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NATIONAL AVIATION UNIVERSITY
FACULTY OF ENVIRONMENTAL SAFETY,
ENGINEERING AND TECHNOLOGIES
DEPARTMENT OF ECOLOGY

APPROVED TO DEFENCE
Head of theGraduate Department
_____________V.F.Frolov
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MASTER THESIS

(EXPLANATORY NOTE)

SPECIALIZATION“ECOLOGY AND ENVIRONMENTAL PROTECTION”

Theme: «Evaluation of allelopathic activity of plants when applying phytoremediation technologies »

Done by: student group 202 m Khakimova Vlada Ruslanivna
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KYIV 2020

МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ,
ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА ЕКОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової
кафедри
_____________В.Ф. Фролов
«_____» ___________ 2020 р.

ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ВИПУСКНИКА ОСВІТНЬОГО СТУПЕНЯ МАГІСТРА

ЗА СПЕЦІАЛІЗАЦІЄЮ «ЕКОЛОГІЯ ТА ОХОРОНА НАВКОЛИШНЬОГО СЕРЕДОВИЩА»

Тема: «Оцінка алелопатичної активності рослин при їх застосуванні у фіторемедіаційних технологіях»

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КИЇВ 2020

NATIONAL AVIATION UNIVERSITY

Faculty of Environmental Safety, Engineering, and Technologies
Ecology Department
Direction (speciality, major): speciality 101ma"Ecology", specialization "Ecology and Environmental Protection"
(code, name)

APPROVED
Head of the Department
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«____» _________ 2020__

BACHELOR THESIS ASSIGNMENT
Khakimova Vlada Ruslanivna

1. Theme «Evaluation of allelopathic activity of plants when used in phytoremediation technologies » approved by the Rector on October 18, 2019, №2612/ст.

2. Duration of work: from 18.10.2019 to 06.02.2020

3. Input data: scientific literature on the use of plants in phytoremediation technologies, experimental studies of the role of allelopathic effects of plants in their application in the processes of soil purification from contamination.

4. Content of explanatory note: analytical review of the literature on the subject of the diploma. Consideration of the problem of soil purification from contamination by phytoremediation methods and the role of allelopathic interactions between plants in the development of phytoremediation technologies. Materials and methods of investigation of allelopathic impact. Evaluation of allelopathic activity of plants when used in phytoremediation.

5. The list of mandatory illustrated material: tables, figures, charts, diagrams.

6. Schedule of Master thesis fulfillment

№ з/п	Task	Term	Advisor's signature
1	Receive themes task, search the literature and legislative frameworks	18.10.2019	
2	Making "draft" version of the explanatory note for the preliminary presentation at the department	18.10.2019	
3	Preliminary presentation of the diploma work at the department	19.10.2019-09.12.2019	
4	Preparing the main part (Chapter I)	10.12.2019	
5	Preparing the main part (Chapter II)	11.12.2019–18.12.2019	
6	Preparing the main part (Chapter III)	19.12.2019–25.12.2019	
7	Preparing the main part (Chapter IV)	26.12.2019–30.12.2019	
8	Preparing the main part (Chapter V)	31.12.2019 – 06.01.2020	
9	Formulating conclusions and recommendations of the thesis	07.01.2020–13.01.2020	
10	Consideration of remarks, recommendations, finalizing of the work, consultation with standards inspector	14.01.2020	
11	Preliminary presentation of the work at the department	15.01.2020	
12	Taking into account the comments and recommendations and training to protect	16.01.2020–05.02.2020	
13	Presentation of the work at the department	06.02.2020	

7. Consultant(s) ofcertain chapter(s):

Chapter	Consultant (academic rank, S.N.P)	Date, signature	
		Given by	Accepted by
Labor Precaution	Ph. D. in Biological Sciences, Associate Professor of Safety of Human Activities Department, Viktoriia V. Kovalenko		

8. Date of task issue: «18» October 2019

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ЗАТВЕРДЖУЮ
Завідувач кафедри
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«____» _________ 2020_ р.

ЗАВДАННЯ
на виконання дипломної роботи
Хакімової Влади Русланівни

1. Тема роботи «Оцінка алелопатичної активності рослин при їх застосуванні у фіторемедіаційних технологіях» затверджена наказом ректора від «18» жовтня 2018р. №2612/ст.

2. Термін виконання роботи: з 18.10.2019 р. по 06.02.2020 р.

3. Вихідні дані роботи: наукова література щодо використання рослин у фіторемедіаційних технологіях, експериментальні дослідження ролі алелопатичного впливу рослин при їх застосуванні у процесах очищення грунтів від забруднення.

