research carried out within the doctoral thesis showed that the Tarnița area located in Ostra commune, Suceava county is extremely polluted. The results of the analysis of mining waste samples taken from the tailings dump, soil and wastewater samples showed that they are heavily contaminated with heavy metals such as iron, lead copper, cadmium, but also with arsenic-type metalloids, and the analysis of soil collected from contaminated areas showed their acidity, their pH being between 2.04 and 7.5. The high acidity of metalliferous waste recorded in these types of substrate could be correlated with the availability of heavy metals and the contamination of water and soils in the vicinity of waste landfills by leaching.

3.1.2. Determination of the concentration of heavy metals and metalloids by inductively coupled plasma optical emission spectroscopy (ICP - OES)

The samples of metalliferous mining waste (DMM) and soil from Tarnița taken from a depth of 20-30 cm were dried in air and then in an oven at a temperature of 40 °C. Prior to analysis by ICP-OES and AAS spectrometry, all samples were stored at 4 °C.

ICP-OES analysis, samples of metalliferous mining waste (DMM) and soil (0.2-0.3 g) were extracted with 15 mL of a mixture of HNO_3 : HCl acids, in a ratio of 1: 3, a mixture maintained at boiling point for 25 minutes, and the solution obtained was filtered and subsequently diluted to 50 mL with Milli-Q ultrapure water.

In parallel, samples of metalliferous mining waste (DMM) and soil (1 g) were subjected to extraction on the ultrasonic bath for 15 minutes at 5000 rpm, once, twice and three times, respectively, with 10 mL of ultrapure water Milli-Q. The toxic supernatant obtained after the first extraction was used to determine the concentrations of contaminants in metalliferous mining waste or soil by ICP-OES or AAS measurements.

3.1.3. Determination of the concentration of heavy metals by atomic absorption spectrometry (AAS)

To determine the concentration of heavy metals by the AAS method, samples with toxic supernatant (ST) obtained after the first extraction of DMM, were treated with 4% HCl, in a ratio of 1: 8 (v / v), HCl : ST . Samples with decontaminated toxic supernatant (DMMD) were precipitated by the addition of 4% NaOH to pH 5.0 and, after filtering the heavy metal residue through filter paper, the clear solution was neutralized with HCl, 1: 8 (v / v), HCl : sample. The supernatants obtained were neutralized with 4% HCl and 4% NaOH to pH 6.9-7.0.

In parallel samples with supernatant obtained after the first extraction of the collected soil in the vicinity of the mining waste dump (approx. 30 m) were filtered through filter paper (SF), and the solution obtained was acidified with 4% HCl, in a ratio of 1: 8 (v / v), HCl : sample.

After ultracentrifugation for 5 minutes at 5000 rpm, to remove the precipitates obtained, all these solutions were samples for measuring the heavy metal content by atomic absorption spectrometry (AAS).

3.1.4. Obtained results

Through the ICP-OES analysis, significant quantities of heavy metals such as Fe, Cu, Pb and Zn were detected in the Tarniţa forest area both in and around the metalliferous mining landfill (Table 3.1). Of all the elements found in the landfills, iron showed the highest concentration and, in addition, this element was found at great distances from the landfill, its quantity becoming even higher than in the original collection *site* . However, the most toxic elements found in the tailings dump were copper (3,119 mg / kg), arsenic (676 mg / kg) and lead (2,672 mg / kg) [17]. The other elements, such as Ba (14 mg / kg) and Zn (432 mg / kg) are either less toxic or were present as trace elements. In the samples of mining waste collected from the landfill, the elements Cr and Mn were present in the form of traces, in quantities less than 0.7 mg / kg.

Table 3.1. Concentration of heavy metals and metalloids in DMM and soil (mg / kg) determined by ICP-OES: (DMM) sample of metalliferous mining waste collected from the tailings dump; (S) soil sample collected from 30m away from the metalliferous mining waste dump.

Total concentration of heavy metals (mg / kg)		
Elements	Sample	
	DMM	S
(Fe)	357,869 ± 280	493,500 ± 405
(Cu)	3,119 ± 65	334 ± 8
(Pb)	2,672 ± 58	137 ± 12
(Zn)	432 ± 73	420 ± 17
(Ba)	14 ± 58	537 ± 5
(Cr)	traces	20 ± 7
(Mn)	traces	661 ± 10
(Ni)	0	29 ± 8
(As)	676 ± 13	96 ± 5

The results obtained were compared with the normal standard values for heavy metals and arsenic in soils, data presented in Table 3.2. .

Table 3.2. Normal standard values for heavy metals and arsenic in soils [57].

Heavy metal concentration (mg / kg dry matter)								
(Fe)	(Cu)	(Pb)	(Zn)	(Ba)	(Mn)	(Ni)	(Cr)	(As)
-	20	20	100	200	900	20	30	5

The concentrations of heavy metals in aqueous extracts with mining residues are shown in Table 3.3. The standard error was less than 5%. The results obtained by atomic absorption spectrometry (AAS) for aqueous extracts containing heavy metal mining residues were compared with the allowable values for surface water samples in Romania. The standard values of surface waters for classes 1, 2 and 3 are presented in Table 3.4 [56, 75].

Table 3.3. Concentration of heavy metals in mining residues (mg / L) determined by AAS: DMM - sample of metalliferous mining waste (1g DMM collected from the dump, in 10 mL H_2O and treated with HCl); DMMD - decontaminated metallic mining waste sample (DMM collected from the dump, in 10 mL H_2O, precipitated with NaOH and neutralized with HCl); SF - filtered soil sample (1 g soil collected from 30 m away from the dump, in 10 mL H_2O, filtered and treated with HCl).

Sample	Total concentration of heavy metals (mg / L)			
	(Fe)	(Cu)	(Zn)	(Mn)
DMM	675.63	32.14	7.07	0.38
DMMD	238.58	0.266	4.28	0.4
SF (30m)	5.86	0.276	3.35	0.009

Table 3.4. Standard values for Class 1, 2 and surface waters 3 [75].

Class	Heavy metal concentration (mg / L)					
	(Fe)	(Cu)	(Zn)	(Mn)	(Ni)	(Cr)
1	0.3	0.05	0.03	0.5	0.1	0.5
2	1	0.05	0.03	0.3	0.1	0.5
3	1	0.05	0.03	0.4	0.1	0.5

Natural water quality problems are mainly caused by heavy metals such as Fe, Cu, Mn and Zn [40].

As can be seen from Table 3.3, there are excessive increases in Fe (675.63 mg / L) and Cu (32.14 mg / L) concentrations in the toxic supernatant obtained by washing the DMM samples collected from the tailings depot, in while Zn (7.07 mg / L) was less present. Mn (0.38 mg / L), which is less toxic and has biological activity, was present in traces as a trace element. Currently the concentration limits allowed in natural waters for Fe, Cu and Zn are 0.3-1 mg / L, 0.05 mg / L and 0.03 mg / L (Table 3.4), values that have been far exceeded in the case of research, a fact that demonstrates that the samples collected from the ore waste dump are extremely toxic and present a real danger to the quality of the environment.

Regarding the concentration of iron in aqueous extracts with tailings decontaminated by hydroxide precipitation (238.58 mg / L), but also in aqueous extracts obtained from soil collected from 30 m away from the tailings dump (5.86 mg / L), the values of this metal are well above the allowable limit of 0.3-1.0 mg / L, found in surface waters. So the Fe concentration continues to be extremely toxic, due to its extremely high values.

The concentration of zinc in both aqueous extracts of mining waste (4.28 mg / L), which have been partially decontaminated, and in soil extracts (3.35 mg / L), are still high, well above the values of 0 .03 mg / L, standard admissible.

The concentration of copper exceeded the standard values allowed for surface waters (0.05 mg / L), while the increases compared to standard values were much lower (sterile decontaminated - 0.266 mg / L and soil - 0.276 mg / L). However, copper ions present in the environment were still considered toxic to human health.

The high concentrations of Fe, Cu and Zn in the aqueous extracts obtained from the DMM and soil samples collected from Tarnița cause an increased toxicity on the food chain. Thus, another contaminant, extremely toxic, detected in the mining waste dump and in the surroundings was arsenic. The concentrations determined by the ICP-OES method were 676 mg / kg in the mining waste dump and 96 mg / kg in the soil samples collected from 30 m distance from the dump [78]. Low levels of arsenic are naturally present in the soil [46]. The concentration of arsenic in the soil allowed worldwide is around 5 mg / kg, with substantial variations depending on the origin of the soil [47]. In the determinations carried out in the Tarnița area, the values recorded for this metalloid were well above the allowed limit, data indicating that these concentrations represent a real danger to human health and the environment.

3.1.5. Preliminary conclusions

ICP-OES measurements showed that metalliferous mining landfills contain large amounts of Fe (358 g / kg), Cu (3,119 mg / kg), As (676 mg / kg), Pb (2,672 mg / kg) and Zn (432 mg / kg). In the Tarnița deposit subjected to spectrophotometric analysis, the quantities of heavy metal and arsenic determined varied as follows: Fe> Cu> Pb> As> Zn> Ba> Cr> Mn> Ni, the most toxic elements found in the dump being Fe (due to the very high quantity), then Cu, Pb and an extremely dangerous metalloid, As. Although Fe was quantitatively determined at the highest value, this metal was also found at a distance from the dump (493.5 g / kg), its quantity obviously exceeding the amount in the metalliferous mining landfill of 358 g / kg .

In the case of soils located at a distance from dumps, the values of these elements, with the exception of Cr and Mn, are well above the limit of the normal values allowed for soils. These elements recorded the following values above the permissible limit: Ba (337 mg / kg), Zn (320 mg / kg), Cu (314 mg / kg), As (91 mg / kg), Pb (117 mg / kg), Ni (9 mg / kg). In this context, we consider that certain quantitatively determined elements (Fe, Cu, As, Pb and Zn) in the Tarnița mining operations (dump, as well as soil taken from 30 m away) continue to be, through the high values of their extremely toxic concentration. for humans and the environment.

Although heavy metals in extremely toxic concentrations form aggregates in metalliferous mining landfills, they could be easily removed by water extraction in a ratio of 1:10, under the action of ultrasound. As a result, after the first extraction with distilled water, the toxic metals passed into the resulting supernatant.

AAS measurements performed on samples of aqueous extracts obtained from samples collected from the mining landfill and from the surrounding soil (1g DMM / soil at 10 mL H_2O) , respectively, showed that some heavy metals such as Fe (DMM – 675.63 mg / L, SF (30m) - 5.86 mg / L), Cu (DMM - 32.14 mg / L, SF (30m) - 0.276 mg / L), Zn (DMM - 7.07 mg / L), SF (30m) - 3.35 mg / L) and Mn (DMM - 0.38 mg / L, SF (30m) - 0.009 mg /L) are present in high concentrations, extremely toxic to the environment. The concentrations of these heavy metals in DMM and soil, determined by AAS, decreased in value after the first water extraction, but these values remained excessively high in the samples, compared to the standard values allowed nationally for surface waters in Romania . This has shown that many amounts of heavy metals are insoluble and cannot be extracted with water in the form of metal ions.

As the supernatant obtained by water extraction is toxic to the environment, further decontamination by hydroxide precipitation was further used. In the case of extracts with decontaminated mining waste by precipitation with sodium hydroxide (DMMD), the concentrations of gel metals continued to be very high, compared to the standard values allowed: Fe (238.58 mg / L) , Cu (0.266 mg / L) , Zn (4.28 mg / L) and Mn (0.4 mg / L).

The AAS measurements showed that the difference between the concentrations determined for these heavy metals and the standard values allowed ordered the contaminants, in descending order, as follows: Fe> Cu> Zn> Mn for the DMM extract; Fe> Zn> Cu> Mn - for the extract obtained from DMMD; Fe> Zn> Cu - for the extract obtained from SF (30 m). The concentration of manganese in the aqueous extract of the soil collected from 30 m away from the metalliferous mining waste dump did not exceed the allowed standard values, of 0.3 - 0.5 mg / L.

The high concentrations of Fe, Cu and Zn determined in the aqueous extracts obtained from the samples of metalliferous mining waste and soil collected from the mining operations Tarnița demonstrate the increased toxicity and the continuous danger that these contaminants present for the food chain in the forest area to which the mining belongs.

3.2 . Assessment of the toxic effect of mining residues in copper and barite mines Tarniţa by caryopsis germination and seedling growth tests on common wheat, *Triticum aestivum* L., Putna variety

3.2.1. Germination tests of wheat caryopsis and seedling growth on soils in the vicinity of the mining waste dump

3.2.1.1. The objective of the research

One of the research objectives proposed in this doctoral thesis was to study the toxicity of soils from the mining area Tarnița [52], by conducting germination experiments on test plant caryopsis such as *Triticum aestivum* L. (common wheat, spring) , experiments performed in laboratory conditions for this purpose.