4. Зміст пояснювальної записки: аналітичний огляд літературних джерел з тематики диплому. Розгляд проблеми очищення грунтів від забруднення методами фіторемедіації та ролі алелопатичних взаємодій між рослинами при розробці фіторемедіаційних технологій. Матеріали та методи дослідження алелопатичного впливу. Оцінка алелопатичної активності рослин при їх застосуванні у фіторемедіації.

5. Перелік обов'язкового ілюстративного матеріалу: таблиці, рисунки, діаграми.

6. Календарний план-графік

№ з/п	Завдання	Термін виконання	Підпис керівника
1	Отримання теми завдання, пошук літературних джерел та законодавчої бази	18.10.2019	
2	Оформлення «чорнового» варіанту пояснювальної записки для попереднього представлення на кафедрі	19.10.2019-09.12.2019	
3	Попереднє представлення роботи на кафедрі	10.12.2019	
4	Підготовка основної частини (Розділ I)	11.12.2019–18.12.2019	
5	Підготовка основної частини (Розділ II)	19.12.2019–25.12.2019	
6	Підготовка основної частини (Розділ III)	26.12.2019–30.12.2019	
7	Підготовка основної частини (Розділ IV)	31.12.2019 – 06.01.2020	
9	Формування висновків та рекомендацій дипломної роботи	07.01.2020–13.01.2020	
10	Оформлення пояснювальної записки до попереднього представлення на кафедрі, консультація з нормоконтролером	14.01.2020	
11	Попереднє представлення роботи на кафедрі	15.01.2020	
12	Урахування зауважень, рекомендацій та підготовка до захисту	16.01.2020–05.02.2020	
13	Представлення роботи на кафедрі	06.02.2020	

7. Консультація розділу «Охорона праці»

Розділ	Консультант (посада, П.І.Б.)	Дата, підпис	
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ABSTRACT

Explanatory note to the thesis on « Impact of oil pollution on freshwater ecosystems » includes pages 77, 16 figures, 4 tables, references 51.

Object of research – the use of plants in phytoremediation technologies to clean up soil from contamination

Subject of research – determination of allelopathic activity of plants when used in phytoremediation.

Aim of the work was to to evaluate the allelopathic effect between phytoremediants and native plants of phytocenosis in the development of phytoremediation soil cleaning.

Methods - analysis of scientific literature, experimental studies of allelopathic effects of plants, statistical methods of processing results.

Actuality. Phytoremediation is acknowledged as the perspective technology of soil decontamination and recovery. Nevertheless, there are certain factors that are supposed to influence this process, decreasing its effectiveness. The introduction of phytoremediation plants as the non-indigenous species into a phytocenosis of contaminated area may initiate the activation of an allelopathic activity of the present species. Allelopathic activity is commonly defined as a process of secretion of the secondary metabolites in a form of volatile compounds, exudates that inhibit development of other plants. Thus, this thesis focuses on the assessment of an allelopathic activity of the indigenous species and its influence on selected phytoremediation plants. Bioassay and germination test have been conducted to measure the effects of water-soluble extracts of *Amaranthus retroflexus L., Cirsium arvense L., Barbarea vulgaris R. Br. And Artemisia absinthium L.* on the introduced species used in phytoremediation.

PHYTOREMEDIATION, CONTAMINATED SOIL CLEANING, ALLELOPATHIC EFFECT OF PLANTS, PHYTOREMEDIATION TECHNOLOGIES

РЕФЕРАТ

Пояснювальна записка до дипломної роботи на тему « Оцінка алелопатичної активності рослин при їх застосуванні у фіторемедіаційних технологіях» містить 77 сторінок , 16 рисунків, 4 таблиці, літературних джерел 51.

Об'єкт дослідження – застосування рослин у фіторемедіаційних технологіях для очищення грунтів від забруднення.

Предмет дослідження– визначення алелопатичної активності рослин при їх використанні у фіторемедіації.

Мета – оцінити алелопатичний вплив між рослинами-фіторемедіантами та аборигенними рослинами фітоценозу при розробці фіторемедіаційних технологій очищення грунтів.

Методи дослідження – аналіз наукової літератури, експериментальні дослідження алелопатичного впливу рослин, статистичні методи обробки результатів.

Актуальність. Фіторемедіація визнана перспективною технологією знезараження та відновлення ґрунтів. Тим не менш, є певні фактори, які можуть впливати на цей процес, знижуючи його ефективність. Введення рослини-фіторемедіанта як некорінного виду у фітоценоз забрудненої ділянки може ініціювати активацію алелопатичної активності корінних видів. Алеллопатичну активність зазвичай визначають як процес секреції вторинних метаболітів у вигляді летких сполук, ексудатів, що гальмують розвиток інших рослин. Таким чином, ця дипломна робота зосереджена на оцінці алелопатичної активності корінних видів та їх впливу на вибрані рослини-фіторемедіанти. Для вимірювання впливу водорозчинних екстрактів *Amaranthus retroflexus L., Cirsium arvense L., Barbarea vulgaris R. Br., Artemisia absinthium L.* було проведено біотестування відносно інтродукованих видів, що використовуються у фіторемедіації.