In a first experiment, the process of germination and growth of common wheat seedlings resulting on the collected soils in the vicinity of the metal mining waste dump was compared, compared to the garden soil (collected from the Iasi area). For this, the number of germinated caryopsis, the number of resulting seedlings, as well as their mass and size were determined 7 days after the beginning of the experiments performed in laboratory conditions.

3.2.1.2. Way of working

In order to determine the effect of soil toxicity located 30 m away from the metal mining waste dump on the germination of caryopsis and the development of common wheat seedlings, batches of 50 caryopsis were made in three repetitions, which were introduced in sufficiently large test tubes (180 x 18 mm), to allow their mixing with 5 mL of ultrapure water (milli-Q), and after an hour they were placed in Petri dishes with a diameter of 9 cm, on double filter paper over 0 g (control), 5 g, 10 g and 20 g of soil, respectively. For comparison, triple control tests were performed, with 5 g of garden soil and 50 test caryopsis.

3.2.1.3. Obtained results

The effect of the collected soil in the vicinity of the metalliferous mining landfill on the germination and growth of wheat seedlings is highlighted in Figure 3.1.

The germination faculty of caryopsis grown on the soil coming from Tarniţa exploitation was 92%, respectively 82%, compared to the test batches grown on garden soil, which registered a germination faculty of 98% (Table 3.5). Decreasing germination may suggest that plants may not grow on the soil near mining heaps.

Table 3.5. Germination of *Triticum aestivum* L. caryopsis, Putna variety and growth of test seedlings under laboratory conditions, on soil from the vicinity of the metalliferous mining waste dump from Tarnița mining (7 days of germination)

Treatment * ⁾	F_g ** ⁾ (%)	Average seedling mass (m_p, mg)	Average seedling height (cm)
Control, 5 mL H_2O	96 ± 1.2	79.25 ± 1.8	12.11 ± 0.6
5 g of garden soil	98 ± 2.0	83.47 ± 1.0	12.53 ± 1.2
5 g Tarnița soil	92 ± 1.3	78.91 ± 0.5	11.98 ± 0.8
10 g Tarnița soil	82 ± 2.2	77.57 ± 1.2	11.86 ± 1.2

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test (p ≤ 0, 05).

**) Fg = percentage of caryopsis germinated after 7 days.

This result indicates that toxic substances in the soil collected in the vicinity of the mining waste dump negatively influence both the germination of caryopsis and the growth of common wheat test seedlings: F_g (%): garden soil (98)> control (96)> 5g Tarnița soil (92)> 10g Tarnița soil (82).

FIG. 3.1. Seedling growth of *Triticum aestivum* L., Putna variety on soil collected from 30m distance from the waste dump from the Tarnița mining operation at 7 days of germination, in laboratory conditions. Treatments: a) 5 g of garden soil, b) 5 g of Tarnița soil.

Both the average mass of seedlings in the variants with 5 g and 10 g of soil collected from Tarnița, respectively, and their average height decreased, compared to those recorded for the working variant using garden soil.

Table 3.6. Germination of *Triticum aestivum* L. caryopsis, Putna variety and growth of test seedlings on soil from the Tarnița mining operation in laboratory conditions, at 7 days of germination.

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p , mg)	Average height of seedlings (cm)
Control, 5 mL H_2O	96 ± 1.2	78.23 ± 1.9	12.13 ± 0.3
5 g Tarniţa soil	93 ± 0.7	75.47 ± 0.5	11.22 ± 0.1
10 g Tarniţa soil	83 ± 1.3	63.36 ± 0.9	10.84 ± 0.1
20 g Tarniţa soil	76 ± 3.1	59.60 ± 0.6	9.47 ± 0.5

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test (p ≤ 0, 05).

**) Fg = percentage of caryopsis germinated after 7 days.

From the analysis of the germination parameters of the test seedlings grown on these soils coming from Tarnița exploitation, it was found that the process of seedling growth and development is affected, compared to the control seedling lots (m_p = 79.25 mg, height by 12.11 cm) and, respectively, with those grown on garden soil (m_p = 83.47 mg, height by 12.53 cm), in the test lots grown on heavy metal soil, the mass and, respectively, the average height of the seedlings with higher values small: 5 g Tarniţa soil: m_p = 78.91 mg, height by 11.98 cm; 10 g Tarniţa soil: m_p = 77.57 mg, height = 11.86 cm.

From Table 3.6 and Figure 3.2 it can be seen that the soil samples collected from the Tarniţa mining used in the experiment show a slight toxicity, because they inhibited the germination process, especially in the case of the sample with 20 g of soil, a variant in which the germination of wheat caryopsis decreased by 20%.

FIG. 3.2. Growing seedlings of *Triticum aestivum* L. , Putna variety on soil from the Tarnița mining operation in laboratory conditions, at 7 days of germination. Treatments: a) 5 g sol, b) 20 g sol.

Both the average mass and the average height of the seedlings decreased with the increase of the amount of polluted soil used as substrate, compared to the control lots as follows: m_p (mg): control (78.23) > 5g Tarnița soil (75.47) > 10g Tarnița soil (63.36) > 20g Tarnița soil (59.60); height (cm): control (12.13) > 5g Tarnița soil (11.22) > 10g Tarnița soil (10.84) > 20g Tarnița soil (9.47).

So higher amounts of support soil for growing test seedlings had a clear tendency to reduce these parameters.

3.2.1.4. Preliminary conclusions

The soils collected on the left bank of the Tărnicioara River, from about 30 m from the metalliferous deposit, determined the decrease of the germination parameters, the growth process of the test seedlings being negatively influenced by the excess of heavy metals in these soils, compared to the control lots, treated with distilled water and, respectively, those grown on garden soil.

The results obtained showed that the biology of *Triticum aestivum* L. (common wheat) plants grown on soil samples collected around the metalliferous mining waste dump from the Tarnița mining operation is significantly influenced by the quality and quantity of heavy metal polluted soil used as substrate, growth quantities of soil and the excess of heavy metals in its composition negatively influencing the process of germination and growth of young seedlings thus resulting.

3.2.2. Germination tests for wheat caryopsis and seedling growth resulting from the toxicity of metalliferous mining waste

3.2.2.1. The objective of the research

Another research objective aimed at testing the toxicity of metalliferous mining waste (DMM) taken from the Tarnița mining area on the germination process of common wheat caryopsis and the growth process of the resulting seedlings, in order to evaluate the possibilities of reducing this toxicity by extraction / washing soluble contaminants in those substrates with distilled water.

Caryopsis germination and seedling growth tests were performed on samples of mining waste collected from the tailings dump (without extraction), as well as on samples of mining waste subjected to double and triple extractions, respectively.

The experiments performed in laboratory conditions aimed at analyzing the germination parameters of the test species caryopsis and analyzing the seedling growth parameters thus resulting for increased variants both under stress conditions caused by the high toxicity of samples collected from the dump and under reduced conditions. of toxicity through the extraction of contaminants.

3.2.2.2. Way of working

In order to determine the effect of the toxicity of metalliferous mining waste (DMM) collected from the tailings dump on the germination of caryopsis and the development of wheat seedlings, triple samples were performed with 1 g of untreated mining waste, 1 g of double extracted mining waste, respectively 1 g of triple extracted mining waste.

One step of soil extraction was to add 10 mL of ultrapure water (milli-Q) to 1 g of mining waste and keep the mixture in the ultrasonic bath for 15 minutes, followed by centrifugation at 5000 rpm for 5 minutes. The toxic supernatant obtained after centrifugation was removed, and the mining waste remaining after extraction was used in the germination tests, in laboratory conditions, as a germination substrate, in Petri dishes.

All samples with extracted mining waste (1 g in each test tube) were mixed with 5 ml of milli-Q ultrapure water, after which 50 test caryopsis were added. For comparison, triple control samples were performed with 5 mL of milli-Q ultrapure water and 50 test caryopsis.

3.2.2.3. Obtained results

During the treatment, lasting one week, the sample with mining waste, not subjected to extraction, completely inhibited the germination of wheat husks (Figure 3.3, treatment b).

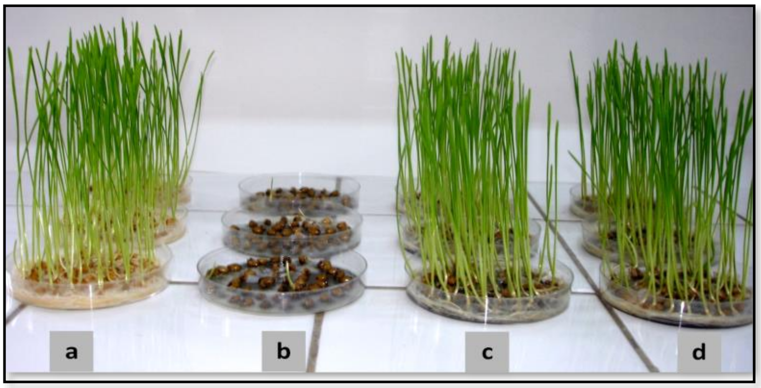

FIG. 3.3. Germination of caryopsis and growth of seedlings of *Triticum aestivum* L., Putna variety in laboratory conditions, using as substrate the metalliferous mining waste (DMM) from the Tarniţa mining operation, at 7 days of germination. Treatments: a) Control, 5 mL distilled water, b) 1 g DMM without extraction, c) 1 g DMM double extract, d) 1 g DMM triple extract.

The germination of wheat caryopsis recovered the difference in value compared to the working variant without extraction process, after the extraction of mining waste two or three times in succession (Figure 3.3, treatment c and d), situations in which the soil used as an experimental substrate it becomes, by repeated washing, less toxic.

The germination capacity of the caryopsis cultivated in the control variant was 95%; this parameter decreased to 3% in the case of the variant cultivated on the substrate represented by mining waste collected from the landfill of the Tarniţa mining operation, and by the extraction of 2 and 3 times, respectively, of the mining waste with 10 mL of ultrapure water (milli- Q) the germination capacity of caryopsis returned to 94% and 97%, respectively (Table 3.7). These results indicate that the toxic substances initially present in the mining waste sample were extracted during extraction, passing into the supernatant resulting from washing.

Table 3.7. Germination of *Triticum aestivum* L. caryopsis, Putna variety and growth of test seedlings on metalliferous mining waste (DMM) collected from the landfill from the Tarnița mining operation in laboratory conditions, at 7 days of germination

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p , mg)	Average height of seedlings (cm)
Blanck, H_2O	95 ± 1.2	63.5 ± 2.3	11.3 ± 0.4
DMM without extraction	3 ± 1.2	10.3 ± 5.6	1.6 ± 0.5
DMM extracted 2 times	94 ± 2.0	58.6 ± 5.7	10.4 ± 0.7
DMM extracted 3 times	97 ± 2.3	65.5 ± 5.1	10.9 ± 0.5

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

Both the average mass of the seedlings and their average height decreased drastically in the samples of wheat germinated on the sample of mining waste and returned to almost normal values (compared to the control variant - germination of caryopsis in ultrapure water Milli-Q) after its contaminants were extracted by washing with water 2 and 3 times, respectively.

This experiment showed that the toxic metals left in the waste after extraction of their water-soluble part are not toxic to plants under the given experimental conditions. However, over time, metal sulfides may become soluble and affect plant germination. Unlike chemical analyzes that indicated the accumulation of a large amount of toxic heavy metals in the analyzed mining waste, biological tests showed that their toxicity is much more obvious, being determined only by soluble species.

3.2.2.4. Preliminary conclusions

The process of germination in laboratory conditions of common wheat caryopsis in the variants containing substrate collected from the mining waste dump was significantly influenced by the presence of excess heavy metals, a phenomenon highlighted by chemical analyzes performed on this working substrate, the results thus obtained supporting practically the statement according to which the samples of metalliferous mining waste, through the specific chemical components contained, can completely inhibit the germination of caryopsis test wheat variety.

Decontamination by successive extractions with distilled water reduced the level of contaminants in the analysis substrate, which were removed by water extraction and centrifugation, processes followed by their concentration in the extraction supernatant. As a result, the parameters of caryopsis germination and growth of test seedlings subsequently reverted to double extraction of mining waste samples (DMM x 2) and triple extraction (DMM x 3) with water, respectively, to values close to those obtained in the control variant, within the norms considered physiologically normal for the biological material analyzed.