ФІТОРЕМЕДІАЦІЯ, ОЧИЩЕННЯ ЗАБРУДНЕНИХ ГРУНТІВ, АЛЕЛОПАТИЧНИЙ ВПЛИВ РОСЛИН, ФІТОРЕМЕДІАЦІЙНІ ТЕХНОЛОГІЇ.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOTIONS

NE – natural environment;

SITE - Superfund Innovative Technology Evaluation;

BOA - benzoxazolinone;

MPC – maximum permissible concentration;

MAC – maximum acceptrable concentration;

UNESCO -United Nations Educational, Scientific and Cultural Organization;

RAL – roughly acceptable standards;

ATP - adenosine triphosphate.

INTRODUCTION

An ecosystem as a community of living organisms is specified by both internal and external mutual interactions. The competition between species is considered to be one form of such interconnections. A species may enter into competition with others for a habitat, resources e.g. water, nutrients, light,etc.

In the scientific literature, the term "allelopathic interaction" is used to describe the chemical competition between living organisms in an ecosystem which reflects the suppressing of germination process, the inhibiting ofgrowth, development, and the reproduction rate of the adjacent organisms . The mentioned physiological phenomenon is based on releasing of inhibitory substances by a plant that causes depression of other species sharing the same habitat. These specific substances have been known as allelochemicals and are represented by the secondary metabolites consisting of organic compounds, e.g. flavonoids, terpenoids, alkaloids, phenolic compounds, benzoxazolinone (BOA) and many others.

In the phytoremediation technology, allelopathic interaction is considered to be the influential aspect that may define the efficiency and success of plants introduction in a phytocenosis of the investigated territory and consequently, the entire process of recovery.

Thus, allelopathic species express the ability to cause the phytotoxical stress on neighboring plants by means of allelochemicals secreting from plumule or radical systems mainly in a form of volatile compounds, exudates or due to their residues decaying in a soil layer .

The entire process starting from the allelochemicals production till their release into the environment and subsequently, mechanisms of influences are found as slightly investigated. Modes of allelochemicals action were described in the asset of scientific publication . From the beginning, allelochemicals may affect the membrane hat activates the subsequent modifications in the biochemical and physiological processes inside a plant-target. The initiated changes are connected mostly with damaging of the important molecular targets, e.g. cells integrity, photosynthesis and biosynthesis processes, production of enzymes, replication, respiration and metabolism etc.

Manifestation of the allelopathic activity can beregarded as a mechanism of organisms defense against not only competitors, but also herbivores, predators and parasites. Moreover allelopathic interactions play the important role in control of biodiversity within an ecosystem via regulation of the species distribution, abundance in communities and limitation of population of the associated.

To develop the efficient and successful strategy of such decontamination technology as phytoremediation, it is crucial to understand a mechanism by which the indigenous species may affect the introduced phytoremediation plants and to define a possible rate of plants suppression and inhibition.

Relevance of the work

The introduction of phytoremediation plants as the non-indigenous species into a phytocenosis of contaminated area may initiate the activation of an allelopathic activity of the present species. Allelopathic activity is commonly defined as a process of secretion of the secondary metabolites in a form of volatile compounds, exudates that inhibit development of other plants. Thus, this thesis focuses on the assessment of an allelopathic activity of the indigenous species and its influence on selected phytoremediation plants.

Aim and tasks of the diploma work

Aim of the work: The aim of the work was to to evaluate the allelopathic effect between phytoremediants and native plants of phytocenosis in the development of phytoremediation soil cleaning.

Tasks of the work:

1. To analyze the importance and necessity of investigating the allelopathic effect of plants in the development of effective soil cleaning technology by phytoremediation.
2. To carry out experimental studies of the effect of indigenous plant extracts on the germination of seeds of herbal plants.
3. To determine the allelopathic effect of plants on growth processes.

4. To compare allelopathic resistance to two plant species promising for use in phytoremediation *A. pani*culatus and *A. caudatus*.

Object of research: the use of plants in phytoremediation technologies to clean up soil from contamination

Subject of research: determination of allelopathic activity of plants when used in phytoremediation.

Methods of research - analysis of scientific literature, experimental studies of allelopathic effects of plants, statistical methods of processing results.