3.2.3. Evidence of toxicity of mining residue extracts by wheat caryopsis germination and seedling growth tests

3.2.3.1. The objective of the research

DMM samples collected from Tarnița exploitation indicated, by determinations performed by atomic absorption spectrometry (AAS), excessive increases of iron (675.63 mg / L) and copper (32.14 mg / L) concentrations in supernatant solutions. obtained by extractions (Table 3.3). Far exceeding the toxicity values allowed by the standards in force, the respective supernatants are practically a real danger to the quality of the environment in the explored area.

In this context, a research objective of the doctoral thesis aimed at studying the toxicity of extracts in the form of supernatant, obtained from metalliferous mining waste (DMM) taken from the Tarnița mining area, on the germination of common wheat caryopsis, used as biological test material.

Laboratory germination and seedling growth tests used toxic supernatant (ST) resulting from the first water extraction of DMM collected from the dump and toxic supernatant decontaminated by dilution (0 to 5 mL) and tracked both germination rate of caryopsis, as well as the level of increase of the dimensions (length, fresh mass) of the seedlings thus resulted in all variants of analysis: stress caused by the high toxicity of the supernatant obtained from DMM, as well as in conditions of decreasing the concentration of contaminants by water dilution.

3.2.3.2. Way of working

Triple samples of 1 g of DMM collected from the tailings from the Tarniţa mining operation were placed in centrifuge tubes and subjected to extraction with 10 mL of ultrapure water (milli-Q) on the ultrasonic bath for 15 minutes. For heavy metal extraction, the resulting suspensions were ultracentrifuged for 5 minutes at 5000 rpm. The toxic supernatant was separated and used in germination experiments to study the effect of heavy metals on the test plant, common wheat - *Triticum aestivum* L., Putna variety.

In order to determine the effect of the mining residues from the Tarniţa mining operation on the germination of caryopsis and the growth of the test seedlings, triple samples were performed with 1, 2, 3, 4 and, respectively, 5 mL of toxic supernatant, mixed appropriately with 4, 3, 2 , 1 and 0 mL of milli-Q ultrapure water, respectively, so as to obtain 5 mL of toxic solution for each treatment variant. All samples were placed in test tubes with 50 grains of test wheat. For comparison, triple control samples were performed, with 5 mL of milli-Q ultrapure water and 50 test caryopsis.

3.2.3.3. Obtained results

The results presented in Figure 3.4 and Table 3.8 show that the germination capacity decreases from 97% in the case of control seedling batches to 67% in the use of 3 mL of toxic supernatant and continues to decrease to 48% in the case of control seedlings. add 5 mL of toxic supernatant over the caryopsis.

The average mass of common wheat seedlings decreased progressively with increasing volume of toxic supernatant added to the treatment solutions. Following this set of experiments, we selected the solution containing 3 mL toxic supernatant and 2 mL distilled water to perform microbiological decontamination experiments of the residual substrate, using as decontamination material the yeast species *Saccharomyces cerevisiae* Meyen ex EC Hansen .

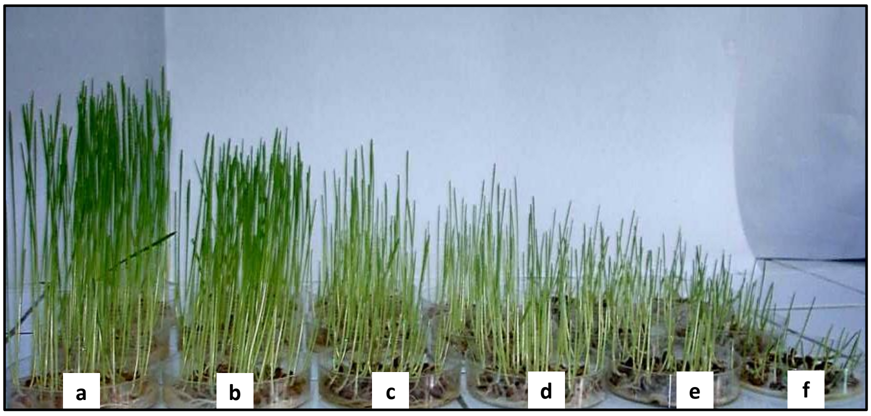

FIG. 3.4. Germination of caryopsis and growth of seedlings of *Triticum aestivum* L., Putna variety in laboratory conditions, using as substrate mining residues from the Tarniţa mining operation, at 7 germination. Treatments: a) Control, 5 mL H_2O, b) 1 mL ST+4 mL H_2O, c) 2 mL ST+3 mL H_2O, d) 3 mL ST+2 mL H_2O, e) 4 mL ST + 1 mL H_2O, f) 5 mL ST. ST = toxic supernatant.

In the process of germination of wheat caryopsis in some of the resulting seedlings was observed the absence or poor development of the root (initial root) and normal growth of adventitious roots [2, 3, 39, 87], when common wheat caryopsis was treated with 5 mL ST (Figure 3.5). This has been correlated with the tendency of plants to accumulate heavy metals, especially in the roots [35].

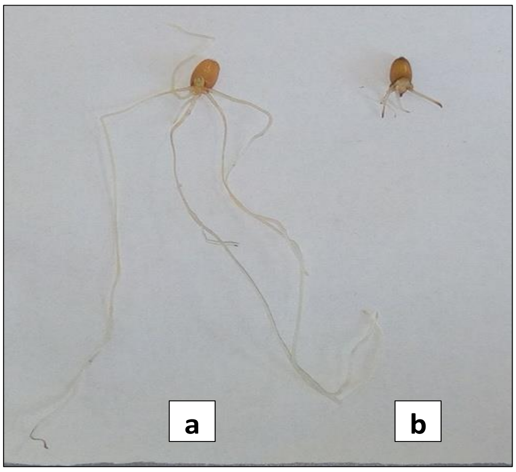

FIG. 3.5. The effect of mining residues in the Tarnița mining area on the formation and growth of the root and adventitious (secondary) roots in the seedlings of *Triticum aestivum* L., Putna variety cultivated in experimental laboratory conditions 7 days after treatment . Treatments: a) Control, 5 mL H_2O , b) 5 mL ST. ST = toxic supernatant.

Some of the results obtained and published [78] showed that the common wheat species is sensitive to mining residues in the Tarnița mining area. Thus, as shown in Table 3.8 and Figure 3.6, the germination capacity decreased continuously and constantly from 97% (Milli-Q ultrapure water control) to only 48% (when using 5 mL of toxic supernatant).

At the same time, the mass of seedlings resulting at 7 days of treatment decreased from 62.1 mg / lot to only 18.9 mg / lot, that is, of 3.3 times: m_p (mg): M (62.1) > 1 ST (41.3) > 2 ST (37.2) > 3 ST (28.8) > 4 ST (21.2) > 5 ST (18.9).

Table 3.8. Germination faculty of *Triticum aestivum* L. caryopsis, Putna variety germinated in laboratory conditions and the average mass of seedlings thus resulting, as a result of the application of treatments with increasing volumes of toxic supernatant (ST), at 7 days of germination.

Treatment *)	F_g **) (%)	Average seedling mass (m_p, mg)
Blamck, H_2O	97 ± 4.6	62.1 ± 0.4
1 mL ST + 4 mL H_2O	91 ± 2.3	41.3 ± 2.0
2 mL ST + 3 mL H_2O	95 ± 3.1	37.2 ± 3.8
3 mL ST + 2 mL H_2O	67 ± 12.2	28.8 ± 2.1
4 mL ST + 1 mL H_2O	51 ± 7.9	21.2 ± 5.6
5 mL ST	48 ± 7.7	18.9 ± 2.1

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

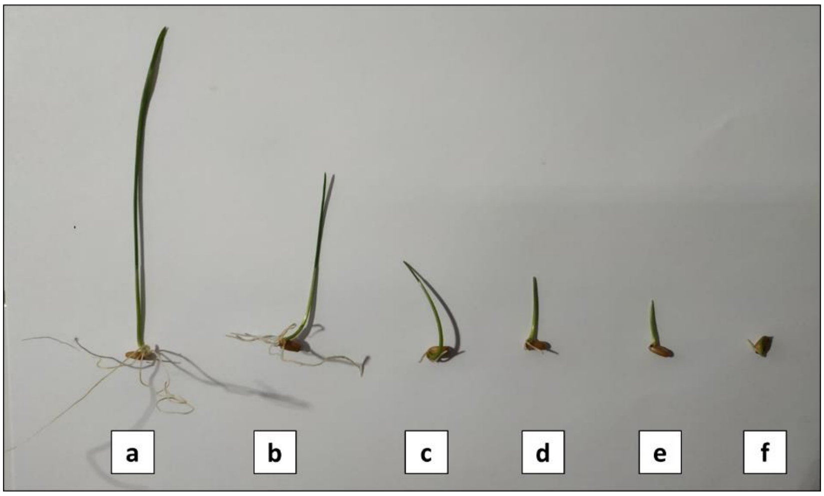

FIG. 3.6. Seedlings of *Triticum aestivum* L., variety Putna at 7 germination, grown in laboratory conditions, on the substrate with mining residues from the Tarniţa mining operation. Treatments: a) Control, 5 mL H_2O, b) 1 mL ST + 4 mL H_2O, c) 2 mL ST + 3 mL H_2O, d) 3 mL ST + 2 mL H_2O, e) 4 mL ST + 1 mL H_2O, f) 5 mL ST. ST = toxic supernatant.

3.2.3.4. Preliminary conclusions

The results obtained in the germination tests showed that the germination capacity of wheat caryopsis decreases from 97% in the control group to 67% in the use of 3 mL of toxic supernatant and continues to decrease up to 48% in the case of added, over the common wheat caryopsis, only a toxic supernatant (5 mL). At the same time, the common wheat seedlings registered a progressive reduction of the average mass, with the increase of the volume of toxic supernatant added in the treatment variants.

In the experiments performed, the toxicity effect of the supernatant obtained from the metalliferous mining waste was reduced by successive dilution, before performing the germination tests, which led to the increase of the analyzed germination parameters.

3.3. Decontamination of mining residues from Tarniţa minings of copper and barite

3.3.1. Decontaminating effect of supernatant of a yeast culture Saccharomyces cerevisiae Meyen ex EC Hansen on toxic supernatants in mining residues

The decontaminating effect of the yeast suspension *Saccharomyces cerevisiae* Meyen ex EC Hansen in its natural, active and inactivated form by boiling was tested on toxic extracts of mining residues collected from the Tarniţa mining area. Germination tests of wheat caryopsis and resulting seedling growth have shown its effectiveness in removing toxic elements from supernatant or soil solutions collected from this area contaminated with various mining residues.

3.3.1.1. The objective of the research

Due to the fact that the literature presents different chemical, biological and microbiological methods for decontamination of soils and waters of heavy metals and metalloids, another research objective proposed in the doctoral thesis aimed to study the reduction of toxicity of supernatants obtained from metalliferous mining waste. (DMM) taken from the Tarnița mining area by decontamination with biologically active material (live) and inactivated by the application of high temperatures, material represented by supernatant obtained from yeast cultures (*Saccharomyces cerevisiae* Meyen ex EC Hansen). For this purpose, germination tests were performed using common wheat caryopsis, to highlight the degree of decontamination of the cultivation substrate by the use of yeast supernatant, as well as its effect on the germination process of wheat caryopsis, under experimental laboratory conditions, experiments that followed the germination parameters of caryopsis and the modification of the morphometric indices of the seedlings thus resulting (length, fresh mass) after biological decontamination.

3.3.1.2. Way of working

In order to determine the heavy metal decontamination effect of the yeast *Saccharomyces cerevisiae* Meyen ex EC Hansen of toxic supernatants / mining residues in the area of the barite mine in question, the method of germination of wheat caryopsis and seedling growth was tested. newly formed on polluted environments, originating from the mentioned mining area, initially subjected to the process of biological decontamination with yeast.

The decontamination experiments with active and inactivated yeast, respectively, used as an experimental substrate the variant with dilute toxic supernatants (0 to 5 mL), namely the mixture of 3 mL ST and 2 mL of H_2O. Thus the effect of the yeast extract was determined. active and, respectively, inactivated on the germination of caryopsis in the presence of mining residues, represented by the toxic supernatant (1 g DMM Tarnița : 10 mL H_2O).

3.3.1.3. Obtained results

The first two experiments looked at the effect of the supernatant obtained from the active and inactivated yeast suspension, respectively, on the germination of common wheat caryopsis.