Personal contribution of the graduate: the analysis of scientific sources has been carried out, the technique for determining of allelopathic effects of plants has been mastered, and statistical analysis of the data has been carried out.

Scientific novelty. As a result of research, it was established that when selecting plants for effective phytoremediation it is necessary to select species resistant to allelopathic influence, such as *A. paniculatus* .

Practical value of the work. The results can be aplied in effective soil cleaning technology using phytoremediation.

CHAPTER 1
ROLE OF ALLELOPATHIC INTERACTION BETWEEN PLANTS IN THE DEVELOPMENT OF PHYTOREMEDIATION TECHNOLOGIES

1.1. Efficiency of phytoremediation technologies

Phytoremediation, can be defined as the use of green plants to remove pollutants from the environment or to render them harmless [1]. It is also referred to as green technology and can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air. In this respect, plants can be compared to solar driven pumps capable of extracting and concentrating certain elements from their environment. This technology is being considered as a new highly promising technology for the remediation of polluted sites [2].

The concept of using plants to clean up contaminated environments is very old and cannot be traced to any particular source. Chaney (1983) was the first to reintroduce it as a remediation technique on metal-contaminated soils. Initially, the concept was based on metal hyper-accumulating plants, which are able to uptake and tolerate extremely high levels of metals. In the past, extensive research has been conducted in the field of phytoextraction: searching for new phytoextractors; providing more fundamental knowledge about metal uptake, translocation, and tolerance by plants as well as, improving plant metal accumulation and tolerance by genetic transformations. Another approach in the concept῾s development was based on high biomass-producing plants used together with chemical agents to enhance metal solubility and uptake by plants [3].

Certain plants, called hyper-accumulators, absorb unusually large amounts of metals in comparison to other plants. More than 400 plant species have been identified to have potential for soil and water remediation. As different plants have different abilities to uptake and withstand high levels of pollutants, many different plants may be used for phytoremediation. The strategies used in developing a phytoremediation plant are (a) screening of hyperaccumulator candidate plants, (b) plant breeding, and (c) development of improved hyperaccumulators using genetic tools. The hyperaccumulators that have been most extensively studied by scientific community include Thlaspi sp., Arabidopsis sp., Sedum alfredii sp. (both genera belong to the family of Brassicaceae and Alyssum). Thlaspi sp. are known to hyperaccumulate more than one metal, that is, T. caerulescens for Cd, Ni, Pb and Zn, T. goesingense for Ni and Zn, T. ochroleucum for Ni and Zn, and T. rotundifolium for Ni, Pb and Zn.

Over the last decade, there has been an increase in both industrial activities and urbanization. Both aquatic and terrestrial habitats are becoming progressively polluted due to the discharge of pollutants generated from various industries, transportation and fossil fuel burning. Many industries discharge their untreated wastewaters and other industrial wastes containing various proportions of heavy metals depending on what the industry produces, into natural body waters and on lands. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe) and the platinum group elements.

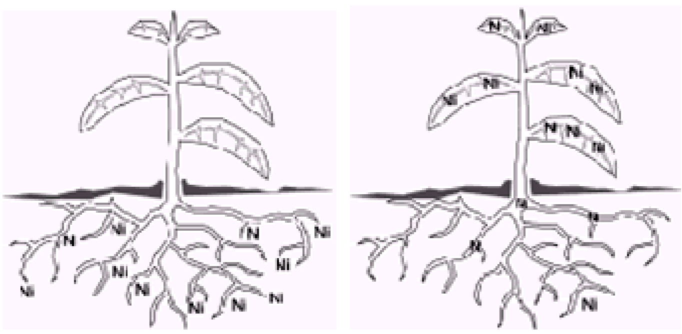

Fig.1.1. Uptake of metals Ni by phytoextraction. Nickel is removed from soil by moving up into plant roots, stems, and leaves. The plant is then harvested and disposed of and the site replanted until the Ni in the soil is lowered to acceptable levels.

Current remediation strategies of heavy metals is primarily based on physicochemical technologies which are meant primarily for intensive in situ or ex situ treatment of relatively highly polluted sites, and thus are not very suitable for the remediation of vast, diffusely polluted areas where pollutants only occur at relatively low concentrations and superficially.

In this context, phytoremediation appears as a valid option since it is best suited for the remediation of these diffusely polluted areas and at much lower costs than other methods [4]. The idea of using plants to remove metals from soils came from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate high concentrations of metals in their foliage.

Phytoremediation offers a cost-effective, nonintrusive, and safe alternative to conventional cleanup techniques (Glick, 2003). Phytoremediation can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatization and rhizofiltration.