The results obtained (figure 3.7 and table 3.9) indicated that, compared to the control variant, there was a decrease in germination capacity, mass and, respectively, the average height of the test seedlings, in the cultivation conditions in which caryopsis were treated with supernatant from incubating the yeast with ultrapure water at room temperature or boiling, followed by centrifugation, probably due to the organic substances released by the active / inactivated yeast culture in the supernatant. Thus, the average seedling mass decreased to 55.6% in the case of caryopsis germinated on medium represented by the supernatant obtained from the active yeast culture and, respectively, to 40.3% in the case of caryopsis germinated on medium represented by supernatant obtained from the culture of yeast. inactivated yeast, compared to the batches of seedlings from the control variant, germinated in ultrapure water.

In turn, the supernatant from the inactivated yeast culture, compared to the one obtained from the active culture, helped less the growth of the common wheat test seedlings, in the presence of the toxicity of heavy metals from the mining residues from Tarnița.

According to the results obtained and already published [78] we can also say that the treatment of common wheat caryopsis with the supernatant obtained from the suspension of active yeast (yeast incubated with water at room temperature - DA) and, respectively, inactivated (yeast incubated with water at boiling point - DI) determined the decrease of the germination capacity, of the mass and of the average height of the resulting test seedlings, respectively, compared to the control batches, treated only with water (C): Fg (%): C (97) > DA (94) > DI (85): m_p (g): C (61.3) > DA (55.6) > DI (40.3); height (cm): C (10.4) > DA (8.1) > DI (4.9).

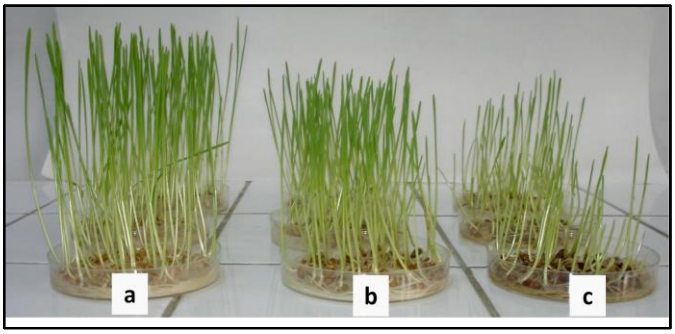

FIG. 3.7. The effect of the supernatant obtained from the active yeast culture on the germination of caryopsis of *Triticum aestivum* L., Putna variety and the growth of seedlings thus resulting in laboratory cultivation conditions, at 7 days of germination : a) Control, H_2O; b) Supernatant with active yeast; c) Supernatant with inactivated yeast.

Table 3.9. The effect of the supernatant obtained from the active and inactivated yeast culture on the germination of caryopsis of *Triticum aestivum* L. and the growth of seedlings thus resulting in laboratory cultivation conditions, at 7 days of germination.

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, g)	Average height of seedlings (cm)
Control, H_2O (C)	97 ± 1.2	61.3 ± 3.8	10.4 ± 0.3
Supernatant active yeast (DA)	94 ± 3.4	55.6 ± 1.7	8.1 ± 0.2
Supernatant inactivated yeast (DI)	85 ± 9.9	40.3 ± 8.2	4.9 ± 1.5

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

At the same time, the toxic solution obtained from the metalliferous mining waste from the Tarnița mining operation, determined the decrease of the germination faculty and affected the growth and development of the resulting seedlings (Figure 3.8).

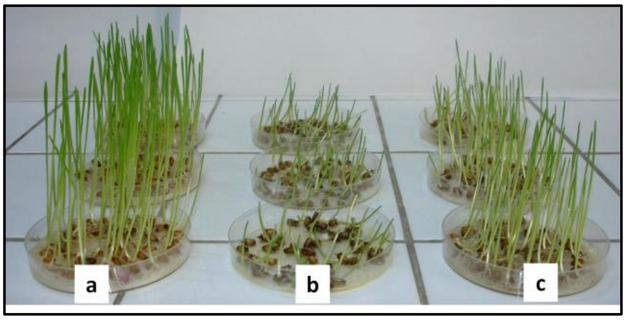

FIG. 3.8. Inhibitory effect of the supernatant obtained from the active yeast culture on the toxicity of heavy metals from mining residues in the Tarnița mining area used as a substrate for the germination of caryopsis of *Triticum aestivum* L., Putna variety grown in laboratory conditions, at 7 days of germination: a) Active yeast supernatant; b) Toxic solution; c) Supernatant with active yeast and toxic solution.

In turn, both the germination parameters and the development of the resulting seedlings increased in the case of inhibition of heavy metal toxicity, probably due to the presence of active yeast in the composition of supernatant a (Figure 3.8, Table 3.10): F_g (%): Sol. T (71) < DA + ST (93) < DA (94); m_p (g): Sol. T (29) < DA + Sol. T (42.5) < DA (55.6); height (cm): Sol. T (2.9) < DA + Sol. T (4.9) < DA (8.1).

Table 3.10. The effect of the supernatant with active yeast and the toxic solution on the germination process of caryopsis of *Triticum aestivum* L. , Putna variety and the growth of the resulting seedlings, in experimental conditions of laboratory cultivation at 7 days of germination

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p , g)	Average height of seedlings (cm)
Active yeast supernatant (DA)	94 ± 3.5	55.6 ± 1.7	8.1 ± 0.2
Toxic solution (Sol. T)	71 ± 13.3	29.0 ± 2.4	2.9 ± 0.4
Supernatant with active yeast and toxic solution (DA + Sol. T)	93 ± 4.2	42.5 ± 1.3	4.9 ± 0.3

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

At the same time, by incubating the toxic solution with boiling inactivated yeast supernatant, an increase was obtained in both the germination capacity and the plant mass and, respectively, in the average height of the common wheat seedlings (Figure 3.9 and Table 3.11): Fg (%): Sol. T (71.3) < DI (84.7) < DI + Sol. T (92); m_p (g): Sol. T (29) < DI + Sol. T (33.3) < DI (40.3); height (cm): Sol. T (2.9) < DI + Sol. T (3.6) < DI (4.9).

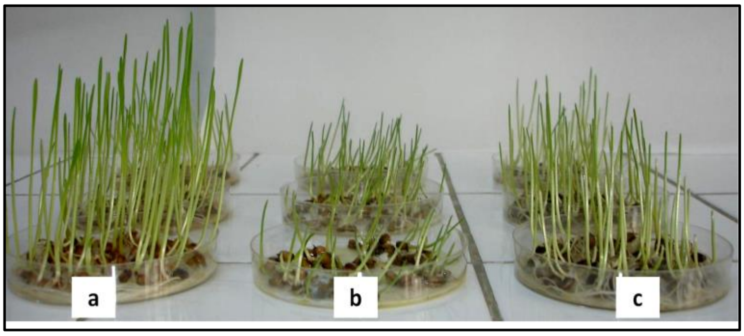

FIG. 3.9. Inhibitory effect of the supernatant obtained from inactivated yeast culture on the toxicity of heavy metals from mining residues in the Tarnița mining area used as germination substrate for caryopsis of *Triticum aestivum* L., Putna variety grown in laboratory conditions, at 7 days of germination: a) Supernatant with inactivated yeast; b) Toxic solution; c) Supernatant with inactivated yeast and toxic solution.

Table 3.11. The effect of the supernatant with inactivated yeast and the toxic solution on the germination process of *Triticum aestivum* L. caryopsis, Putna variety and the growth of the resulting seedlings, in experimental conditions of laboratory cultivation at 7 days of germination.

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, g)	Average height of seedlings (cm)
Inactivated yeast (DI)	84.7 ± 9.9	40.3 ± 8.2	4.9 ± 1.6
Toxic solution (Sol. T)	71.3 ± 13.3	29.0 ± 2.4	2.9 ± 0.4
Inactivated yeast and toxic solution (DI + Sol. T)	92.0 ± 1.9	33.3 ± 1.8	3.6 ± 0.2

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

According to the data presented in the literature [24], it is known that yeasts can absorb microelements from the environment, including iron. However, special physico-chemical conditions are required to achieve an environment that promotes both the development of yeast and the absorption of iron. Various sources of iron do not affect the yeast biomass but, given the efficacy, cost, stability and compatibility with the metabolism of *Saccharomyces cerevisiae* Meyen ex EC Hansen, this yeast species has been used in biological decontamination experiments of toxic supernatants obtained from metal mining waste, before carrying out germination tests on common wheat species.

Yeast *Saccharomyces cerevisiae* Meyen ex EC Hansen can easily grow in different environments when iron is abundant, because it has the ability to consume this chemical element and keep it in its essential metabolic pathways [5, 59].

Therefore, the decontamination of soils contaminated with heavy metals around old mining operations has been and will continue to be a challenge for the scientific world for a long time to come.

In the experiments carried out, the yeasts proved to be an effective microorganism in the decontamination of the toxic supernatant rich in heavy metals, favoring the germination of wheat caryopsis and the development of test seedlings resulting in the germination process.

3.3.1.4. Preliminary conclusions

During treatment with toxic supernatants / mining residues from the Tarnița mining area, the toxic solution (3 mL toxic supernatant and 2 mL ultrapure water Milli-Q, Sol. T) caused the germination parameters to decrease and affected the growth and development of test seedlings, compared to batches treated with the active yeast supernatant (DA). In this sense we can consider that both the germination parameters and the development of seedlings have increased in the case of inhibition of heavy metal toxicity, probably due to the specific composition of the supernatant with active yeast (DA + Sol.T).

Also, by incubating the toxic solution with supernatant with inactivated boiling yeast (DI + Sol. T) an increase was obtained both in the germination capacity and in the weight and, respectively, the average height of the common wheat test seedlings.

In the experiments performed, the supernatant with active yeast was more efficient, compared to the one prepared from inactivated yeast, regarding the decontamination and subsequent use of the solutions resulting from the extraction of metalliferous mining waste (DMM) in the germination tests of wheat caryopsis and new seedling growth. formed, probably because the latter releases large amounts of organic matter, which can disrupt the germination process of wheat caryopsis. We consider, however, that this technical solution can be used in the case of microbiological decontamination and soil cultivation in the coming years, until the total degradation (mineralization) of those compounds released, probably, by yeasts.

3.3.2. Protective effect of glutathione and toxic mining residues

3.3.2.1. The objective of the research

Glutathione, a tripeptide derived from cysteine, is an amino acid with the -SH group in the molecule present in all living cells, with their role of protection against heavy metal ions, organochlorine compounds, oxidants, etc. As a result of the knowledge of its properties, the objective of the experiments performed in laboratory conditions in this thesis is to highlight the role of glutathione in the protection of plant organisms against the aggression of contaminants in the mining area Tarnița. In this sense we used as a model of living organisms the common wheat caryopsis, *Triticum aestivum* L., Putna variety, following their germination parameters, as well as the tolerance of seedlings thus resulting in abiotic stress induced by the presence of heavy metals in wastewater.

3.3.2.2. Way of working

In order to determine the protective effect of glutathione in the germination process of wheat caryopsis in the presence of mining residues from Tarnița exploitation and the development in the first days of the resulting seedlings, treatments were performed on batches of 50 caryopsis, in three repetitions (Table 3.12).

Table 3.12. Working model in evaluating the protective role of glutathione on the process of germination and growth of wheat seedlings, *Triticum aestivum* L., Putna variety

Treatments and working conditions	Reagents (mL)	Stirring time (min.)	Reagents (mL)	Stirring time *) (min.)
Control, H_2O	5	60	-	-
H_2O + ST	2.5 (H_2O)	30	2.5 (ST)	30
GSH 10^{-2} M + H_2O	2.5 (GSH)	30	2.5 (H_2O)	30
GSH $5 \cdot 10^{-3}$ M + ST	2.5 (GSH)	30	2.5 (ST)	30
GSH 10^{-2} M + ST	2.5 (GSH)	30	2.5 (ST)	30

*) From time to time; GSH = glutathione; ST = toxic supernatant;
GSH 10^{-2} M + H_2O = GSH control.

3.3.2.3. Obtained results

During the 7-day treatment period, the sample with toxic solution (water and toxic supernatant) reduced the germination capacity of caryopsis, compared to the control groups treated with ultrapure water Milli-Q (Control: Eg = 95%, Fg = 96 Toxic supernatant: (Eg = 88%, Fg = 90%) and, respectively, with the groups treated with GSH 10^{-2} M (Eg = 97%, Fg = 97%). the action of the toxic solution was affected, so that the total mass and the average height of the seedlings, respectively the total mass of the roots registered decreases (m_p = 1.41 g, height = 3.375 cm, m_r = 0.57 g), compared to batches treated with Milli-Q ultrapure water (m_p = 2.28 g, height = 8.916 cm, m_r = 1.873 g), and, respectively, with the lots treated with GSH 10 $^{-2}$ M (m_p = 1.92 g, height = 6.974 cm, m_r = 0.664 g). These reductions in germination values could be due to the high levels of copper, iron and arsenic in the tested supernatant, as indicated by the ICP-OES (Table 3.1) and AAS (Table 3.3) measurements.