Phytoextraction: the use of plants to remove contaminants from soils. Pollutant-accumulating plants are utilized to transport and concentrate contaminants (metal or organic) from the soil into harvestable parts of the roots and aerial parts of the plant; the term is mostly used to refer to metal removal from soils.

Phytoextraction, or phytomining, is the process of planting a crop of a species that is known to accumulate contaminants in the shoots and leaves of the plants, and then harvesting the crop and removing the contaminant from the site. Unlike the destructive degradation mechanisms, this technique yields a mass of plant and contaminant (typically metals) that must be transported for disposal or recycling. This is a concentration technology that leaves a much smaller mass to be disposed of when compared to excavation and landfilling. This technology is being evaluated in a Superfund Innovative Technology Evaluation (SITE) demonstration, and may also be a technology amenable to contaminant recovery and recycling. Phytoextraction is the name given to the process where plant roots absorb metal contaminants from the soil and translocate them to their above soil tissues. Phytoextraction, also called phytoaccumulation, refers to the uptake of metals from soil by plant roots into above-ground portions of plants [5].

Phytostabilization: the use of plants to reduce the bioavailability of pollutants in the environment. Plants stabilize pollutants in soils by chemically immobilizing the contaminants, thus rendering them harmless and reducing the risk of further environmental degradation by leaching of pollutants into the ground water or by airborne spread.

Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges (United States Protection Agency, 2000). It is the use of plant roots to limit contaminant mobility and bioavailability in the soil and water. Contaminants are absorbed and accumulated by roots, adsorbed onto the roots, or precipitated in the rhizosphere. This reduces or even prevents the mobility of the contaminants preventing migration into the groundwater or air, and also reduces the bioavailability of the contaminant thus preventing spread through the food chain. This technique can also be used to re-establish a plant community on sites that have been denuded due to the high levels of metal contamination. Once a community of tolerant species has been established, the potential for wind erosion (and thus spread of the pollutant) is reduced and leaching of the soil contaminants is also reduced. The plants primary purposes are to (1) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, (2) act as a barrier to prevent direct contact with the contaminated soil and (3) prevent soil erosion and the distribution of the toxic metal to other areas. Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is useful for the treatment of Pb as well as As, Cd, Cr, Cu and Zn. Some of the advantages associated with this technology are that the disposal of hazardous material/ biomass is not required (United States Protection Agency, 2000) and it is very effective when rapid immobilization is needed to preserve ground and surface waters. The presence of plants also reduces soil erosion and decreases the amount of water available in the system (United States Protection Agency, 2000). Phytostabilization has been used to treat contaminated land areas affected by mining activities and Superfund sites [6].

Phytovolatization: the use of plants to volatilize pollutants. Plants extract volatile pollutants (e.g. selenium, mercury and arsenic) from the soil and biologically converts them to a gas which is released via transpiration from the foliage.

Phytovolatilization refers to the uptake and transpiration of contaminants, primary organic compounds by plants. The contaminant, present in the water taken up by the plant, passes through the plant or is modified by the plant, and is released to the atmosphere (evaporates or vaporizes). The contaminant may become modified along the way, as the water travels along the plant"s vascular system from the roots to the leaves, whereby the contaminants evaporate or volatilize into the air surrounding the plant.

Phytodegradation: the use of plants to degrade organic pollutants. Plant roots are utilized to remediate contaminated soils by the breakdown of organic contaminants to simpler molecules which are stored in the plant tissue (Ghosh and Singh, 2005b).

Phytodegradation is the breakdown of organic contaminants within plant tissue. Plants produce enzymes, such as dehalogenase and oxygenase that help catalyze degradation. It appears that both the plants and the associated microbial communities play a significant role in attenuating contaminants.It is referred to the degradation or breakdown of organic contaminants by internal and external metabolic processes driven by the plant (Prasad and Freitas, 2003). Ex planta metabolic processes hydrolyse organic compounds into smaller units that can be absorbed by the plant. Some contaminants can be absorbed by the plant and are then broken down by plant enzymes. These smaller pollutant molecules may then be used as metabolites by the plant as it grows, thus becoming incorporated into the plant tissues.

Plant enzymes have been identified that breakdown ammunition wastes, chlorinated solvents such as TCE (Trichloroethylene), and others which degrade organic herbicideds. Plant enzymes that metabolise contaminants may be released into the rhizosphere, where they may play active role in transformation of contaminants. Enzymes, like dehalogenase, nitro-reductase, peroxidase, laccase and nitrilase, have been discovered in plant sediments and soils. Organic compounds such as munitions, chlorinated solvents, herbicides and insecticides and the inorganic nutrients can be degraded by this technology

Rhizofiltration: the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants. This contaminated water is either collected from a waste site and brought to the plants, or the plants are planted in the contaminated area, where the roots then take up the water and the contaminants dissolved in it (Dushenkov et al., 1995).

Rhizofiltration („rhizo‟ means „root‟) is the adsorption or precipitation onto plant roots (or absorption into the roots) of contaminants that are in solution surrounding the root zone. It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots. Rhizofiltration is similar to Phytoextraction but is concerned with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. The plants to be used for clean-up are raised in greenhouses with their roots in water. Contaminated water is both collected from a waste site and brought to the plants, or the plants are planted in the contaminated area, where the roots then take up the water and the contaminants dissolved in it. As the roots become saturated with contaminants, they are harvested and disposed of safely. Rhizofiltration remediates metals like As, Pb, Cd, Ni, Cu, Cr, V and radionucliides (U, Cs and St).

The ideal plants should produce significant amounts of root biomass or root surface area, be able to accumulate and tolerate significant amounts of target metals, involve easy handling and a low maintenance cost, and has a minimum of secondary waste that requires disposal. Terrestrial plants are more suitable for rhizofiltration because they produce longer, more substantial and often fibrous root systems with large surface areas or metal adsorption. Pteris vittata, commonly known as Chinese brake fern, is the first known As-hyper accumulator (Ma et al., 2001). Several aquatic species have the ability to remove HMs from water, including Water Pennywort (Hydrocotyle umbellate L.) (Dierberg et al., 1987), Duckweed (Lemna minor L.) (Mo et al., 1989) and Water Hyacinth (Eichhornia crassipes).

Table 1.1 summarises the uses and mechanisms used for phytoextraction, phytovolatization, phytodegradation, phytostabilisation and rhizofiltration.

Table 1.1

Applications of phytoremediation

Technique	Plant mechanism	Surface medium
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plants	Soils
Phytodegradation	Enhances microbial degradation in Rhizosphere	Soils, groundwater within rhizosphere
Phytostabilisation	Root exudates cause metal to precipitate and become less available	Soils, groundwater, mine tailing
Phytovolatization	Plants transpire selenium, mercury, and volatile hydrocarbons	Soils and groundwater
Rhizofiltration	Uptake of metals into plant roots	Surface water

Phytoremediation is not a ready fix method for contaminated soils. As with all new technologies it is still poorly understood, and before this technology can become efficient and cost effective on a commercial scale there are limitations that need to be overcome (Khan et al., 2000). For example, there is very little known about the biochemical, molecular and physiological processes involved in the hyper-accumulation process (Salt et al., 1994). For phytoremediation to be effective and to occur within a reasonable time frame, plant yield (biomass production) and contaminant accumulation have to be dramatically enhanced. This may be achieved by cultivation of rapidly growing plants or genetic engineering of plants with as yet unidentified hyper-accumulating genes. Other limitations of phytoremediation include the possible contamination of the food chain as a result of grazing on heavy metal contaminated vegetation. However, phytoremediation interests in the mining sector is growing due to the recovery of rare and expensive trace metals from harvested biomass (phytomining) and the low cost use of plants to remediate mining areas (Tyrer, 2006). Other limitations and advantages of phytoremediation can be seen in Table 1.2.

Table 1.2

Advantages and limitations of phytoremediation

Advantages	Limitations
• Low input costs & aesthetically pleasing (no excavation) • Soil stabilization & reduced leaching of water and transport of inorganics in the soil • Generation of a recyclable metal rich plant residue • Applicability to a range of toxic metals and radionuclides • Minimal environmental disturbance • Elimination of secondary air or water-borne wastes • Enhanced regulatory and public acceptance	• The plant must be able to grow in the contaminated soil or material • The plant can accumulate inorganics that it can reach through root growth and are soluble in soil water • Process can take years for contaminant concentrations to reach regulatory levels (long-term commitment) • The contaminant must be within (or drawn toward) the root zones of plants that are actively growing • It must not present an imminent danger to human health or further environmental harm • Climatic conditions are a limiting factor • Introduction of non-indigenous species may affect biodiversity

1.2. Prospects for the application of allelopathy

The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Statements as early as 300 years BC points to the phenomenon that many crop plants, including chick pea (*Cicer arietinum*) and barley (*Hordeum vulgare*), inhibit the growth of weeds and crop plants other than barley .

The term allelopathy, was introduced by Molisch in 1937, and is derived from the Greek words allelon 'of each other' and pathos 'to suffer' and mean the injurious effect of one upon the other. However, the term is today generally accepted to cover both inhibitory and stimulatory effects of one plant on another plant Some use the term in a wider sense, for instance entomologists,who include the effects of secondary compounds on plant-insect interactions.