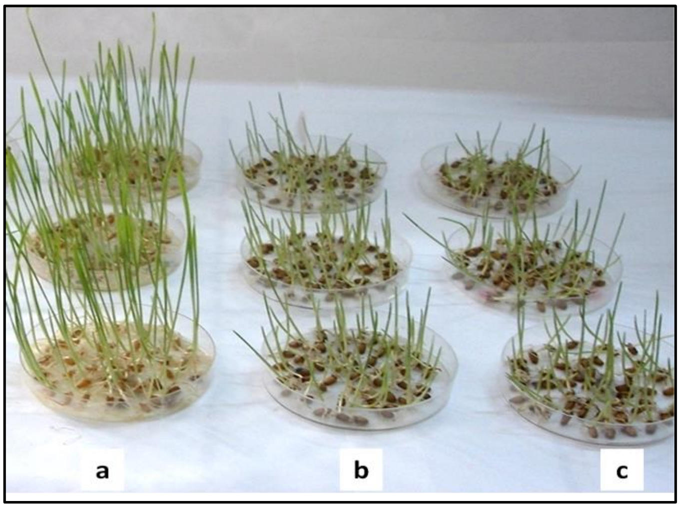

FIG. 3.10. The effect of glutathione on the growth of test seedlings of *Triticum aestivum* L., Putna variety resulting from germinated caryopsis in the presence of mining residues in the Tarnița area, in experimental laboratory conditions (7 days of germination). Test seedling treatments:
a) GSH control, 2.5 mL sol. GSH 10 $^{-2}$ M, 30 min. + 2.5 mL H_2O, 30 min .;
b) 2.5 mL sol. GSH 5 · 10 $^{-3}$ M, 30 min. + 2.5 mL ST, 30 min. ;
c) 2.5 mL sol. GSH 10 $^{-2}$ M, 30 min. + 2.5 mL ST, 30 min. .
GSH = glutathione; ST = toxic supernatant.

Table 3.13. Protective effect of glutathione in mining residue poisoning on test seedlings of *Triticum aestivum* L., Putna variety, determined at 7 days of germination, under experimental laboratory conditions

Treatment *)	E_g **) (%)	F_g ***) (%)	Total weight of seedlings (m_p , g)	Average height of seedlings (cm)	Total weight of roots (m_r , g)
Blanck, H_2O	95 ± 0.6	96 ± 2.0	2.28 ± 0.12	8.916 ± 2.5	1.873 ± 0.09
H_2O + ST	88 ± 2.0	90 ± 2.1	1.41 ± 0.02	3,375 ± 2.1	0.570 ± 0.06
Blanck GSH	97 ± 1.3	97 ± 2.4	1.92 ± 0.09	6,974 ± 1.6	0.644 ± 0.1
GSH 5·10 $^{-3}$ M + ST	96 ± 2.0	97 ± 1.3	1.12 ± 0.03	2,795 ± 2.0	0.697 ± 0.01
GSH 10 $^{-2}$ M + ST	95 ± 1.7	95 ± 0.6	1.29 ± 0.05	3,158 ± 2.0	0.813 ± 0.03

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Eg = percentage of germinated caryopsis after 3 days.

***) Fg = percentage of caryopsis germinated after 7 days.

GSH = glutathione; ST = toxic supernatant.

It should be noted that the germination parameters of caryopsis showed an increasing trend, as a result of the treatment of common wheat caryopsis with GSH of concentration $5 \cdot 10^{-3}$ M (Eg = 96%, Fg = 97%) and, respectively, 10^{-2} M (Eg = 95%, Fg = 95%). The growth of seedlings was thus affected, the total mass and average height of seedlings registering some reductions, which can be explained taking into account the biology of wheat plants, as well as the role of organic substances in plant development in general, observations that can be correlated with results. and statements in the literature [69] .

Compared to the batches of control seedlings treated with GSH 10^{-2} M, for the batches treated with GSH $5 \cdot 10^{-3}$ M, respectively 10^{-2} M and toxic supernatant both the germination parameters and the development of the test seedlings registered decreases in value (Figure 3.10 and Table 3.13), only the total mass of the root register increasing.

This technique of chemical decontamination of mining residues with GSH was used, because it was found that after the first extraction with water of metalliferous mining waste collected from the Tarnița dump in the toxic supernatant obtained (wastewater) large amounts of heavy metals remain.

3.3.2.4. Preliminary conclusions

The obtained results showed the role of glutathione in the germination processes of wheat caryopsis, Putna variety and allowed an evaluation of its tolerance to abiotic stress induced by heavy metals (Cu, Fe, Pb and Zn) present in the mining residues from the Tarnița area.

Compared to control batches treated with ultrapure water Mili-Q (C) and control batches treated with GSH 10^{-2} M, respectively, caryopsis of the test species treated with toxic solution (toxic supernatant and water ultrapure Mili-Q, ST) recorded a decrease in germination parameters: E_g (%): GSH 10^{-2} M (97) > C (95) > ST (88); F_g (%): GSH 10^{-2}M (97) > C (96) > ST (90).

Also, the growth and development of wheat seedlings treated with toxic solution was affected, the total mass and the average height of the seedlings, respectively the total mass of the roots registering decreases : m_p (g): C (2,28) > GSH 10^{-2} M (1.92) > ST (1.41); height (cm): C (8,916) > GSH 10^{-2} M (6,974) > ST (3,375); m_r (g): C (1.873) > GSH 10^{-2} M (0.644) > ST (0.57).

However, the germination capacity of caryopsis increased after treatment with GSH of $5 \cdot 10^{-3}$ M and 10^{-2} M, respectively, 30 minutes before treatment with toxic supernatant:

E_g (%) : ST (88) < GSH 10^{-2} M + ST (95) < GSH $5 \cdot 10^{-3}$ M + ST (96); F_g (%): ST (90) < GSH 10^{-2} M + ST (95) < GSH $5 \cdot 10^{-3}$ M + ST (97).

Seedling growth in the presence of the toxic supernatant was affected, with total mass and average seedling height decreasing. In contrast, root growth in the presence of contaminants in the toxic supernatant, compared to the control group of GSH-grown seedlings, was not affected:m_p (g): ST (1.41) > GSH 10^{-2} M + ST (1.12) > GSH $5 \cdot 10^{-3}$ M + ST (1.12); height (cm): ST (3,375) > GSH 10^{-2} M + ST (3,158) > GSH $5 \cdot 10^{-3}$ M + ST (2,795); m_r (g): GSH 10^{-2} M + ST (0.813) > GSH $5 \cdot 10^{-3}$ M + ST (0.697) > ST (0.57).

The results of the experiment showed that seedling germination and development were strongly inhibited when wheat caryopsis was treated with toxic supernatant obtained by extraction with Mili-Q ultrapure water from mining waste samples taken from the contaminated area (1g DMM to 10 mL of water), followed by ultrasonication and centrifugation. Treatment of wheat caryopsis with different concentrations of glutathione before treatment with toxic supernatant led to increased seedlings and reduced abiotic stress induced by the presence of heavy metals.

3.3.3. Decontamination of mining residues by hydroxide precipitation

3.3.3.1. The objective of the research

The objective of this research was to highlight the decontaminating role of sodium hydroxide on toxic supernatants obtained by extraction from samples collected from areas contaminated with heavy metals. Germination tests using common wheat performed *in vitro*, in laboratory conditions, were previously recommended to highlight the degree of decontamination of solutions containing heavy metals [12, 18].

3.3.3.2. Way of working

To remedy the content of heavy metals in wastewater obtained by extraction from DMM collected from landfills in the Tarnița mining area, the hydroxide-based precipitation method was used [9, 80]. Thus, 1 g of contaminated solid material was sonicated with 10 ml of Mili-Q ultrapure water , and the suspension was centrifuged to obtain the toxic supernatant (ST). It was decontaminated (STD) by precipitating heavy metal ions with a solution of sodium hydroxide (4% NaOH) to pH 5 and pH 12.0, respectively, then ultracentrifuged at 5000 rpm for 5 minutes and treated. subsequently with 4% NaOH or 4% HCl, to neutralize to pH 7.0.

Table 3.14 summarizes the experimental conditions provided for determining the effect of mining residues from the Tarniţa mining area decontaminated by NaOH precipitation on the germination processes of wheat caryopsis and the growth process of the resulting seedlings.

Table 3.14. Experimental conditions provided for the determination of the decontaminating effect, by precipitation with NaOH, of the mining residues from the Tarnița mining area (3 repetitions).

Treatments (wheat caryopsis)	Reagent 1 (mL)	Reagent 2 (mL)	Stirring time (min.)
Control, H_2O	5	-	60
ST	5	-	60
ST + H_2O	3	2	60
STD	5	-	60
STD + H_2O	3	2	60

ST = toxic supernatant; STD = decontaminated toxic supernatant.

3.3.3.3. Obtained results

Germination experiments have shown that soil / metal samples collected from the metal mining waste dump are highly toxic to plants, almost completely inhibiting the germination of wheat caryopsis and seedling growth (Figure 3.11, a-2 and b-2).

In the experiments performed, the germination capacity of wheat caryopsis increased in value after diluting the toxic supernatant with ultrapure water Mili-Q (Figure 3.11, a-3 and b-3), respectively after its precipitation with NaOH and neutralization with HCl (Figure 3.11, a). -4.5 and b-4.5).

The solution obtained after the reaction of the toxic supernatant with sodium hydroxide, and respectively the removal of the precipitate, was used as germination substrate and led to a significant increase in germination parameters as well as the average mass and the average height of the resulting common wheat seedlings. (Figure 3.11 and Table 3.15).

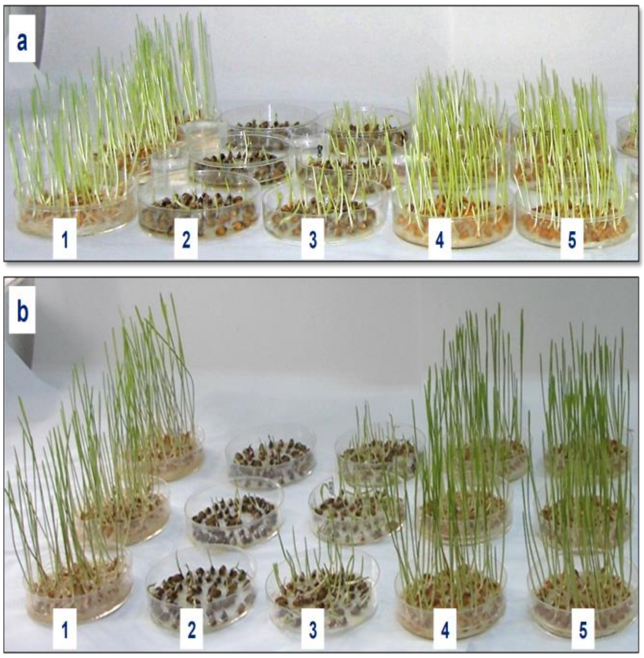

FIG. 3.11. The effect of decontamination of the supernatant obtained from mining residues from the Tarnița mining area by precipitation with NaOH and neutralization on the germination process of *Triticum aestivum* L. caryopsis, Putna variety and seedling growth as follows, under experimental laboratory conditions: (a) Test seedlings at three days of treatment; (b) Test seedlings at seven days of treatment. Treatments applied: 1) Control, 5 mL H_2O; 2) 5 mL ST; c) 3 mL ST + 2 mL H_2O; 4) 5 mL STD; 5) 3 mL STD + 2 mL H_2O.
ST = toxic supernatant; STD = decontaminated toxic supernatant.

Table 3.15. Reduction of supernatant toxicity by precipitation with NaOH and induced effect on the germination process of caryopsis of *Triticum aestivum* L., Putna variety and the growth of seedlings thus resulting, under experimental laboratory conditions

Treatment *)	E_g **) (%)	F_g ***) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
Blanck, H_2O	97 ± 2.1	97 ± 1.2	48.7 ± 3.6	7.4 ± 1.6
ST	25 ± 1.6	28 ± 3.6	11.1 ± 1.6	1.2 ± 2.3
ST + H_2O	56 ± 1.3	75 ± 1.1	22.9 ± 1.0	2.3 ± 1.2
STD	91 ± 2.6	91 ± 2.0	57.1 ± 2.2	6.8 ± 2.0
STD + H_2O	88 ± 2.0	95 ± 1.0	58.4 ± 1.2	7.2 ± 2.1

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).
**) Eg = percentage of germinated caryopsis after 3 days.
***) Fg = percentage of caryopsis germinated after 7 days.
ST = toxic supernatant; STD = decontaminated toxic supernatant.

3.3.3.4. Preliminary conclusions

The precipitation with hydroxide of the supernatant obtained from the mining waste collected from Tarnița, followed by neutralization, led to a significant increase of the germination parameters (germination capacity), as well as of the mass and, respectively, the average height of resulting common wheat seedlings; therefore, the negative effect of the toxic supernatant on seedling germination and growth was inhibited by precipitating some of the contaminants with sodium hydroxide.

Chapter 4. Relationship of Iron, Copper, Arsenic ions from copper and barite mines Tarniţa with common wheat test seedlings, *Triticum aestivum* L., Putna variety

4.1. The effect of iron and copper ions on the germination process of wheat

4.1.1. The objective of the research

The objective of the experiments carried out practically, during the present thesis in laboratory conditions, is to investigate the effect of Fe^{2+} and Cu^{2+} ions identified in excessive quantities in the Tarnița area on the germination processes of common wheat, chosen as a test species and interpretation of seedling tolerance. thus resulting in the chemical stress induced by the respective ions in the culture medium.

4.1.2. Way of working

To determine the effect of Fe^{2+} and Cu^{2+} ions in low concentrations by germination tests, the working method presented in Chapter 2.2.2 was used, using common wheat caryopsis (*Triticum aestivum* L., Putna variety). In the first experiment, 5 mL of $CuSO_4$ and $FeSO_4$, respectively, were used as treatment solutions, applied on 50 common wheat caryopsis, respectively of concentration $5 \cdot 10^{-3}$ M. In the second germination experiment, 5 mL solution of different salts ($CuCl_2$ and $FeSO_4$) were applied on 50 test caryopsis of concentration $5 \cdot 10^{-4}$ M. For comparison, triple control samples were performed with 5 mL milli-Q ultrapure water applied on 50 test caryopsis.

4.1.3. Obtained results

The results obtained in the first germination experiment performed with heavy metal solutions with a concentration of $5 \cdot 10^{-3}$ M (Table 4.1), showed that during the 7-day treatment period the sample with Cu^{2+} ions strongly reduced the germination of caryopsis (Fg = 31%), compared to batches of caryopsis treated with Fe^{2+} ions (Fg = 84%) and, respectively, with batches of caryopsis treated, treated only with ultrapure water Mili-Q (Fg = 97%).

Also, the growth of wheat seedlings from caryopsis treated with Cu^{2+} ions with a concentration of $5 \cdot 10^{-3}$ was affected, the average mass and height of the seedlings being lower (m_p = 53.71 mg, height = 7.84 cm), compared to batches of seedlings treated with Fe^{2+} ions (m_p = 66.33 mg, height = 10.74 cm) and, respectively, with the control groups from the Mili-Q ultrapure water treatments (m_p = 77.31 mg, height = 11.97 cm).

Table 4.1. Effect of Cu^{2+} and Fe^{2+} ions in germination experiments on the test species *Triticum aestivum* L., Putna variety (measurements performed on the 7th day of treatment).

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
Control, H_2O	97 ± 0.7	77.31 ± 1.3	11.97 ± 1.3
$CuSO_4$, $5 \cdot 10^{-3}$ M	31 ± 1.3	53.71 ± 1.6	7.84 ± 1.6
$FeSO_4$, $5 \cdot 10^{-3}$ M	84 ± 2.3	66.33 ± 0.5	10.74 ± 0.5

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

The results obtained in the second germination experiment, performed with different salts of copper and iron ions at a concentration of $5 \cdot 10^{-4}$ M, showed that during the 7-day treatment period, the $FeSO_4$ sample reduced easy germination of caryopsis (Fg = 95%), compared to batches of seedlings treated with $CuCl_2$ (Fg = 97%) and, respectively, with batches of control seedlings treated with ultrapure water Mili-Q (Fg = 99%). Thus, this concentration can be considered a toxicity limit on wheat. At the same time, the soluble ions of these metals in the Tarniţa mining area showed values of clearly higher concentrations.

Table 4.2. The effect of low concentrations of copper and iron salts on the germination process of *Triticum aestivum* L. caryopsis, Putna variety under experimental laboratory cultivation conditions (measurements performed on the 7th day of treatment)

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
Control, H_2O	99 ± 0.7	55.86 ± 0.2	10.37 ± 0.7
$CuCl_2$, $5 \cdot 10^{-4}$ M	97 ± 1.3	53.29 ± 0.9	9.73 ± 0.3
$FeSO_4$, $5 \cdot 10^{-4}$ M	95 ± 2.4	48.08 ± 1.4	9.01 ± 0.4

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

Analyzing the data presented in Table 4.2, it can also be seen that the growth process of wheat seedlings treated with $CuCl_2$ solution at a concentration of $5 \cdot 10^{-4}$ M was not deeply affected, the mass and average height of the seedlings formed being slightly lower (m_p = 53.29 mg, height = 9.73 cm), compared to that recorded in the batches of control seedlings, treated with ultrapure water Mili-Q (m_p = 55.86 mg, H = 10.37 cm). In contrast, batches of seedlings treated with $FeSO_4$ solution at the same concentration of $5 \cdot 10^{-4}$ M recorded higher decreases than in the case of copper ions (m_p = 48.08 mg, height = 9.01 cm) . In turn, although the chlorine ion present in the composition of copper chloride used experimentally is harmful at high concentrations, at the concentration of $5 \cdot 10^{-4}$ M can not be incriminated for possible toxicity.

4.1.4. Preliminary conclusions

The toxicity of copper ions was manifested by the significant decrease in the germination parameters of wheat caryopsis, as well as by affecting the growth of seedlings thus resulting, at a concentration of $5 \cdot 10^{-3}$ M.

Germination tests performed with chlorides and heavy metal sulfates at low concentrations of $5 \cdot 10^{-4}$ M showed that the copper chloride solution inhibits less germination of common wheat caryopsis and seedling development, compared to the test batches treated with ferrous sulfate. The phenomenon thus observed can be explained by the physiological role of the metal ions investigated in the plant body, the copper ion being considered an indispensable microelement in plant development [60, 86].

The practically obtained results showed that Fe^{2+} and Cu^{2+} ions at low concentrations of $5 \cdot 10^{-4}$ M have a slightly inhibitory effect on the germination of common wheat caryopsis, the values of the respective parameters obtained when applying these heavy metals showing very different differences. small, regardless of the type of salt used in performing germination tests. However, at concentrations higher than $5 \cdot 10^{-3}$ M, Cu^{2+} ions show a more pronounced inhibitory effect on the germination of wheat caryopsis, as well as on the growth of the resulting seedlings, compared to Fe^{2+} ions , proving high toxicity. theirs. These results obtained in the research carried out have shown that the toxicity of iron and copper ions can be highlighted by germination tests. Thus, our research suggests a correlation between the germination process of common wheat caryopsis, the growth and development of the resulting seedlings and the existence of heavy metals in polluted areas in the Tarnița mining area, namely: the germination process may be low with increasing ion concentrations investigated metals, but also the volume of toxic supernatant applied (results of previous experiments with supernatant).

4.2. Evidence of arsenite toxicity by wheat caryopsis germination tests

4.2.1. The objective of the research

This subchapter focused on investigating the negative impact of arsenic (in the form of arsenite and arsenate ions) on the germination parameters of *Triticum aestivum* L. caryopsis, Putna variety and the growth of wheat seedlings resulting from the germination process under conditions experimental laboratory, in order to determine the toxicity of arsenite ions at concentrations comparable to those of these ions in the mining waste dumps in the Tarniţa mining area.

4.2.2. Way of working

To determine the effect of arsenic from metalliferous mining waste from the Tarnița area on the germination of wheat caryopsis and the development of newly formed seedlings, sodium arsenite solutions were prepared in four replicas, as follows: 1) 0.25 mM NaH_2AsO_3; 2) 0.826 mM NaH_2AsO_3; 3) 1.8 mM NaH_2AsO_3; 4) 5 mM NaH_2AsO_3. Samples of 5 mL of each sodium arsenite solution were used as a treatment solution for batches of 50 common wheat test caryopsis mounted in three repetitions, the caryopsis being completely immersed in the respective solutions. For comparison, three control samples were performed with 5 mL of ultrapure milli-Q water and 50 caryopsis tests, respectively.

4.2.3. Obtained results

The percentage of germinated caryopsis after 7 days decreased from 100% (control, H_2O) to 96% (AsO_3^{3-}, 0.25 mM), continuously decreasing to 12%, with the increase of the concentration of arsenite ions from 0.25 mM to 5 mM (Figure 4.3).

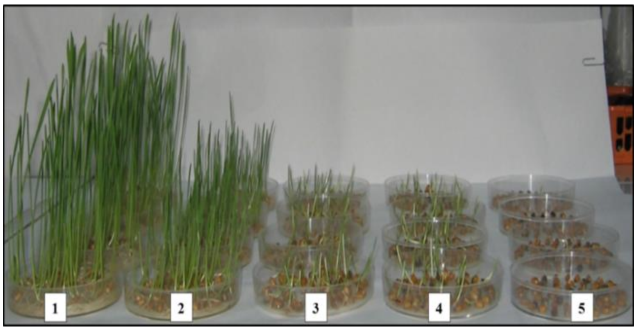

FIG. 4.3. The effect of arsenite ions (AsO_3^{3-}) on the germination of caryopsis *Triticum aestivum* L., Putna variety and on the growth of seedlings resulting after 7 days of germination.
Caryopsis test treatments: 1) control (5 mL H_2O); 2) 5 mL 0.25 mM AsO_3^{3-}; 3) 5 mL 0.826 mM AsO_3^{3-}; 4) 5 mL 1.8 mM AsO_3^{3-}; 5) 5 mL 5mM AsO_3^{3-}.

Table 4.3. The effect of arsenite ions on the germination of caryopsis of *Triticum aestivum* L., Putna variety and the growth of seedlings resulting in experimental laboratory conditions after 7 days of germination

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
Control, H_2O	100 ± 0.5	46.8 ± 0.1	9.3 ± 0.1
AsO_3^{3-}, 0.25 mM	96 ± 1.3	26.8 ± 0.1	4.6 ± 0.3
AsO_3^{3-}, 0.826 mM	90 ± 1.9	7.1 ± 0.0	1.5 ± 0.1
AsO_3^{3-}, 1.8 mM	64 ± 3.0	6.9 ± 0.0	1.4 ± 0.1
AsO_3^{3-}, 5 mM	12 ± 1.0	1.6 ± 0.0	0.6 ± 0.2

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

Similarly, both the height and mass of the seedlings decreased with increasing arsenite concentration (Table 4.3) suggesting that, after the germination process, newly formed seedlings are still affected by increased concentrations of the pollutant, a predictable phenomenon because caryopsis were kept in Petri dishes together with arsenite solutions throughout the experiment.

4.2.4. Preliminary conclusions

The analysis of the results obtained showed that the stress caused by sodium arsenite led to inhibition of plant germination by reducing the germination capacity, decreased the number of resulting seedlings and disrupted their growth, the results decreasing in value directly proportional to increasing the concentration of sodium arsenite in solutions. treatment (Table 4.3).

The results obtained showed that the toxicity of arsenic ions, ions in metalliferous waste and in the soils around the existing mining dumps in the researched mining area can be highlighted by germination tests.

4.3. Protective effect of glutathione on wheat to arsenite ion poisoning

4.3.1. The objective of the research

The research presented in this subchapter focused on investigating the protective effect of glutathione in plant intoxication with arsenic compounds, using tests for germination of common honeysuckle caryopsis, *Triticum aestivum* L., Putna variety and seedling growth under experimental conditions. laboratory.

4.3.2. Way of working

To determine the protective effect of glutathione in sodium arsenite intoxication, in the *first experiment* the germination tests were performed on wheat caryopsis, the sodium arsenite and glutathione solutions being prepared in four repetitions, according to the variants listed in Table 4.4.

In the *second experiment* the sodium arsenite and glutathione solutions required for the treatment of common wheat test caryopsis were prepared in four replicas, as follows: 1) 5 mL 10 mM GSH (considered as a Control sample); 2) 5 mL solution consisting of 2.5 mL NaH_2AsO_3 0.25 mM and 2.5 mL 10 mM GSH; 3) 5 mL solution consisting of 2.5 mL NaH_2AsO_3 0.826 mM and 2.5 mL 10 mM GSH; 4) 5 mL solution consisting of 2.5 mL NaH_2AsO_3 1.8 mM and 2.5 mL 10 mM GSH. In treatment schemes (2), (3) and (4), 2.5 mL of 0.5 mM NaH_2AsO_3 , 2.5 mL of 1.652 mM NaH_2AsO_3 and, respectively, were pipetted into tubes 2.5 mL NaH_2AsO_3 3.6 mM, stirring the contents of each test tube for 30 minutes, and then 2.5 mL of 20 mM GSH was added, stirring occasionally for another 30 minutes. .