In 1996 The International Allelopathy Society defined allelopathy as follows: “Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural biological systems (excluding animals), including positive and negative effects” (Torres et al. 1996).

Therefore, allelopathy is now a fact the direction of science by which to solve global problems day. And this is exactly what he saw as the destination allelopathy academician A.M. Grodzinskij - founder of an allelopathic school in Ukraine.

In the following, the term is used in accordance with Rice (1984), but effects of the chemical compounds involved in plant-plant interactions and the effects of allelopathic plants are discussed in a broader perspective than strictly related to the plant-plant interactions. Chemicals released from plants and imposing allelopathic influences are termed allelochemicals or allelochemics. Most allelochemicals are classified as secondary metabolites and are produced as offshoots of the primary metabolic pathways of the plant. Often, their functioning in the plant is unknown, but some allelochemicals are known also to have structural functions (e.g. as intermediates of lignification) or to play a role in the general defence against herbivores and plant pathogens.

In the scientific literature, the term “allelopathic interaction” is used to describe the chemical competition between living organisms in an ecosystem which reflects the suppressing of germination process, the inhibiting of growth, development, and the reproduction rate of the adjacent organisms.

Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts, and volatilisation and/or by decomposition of plant material (Rice 1984). When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. The most frequent reported gross morphological effects on plants are inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development.

Allelopathic interactions between plants have been studied in both managed and natural ecosystems. In agricultural systems allelopathy can be part of the interference between crops and between crops and weeds and may therefore affect the economical outcome of the plant production. Both crop and weed species with allelopathic activity are known .