Table 4.4. How to prepare the reagents used in the experiment to evaluate the protective role of glutathione in the intoxication with sodium arsenite ions of caryopsis and common wheat seedlings, *Triticum aestivum* L., Putna variety.

Treatments and working conditions	Reagents (mL)	Stirring time (min)	Reagents (mL)	Stirring time *) (min.)
Control, H_2O	5	60	-	-
NaH_2AsO_3 1 mM	5	60	-	-
GSH 2 mM + NaH_2AsO_3 2 mM	2.5 (GSH)	30	2.5 (NaH_2AsO_3)	30
GSH 4 mM + NaH_2AsO_3 2 mM	2.5 (GSH)	30	2.5 (NaH_2AsO_3)	30
GSH 10 mM + NaH_2AsO_3 2 mM	2.5 (GSH)	30	2.5 (NaH_2AsO_3)	30
GSH 20 mM + NaH_2AsO_3 2 mM	2.5 (GSH)	30	2.5 (NaH_2AsO_3)	30

*) From time to time; GSH = glutathione; NaH_2AsO_3 = sodium arsenite

The *third experiment* looked at the comparative effect of arsenite and arsenate ions, as well as the protective effect of glutathione in arsenite ion intoxication in germination tests performed on common wheat caryopsis. In this experiment samples with sodium arsenate, sodium arsenite and glutathione solution were prepared and used in three repetitions, as follows: 1) 5 mL H_2O (control); 2) 5 mL NaH_2AsO_4 1 mM; 3) 5 mL solution consisting of 2.5 mL NaH_2AsO_3 2 mM and 2.5 mL 20 mM GSH (that is 5 mL NaH_2AsO_3 1 mM + 10 mM GSH); 4) 5 mL of 1 mM NaH_2AsO_3. 2.5 mL of 2 mM NaH_2AsO_3 solution was pipetted into the treatment solution (3), the contents of the tube being stirred for 30 minutes, and then 2.5 mL of 20 mM GSH was added, stirring occasionally for another 30 minutes.

The 5 mL samples were used as a treatment solution for batches of 50 common wheat test caryopsis, the experiment being performed in three repetitions. For comparison, three control samples were performed with 5 mL of milli-Q ultrapure water and 50 test caryopsis.

4.3.3. Obtained results

Experiment 1. The results obtained in the first germination experiment, performed with a 1 mM solution of sodium arsenite, showed that during the 7-day treatment period, in which the caryopsis were in contact with arsenite ions, the treatment with this type of ion strongly reduced the germination of caryopsis (Fg = 19%), compared to the groups of control caryopsis, treated only with ultrapure water Mili-Q (Fg = 97%).

Table 4.5. The effect of glutathione and sodium arsenite treatment on caryopsis germination and the growth of *Triticum aestivum* L. seedlings, Putna variety in experimental laboratory conditions, after 7 days of germination

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
Control, 5 mL H_2O	97 ± 1.8	53.3 ± 0.2	9.3 ± 0.1
5 mL NaH_2AsO_3 1 mM	19 ± 1.3	15.7 ± 2.9	1.7 ± 0.2
2.5 mL 2mM GSH + 2.5 mL NaH_2AsO_3 2 mM	21 ± 2.4	16.5 ± 0.4	1.8 ± 0.1
2.5 mL 4 mM GSH + 2.5 mL NaH_2AsO_3 2 mM	31 ± 3.7	18.6 ± 1.7	1.9 ± 0.1
2.5 mL 10 mM GSH + 2.5 mL NaH_2AsO_3 2 mM	42 ± 2.0	19.5 ± 0.7	2.1 ± 0.1
2.5 mL 20 mM GSH + 2.5 mL NaH_2AsO_3 2 mM	87 ± 1.7	20.7 ± 0.4	2.3 ± 0.1

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

After applying GSH solutions (2.5mL) of concentration between 2 and 20mM for 30 minutes on the analysis material, an operation that allowed its partial absorption by the caryopsis taken, the subsequent treatment, for another 30 minutes, with the solution of sodium arsenite (2.5 mL) of 2 mM concentration caused a slight increase in the percentage of germination capacity (Table 4.5). This tendency to increase the germination capacity continued with the increase of glutathione concentration. It should be noted that both the germination parameters of caryopsis and the average mass of the resulting seedlings and their height showed an upward evolution, as a result of the treatment of common wheat caryopsis with GSH concentration 2 - 20 mM (Table 4.5).

Experiment 2. In this experiment, the arsenite concentrations were chosen so as to better capture the inhibitory effect of arsenite and the role of glutathione in modifying its inhibitory effect on some germination parameters of wheat caryopsis.

If we compare the data obtained practically (Table 4.6) for the treatment with 0.25 mM sodium arsenite with those in table 4.3, it can be considered that glutathione practically cancels the toxic effect of arsenite.

While arsenite ions with a concentration of 1.8 mM reduce the germination capacity of wheat seeds to 64%, in the absence of glutathione (Table 4.3), when added to it at a concentration of 10 mM, this physiological parameter increased in value by 24% (Table 4.6).

Table 4.6. Protective effect of glutathione against the toxicity of sodium arsenite during the germination process of caryopsis of *Triticum aestivum* L., Putna variety and seedling growth resulting in experimental laboratory conditions (after 7 days of treatment).

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p , mg)	Average height of seedlings (cm)
5 mL GSH 10 mM (Control)	99 ± 0.5	36.7 ± 1.2	7.7 ± 0.0
2.5 mL NaH_2AsO_3 0.25 mM + 2.5 mL 10 mM GSH	98 ± 1.4	38.0 ± 0.4	7.8 ± 0.1
2.5 mL NaH_2AsO_3 0.826 mM + 2.5 mL 10 mM GSH	97 ± 0.6	17.3 ± 0.4	2.4 ± 0.1
2.5 mL NaH_2AsO_3 1.8 mM + 2.5 mL 10 mM GSH	88 ± 0.9	12.5 ± 0.5	1.7 ± 0.1

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test (p ≤ 0, 05).

**) Fg = percentage of caryopsis germinated after 7 days.

Significant effects were also observed in terms of total mass and average height of the resulting seedlings. Thus, their average height increased by 0.3 units (cm) at the same concentration of 1.8 mM sodium arsenite, the height being 1.4 cm (see Table 4.3), and by inhibiting the toxicity of arsenite ions by the solution The 10 mM glutathione height was 1.7 cm (see Table 4.6). In turn, the average mass of seedlings grown in the presence of arsenite ions at a concentration of 1.8 mM also increased by 5.6 units (mg).

The results obtained in the second experiment suggested that glutathione, at a concentration of 10 mM, greatly reduces the toxicity of sodium arsenite at a concentration of 1.8 mM. To validate these results, another experiment was initiated, in which a control with ultrapure water Mili-Q, the treatment with arsenite and the one with arsenite plus glutathione was used.

Experiment 3. Sodium arsenate treatment was also included in this experiment to compare the toxic activity of the arsenite ion with that of the arsenate ion, and a higher toxicity of the latter was observed (Figure 4.5), contrary to the data specified by specialized literature [36]. Thus, compared to the control, the number of newly formed seedlings decreased, in the case of treatment with arsenic ion, from an average of 48 per batch, to one of only 40 seedlings.

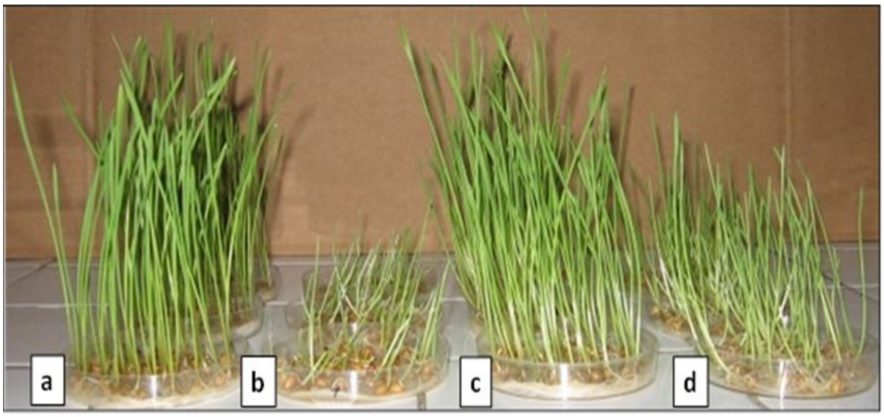

FIG. 4.5. The effect of arsenate and sodium arsenite ions applied in the presence of glutathione on the germination process of caryopsis of *Triticum aestivum* L., Putna variety and the growth of newly formed seedlings after 7 days of germination. Treatments applied : a) Control, H_2O; b) NaH_2AsO_4 , 1 mM; c) NaH_2AsO_3 , 1 mM + GSH, 10 mM; d) NaH_2AsO_3 , 1 mM.

Compared to the batches of control seedlings treated with Mili-Q ultrapure water , the average height of the seedlings treated with arsenate ions decreased from 11.6 cm to 2.5 cm. (Figure 4.5 and Table 4.7). At the same time, compared to the action of arsenate ions, sodium arsenite did not affect the number of seedlings formed, and their average height decreased by 46% and not by 78.4% (from 11.6 cm to 6.2 cm).

In turn, g lutation showed a protective role in the intoxication of plant material with arsenite ions, the decrease of seedling height not being significant (9.9 cm compared to 11.6

cm). The average height of the resulting seedlings was recovered by a percentage of 37% when, after the treatment with sodium arsenite followed by one with glutathione, the values thus recorded of this parameter varying from 6.2 cm (variant without GSH) to 9.9 cm (variant with GSH). At the same time, the average mass of newly formed seedlings through the germination process also increased by 32% in the presence of arsenite and glutathione ions: 37.0 mg (variant without GSH) to 54.5 cm (variant with GSH).

Table 4.7. The effect of arsenate and sodium arsenite ions applied in the presence of glutathione on the germination process of *Triticum aestivum* L. caryopsis, Putna variety and the growth of newly formed seedlings after 7 days of germination.

Treatment *)	F_g **) (%)	Medium mass of seedlings (m_p, mg)	Average height of seedlings (cm)
H_2O (Control)	97 ± 1.8	58.0 ± 1.3	11.6 ± 0.1
NaH_2AsO_4 1 mM	90 ± 0.6	19.0 ± 0.9	2.5 ± 0.1
NaH_2AsO_3 1 mM + GSH 10 mM	100 ± 0.0	54.5 ± 0.8	9.9 ± 0.1
NaH_2AsO_3 1 mM	97 ± 1.3	37.0 ± 1.8	6.2 ± 0.2

*) The values are arithmetic means of three values ± standard error (50 caryopsis / sample); according to the Tukey test ($p \leq 0, 05$).

**) Fg = percentage of caryopsis germinated after 7 days.

4.3.4. Preliminary conclusions

The first germination experiment of wheat caryopsis performed by the application of sodium arsenite ions showed that during the 7-day germination period the applied 1 mM arsenite concentration determined a toxicity manifested by the significant decrease of the germination parameters and by affecting the growth of newly formed wheat seedlings . If this treatment of caryopsis led to impairment of germination parameters, as well as morphological parameters of seedlings, the additional treatment applied to caryopsis thus intoxicated with glutathione concentrations of 2 - 20 mM significantly improved the germination process .

The *second germination experiment,* performed by applying sodium arsenite with concentrations of 0.25 - 1.8 mM, determined a decrease in the values of the analyzed germination parameters, and the concomitant application of 10 mM glutathione with arsenite ions had protective role on wheat caryopsis, which thus successfully passed the germination interval, in conditions of toxicity of arsenite in the culture medium.

The results obtained in the *third experiment* showed that the arsenate ion showed a higher toxicity, compared to the arsenite ion, at the same concentration of 1 mM. In this experiment too, 10 mM glutathione highlighted its protective role in arsenite poisoning.

The concentrations used in the three experiments were well below the concentrations of arsenic species in the Tarniţa area and, in the organized experimental laboratory conditions, these concentrations showed a high toxicity on the biological material under test (newly formed seedlings of germinated caryopsis, which significantly reduced root size, sometimes to extinction).

In turn, glutathione has been shown to have a protective role against low concentrations of arsenite ions applied simultaneously in the culture medium, in experiments organized in laboratory conditions.

GENERAL CONCLUSIONS

The research that was the subject of this thesis aimed at the physiological response of common wheat plants, *Triticum aestivum* L., Putna variety, represented by the intensity of the caryopsis germination process and the growth of the resulting seedlings, as well as the activity level of some enzymatic compounds specific with antioxidant role, involved in the metabolism of young seedlings newly formed both in excess of heavy metals and arsenic from metalliferous mining material (soils) and mining residues (toxic supernatant) from the area of barite mine Tarnița, Ostra commune, Suceava county, and and to residues resulting from experimental decontamination, by microbiological and chemical methods, respectively, of the cultivation substrates taken from the respective mining area.

The analysis of the practically obtained data allows us to state some conclusions, which aim to meet the proposed research objectives, as follows:

I. Concentrations of heavy metals and arsenic detected in mining and soil waste samples from copper and barite mines Tarniţa

- Inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements have shown that landfills in the Tarnița mining area contain large amounts of heavy metals such as iron, copper, lead and zinc, as well as metalloids such as arsenic. Among the heavy metals thus identified, iron recorded the highest concentrations in and around the waste dump, followed by copper and lead, these values being probably determined by the specific climatic conditions in the mining area in question. In turn, the soils collected at a distance of about 30 m from the landfill of metalliferous mining waste also proved to have large amounts of copper, lead and arsenic.
- Atomic absorption spectroscopy (AAS) measurements have shown that in aqueous extracts obtained from metalliferous mining waste and from soils taken from approximately 30m distance from the waste dump heavy metals such as iron, copper, zinc and manganese are present in high concentrations. The values of the concentrations thus determined far exceeded the values allowed by the national standards for surface waters in Romania, proving that in the Tarnița area many heavy metals were insoluble and could not be extracted with water in the form of metal ions, deeply affecting the forest vegetation. investigation area . Experimental decontamination of extracts with metalliferous mining waste by precipitation with sodium hydroxide did not significantly reduce the concentrations of iron, copper, zinc and manganese, which are still kept at very high values, compared to the standard limits allowed.
- The ICP-OES and AAS analysis methods allowed the determination of heavy metal and arsenic concentrations in soil samples and mining residues (aqueous extracts) with high sensitivity. The results obtained in this way highlighted the existence, in the work area, of high concentrations of iron, copper, zinc, manganese and arsenic, some of these components being extremely toxic, even at low values, for the environment, a reality that suggests that deposits of metalliferous waste from the

mining and forestry area Tarniţa is still a strong source of contamination and a continuous danger for the food chain at the local level .

II. Assessment of the toxic effect of mining residues from copper and barite mines Tarniţa by germination tests of common wheat caryopsis, *Triticum aestivum* L., Putna variety

- The excess of heavy metals and arsenic determined in the samples collected from the mining waste dump and from the soils located at about 30 m from the landfill significantly reduced the germination parameters of common wheat caryopsis (up to a complete inhibition of it), while also reducing the process of growing newly formed seedlings, compared to the control lots, treated with distilled water and, respectively, with those grown on garden soil.
- The successive dilution of the supernatant obtained from the metalliferous mining waste by its extraction twice and respectively three times with water, reducing the level of toxic contaminants contained in the extracts thus prepared, progressively improved the germination parameters of wheat caryopsis and seedling development. new format.

III. Decontamination of mining residues from copper and barite Tarniţa mines

- Germination experiments of common wheat caryopsis using an environment with toxic supernatants / mining residues in the mining area Tarnița (3 mL toxic supernatant and 2 mL water) led to decreased germination parameters and affected the growth and development of wheat seedlings as a result, compared to batches treated with *Saccharomyces cerevisiae* Meyen yeast ex EC Hansen active and inactivated by treatment, respectively. thermally, the active microorganism proving to be able to inhibit, to a greater extent, compared to the inactivated variant, the toxicity of heavy metals existing in the toxic supernatants / mining residues for analysis. According to the data specified in the literature, this microorganism can be used in the case of microbiological decontamination of soils contaminated with heavy metals in two stages: a first extraction with water, followed by the passage of waters with heavy metals above the mass of yeast that retains, the yeast resulting from the decontamination process being subsequently dried and incinerated, and the heavy metals recovered by chemical methods of electrolysis or precipitation.
- Germination experiments of wheat caryopsis in the presence of glutathione on medium with toxic supernatants from the Tarnița area demonstrated its role in reducing the abiotic stress induced by heavy metals present in the respective metalliferous mining waste: the toxic supernatant obtained by water extraction of samples Mining waste from the contaminated area strongly inhibited the germination and development of common wheat seedlings, but treating wheat caryopsis with different concentrations of glutathione before using the toxic supernatant, reducing abiotic stress induced by the presence of heavy metals in the culture medium, led to increased parameters of germination and growth of newly formed seedlings, suggesting the antitoxic role of this thiol tripeptide.

- The process of precipitation with sodium hydroxide of the toxic supernatants obtained by water extraction of the samples collected from the areas contaminated with heavy metals in the study area, followed by neutralization, led to a significant increase in the germination parameters of the test caryopsis. and, respectively, to the average height of the newly formed seedlings, the sodium hydroxide solution can thus be recommended as a good decontamination agent for some substrates in similar pollution conditions to those existing in the Tarniţa mining area.

IV. Relationship of Iron, Copper and Arsenic ions from Tarniţa copper and barite mines with common wheat seedlings, *Triticum aestivum* L., Putna variety

Relationship of iron and copper ions with common wheat seedlings:

- Treatment solutions containing Fe^{2+} and Cu^{2+} sulphates at a concentration of $5 \cdot 10^{-3}$ M used in the germination experiments of common wheat caryopsis showed that copper ions significantly decreased the values of germination parameters compared to iron ions. and more strongly affected the growth and development of the resulting wheat seedlings, supporting the high toxicity of this type of ions in the existing metalliferous mining waste in the area of the Tarnița barite mine.
- Germination tests performed in the presence of different salts, in the form of cupric chloride and ferrous sulphate in low concentrations, of $5 \cdot 10^{-4}$ M, showed that Cu^{2+} ions less inhibit the germination of common wheat caryopsis and seedling development, compared to Fe^{2+} ions .
- The process of germination of wheat caripses and growth of young seedlings newly formed under experimental conditions, laboratory, *in vitro* cultures, on solutions containing iron and copper ions existing, practically, in the samples of metalliferous mining waste and soil collected from the mining area Tarniţa in much higher concentrations, compared to those tested in this paper, demonstrates their particularly toxic effects on the environment and raises an alarm regarding the risks that their presence poses to the health of the population in the area.

Relationship of arsenic ions with common wheat seedlings:

- Arsenite ions, identified in turn in metalliferous waste and soils in the Tarniţa mining area, applied experimentally in the form of sodium arsenite in concentrations higher than 5 mM induced a pronounced inhibitory effect on the germination of wheat caryopsis, as well as growth and the development of young seedlings, the latter primarily inhibiting the growth and development of roots.
- Germination and growth tests of test seedlings performed in the presence of arsenite and arsenate ions showed that, at the same concentration of 1 mM, the arsenate ion showed a higher toxicity compared to the arsenite ion. Further assuming that the arsenate present in the metalliferous mining waste from the Tarnița mining area may increase the toxicity together with the other toxic elements such as copper, lead, iron, aluminum, etc., present, in turn, in this waste and from the desire to know the contribution of arsenic to the global toxicity of the metalliferous material, the toxic effect of some samples of arsenite or arsenate of different concentrations was tested much lower, compared to the concentrations of arsenic species identified in the

Tarniţa area; the practical results obtained also highlighted this time highly toxic effects of the respective solutions, prepared experimentally, on the test plants.

- Experiments that tracked the interaction between glutathione and sodium arsenite during the process of caryopsis germination and growth of common wheat seedlings showed that glutathione provided, at a concentration of 10 mM, protection against the toxicity of sodium arsenite and had a protective role on seedling growth.

DISSEMINATION OF RESULTS OBTAINED

PUBLICATIONS

I.1. Extensive scientific papers in ISI journals with impact factor:

1. Necula R., Zaharia M., **Butnariu A.** , Zamfirache M.-M., Surleva A., Ciobanu C. I., Pintilie O., Iacoban C., Drochioiu G. - Heavy metals and arsenic in an abandoned barite mining area: ecological risk assessment using biomarkers, *Environmental forensics* , 1 - 13, DOI: 10.1080 / 15275922.2021.1976315, **2021** . (Impact factor **1,328**).

2. Ştefănescu R., **Butnariu A.-E.** , Zamfirache M.-M., Surleva A., Ciobanu C. I., Pintilie, O., Drochioiu G. – Yeast-based microbiological decontamination of heavy metal contaminated soils of Tarniţa, *Carpathian Journal of Earth and Environmental Sciences* , 12 (1), 153 - 159, **2017** . (Impact factor **1,347**).

I.2. Scientific articles published *in extenso*, in ISI-listed conference volumes:

1. Zaharia M., Drochioiu G., **Butnariu A. E.**, Ilieva D., Surleva A. – Heavy metal toxicity and decontamination. Tarnita closed mine pollution case, 17 th International Multidisciplinary Scientific GeoConference SGEM 2017, Energy and Clean Technology, Section Air Pollution and Climate Change, vol 17 (43), pp. 397-404, **2017**.

2. Drochioiu G., **Butnariu A.E.** , Ştefănescu R., Necula R. and Iacoban C. – Possible heavy metal bioremediation of Tarnita forestry area, 16 th International Multidisciplinary Scientific GeoConference SGEM 2016, Water Resources. Forest, Marine and Ocean Ecosystems, vol 3 (II), pp. 609 -616, **2016** .

3. Murariu M., Ciobanu CI, Bunia I., Surleva A., **Butnariu A. E.** – Wheat seeds as environmental markers in heavy metal and arsenic pollution of Tarnita mining area, 16th International Multidisciplinary Scientific GeoConference SGEM 2016, Water Resources. Forest, Marine and Ocean Ecosystems, vol 3 (II), pp. 685 -692, **2016** .

I.3. Scientific papers in journals included in International Databases (BDI):

1. **Butnariu A.** , Zamfirache M.-M., Drochioiu G. – Toxicity assessment of arsenite and its relationship with arsenic polluted area of Tarnița: the protective effect of glutathione, *Acta Chemica* Iași, vol. 28 (1), pp. 95 - 112, **2020**.

2. Pintilie, O., Zaharia, M., Cosma, A., **Butnariu, A.,** Murariu, M., Drochioiu, G., Sandu, I., Effect of heavy metals on the germination of wheat seeds: enzymatic assay , *The annals of "Dunarea de Jos" University of Galati. Fascicle IX. Metallurgy and Materials Science,* B category, 215 code (http://www.cncsis.ro/2006_evaluare_rev.php), ISSN 1453–083x, **2016**.

PARTICIPATIONS IN SCIENTIFIC EVENTS

I.4. International conferences:

1. **Butnariu A. E.**, Ştefãnescu R., Pintilie O., Murariu M., Bunia I., Surleva A., Ciobanu C. I. , Iacoban C. and Zamfirache M.-M. – Wheat seeds as markers of heavy metal pollution and decontamination of Tarnita mining area , 12th International Conference on Colloid and Surface Chemistry, Iași, May, **2016** .

2. Pintilie O., Zaharia M., Cosma A., **Butnariu A. E.**, Murariu M., Drochioiu G. and Sandu I. – The study of the effect of heavy metals on the germination of wheat seeds by enzymatic measurements, *Scientific, Technological and Innovative Research in Current European Context* (International Workshop EUROINVENT), Alexandru Ioan Cuza University Publishing House, (ISBN: 978 - 973 - 703 - 891 - 3), 643-653, **2016** .

I.5. National conferences:

1. O. Pintilie, M. Zaharia, A. Cosma, **A. Butnariu**, M. Murariu, G. Drochioiu, I. Sandu. Effect of heavy metals on the germination of wheat seeds: enzymatic assay, The 7 th Conference on Material Science & Engineering, UgalMat, Galaţi, May, **2016.**

2. **A. E. Butnariu** , M.-M. Zamfirache, A. Lobiuc, G. Drochioiu, O. Pintilie, M. Murariu, C. I. Ciobanu, C. Iacoban, Z. Olteanu – The effect of glutathione on the test species *Triticum aestivum L.* in the presence of heavy metals from mining residues from Tarnița-Ostra, Scientific Communications Session “D. Cheese ”, Bucharest, November, **2016** .

3. **Butnariu A. E.**, Ştefãnescu R., Pintilie O., Zamfirache M.-M., Surleva A., Ciobanu C. I. and Drochioiu G. – Yeast-based microbiological decontamination of heavy metal contaminated soils: Tarnita area case, "Alexandru Ioan Cuza" University Days, Iaşi, October, **2015** .

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