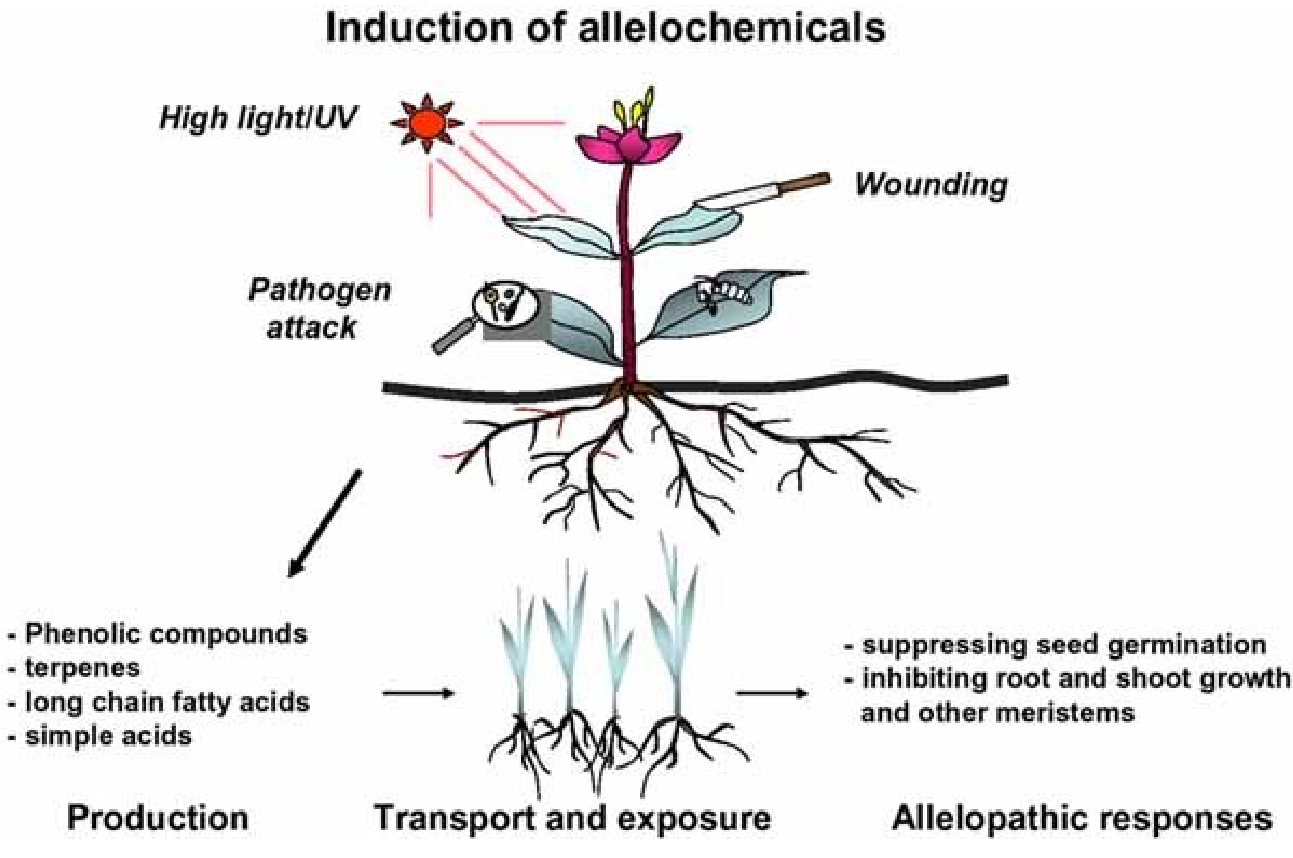

Fig.1.2. Induction of allelochemicals

Recently, several papers have suggested that allelopathy holds great prospects for finding alternative strategies for weed management. Thereby, the reliance on traditional herbicides in crop production can be reduced. Today, the allelopathic activity of some crops, for example rye, is to some extent used in weed management .

The search for genes involved in the production of allelopathic compounds in crops has begun. This widens the opportunity for improving the allelopathic activity of crops through traditional breeding strategies or by genetic engineering.

Biotechnological transfer of allelopathic traits between species has been suggested as a possibility and this could for example be from wild or cultivated plants into commercial crop cultivars. So far, a genetically modified plant with enhanced allelopathic activity has not been marketed.

Another research area within allelopathy is the search and development of new herbicides through the isolation, identification and synthesis of active compounds from allelopathic plants. These compounds are often referred to as 'natural herbicides'.

From the agronomic point of view, the research in allelopathy provides perspectives of a reduced reliance on traditional herbicides if weed control can be achieved by the release of allelochemicals from the crop. Also, in cropping systems where herbicides are not used, for example in organic farming, crop cultivars with enhanced allelopathic activity could be part of the weed management strategy.

Weed control mediated by allelopathy - either as natural herbicides or through the release of allelopathic compounds from a living crop cultivar or from plant residues - is often assumed to be advantageous for the environment compared to traditional herbicides. Due to their origin from natural sources, some authors suggest that the allelopathic compounds will be biodegradable and less polluting than traditional herbicides.

However, other authors emphasise that even though most compounds derived from natural sources appear to have short half live compared to synthetic pesticides, some of these products also have toxicologically undesirable target effects . The need of ecotoxicological studies to unveil the consequences of growing allelopathic cultivars on large scale has also been stressed.

With the possibility for development of genetically modified crops with enhanced allelopathic effect, the ecological consequences of the growth of such crops must be considered. This includes the possible spread of allelopathic plants to other ecosystems than the agricultural and spread of allelopathic traits to other plants.

1.3. Indications of allelopathic interactions between plants

Investigations of allelopathic activity have often been initialised by field observations mainly related to changes in agricultural, horticultural or silvicultural productivity or to changes in vegetation patterns in natural habitats.

Problems of growing the same crop in succeeding years because of poor establishment and stunted growth has lead to investigations of possible causes, including allelopathy. Allelopathy occurring among individuals of the same species is termed autotoxicity. Autotoxicity is known for example in *Medicago sativa* (alfalfa), *Trifolium* spp. (clovers) and *Asparagus officinalis* (asparagus).

Inhibitory effects on germination and establishments of crop caused by residues of either crops or weeds have lead to investigation of the release of toxic compounds from such residues. For example, the allelopathic interference of both living plant and of plant residues of the highly aggressive weed *Elytrigia repens*, quackgrass, has been strongly indicate. Residues from several crop species have been examined for their potential to reduce weed germination.

In cases where the success of a plant, typically a weed, can not be explained by the competitive ability, allelopathy has been suspected to play a role. Investigations of such observations have established or strongly indicated an allelopathic activity of weeds, e.g. *Avena fatua* (wild oat), *E. repens* (quackgrass), *Cirsium arvense* (Canada thistle) and *Stellaria media* (common chickweed).

Reduced weed problems within a crop may indicate that the seed germination or development of weedy species is inhibited by the release of allelochemicals from the crop. This has for example been reported in cultivated fields of some *Brassica* species, where no herbicides were applied. Also in fields of cultivated sunflower, the weed biomass was equally reduced in plots with or without herbicide treatments.

Reduced weed densities following the growth of some crops has been observed and has naturally stimulated the research in allelopathy.

The observation of a weed-free zone around some up-land rice cultivars in a germplasm collection growing in a weed infested field has initiated an extensive research programme with the aim of finding allelopathic rice cultivars for weed control. Weed free zones (80-90% weed control) with a radius of up to 20 cm has been observed. “Fairy rings” has also been observed both in fields with wild and cultivated sunflower (*Helianthus rigidus* and *H. annuus*, respectively). These rings are characterised by a decrease in the number of plants, and inflorescences as well as smaller size of individual plants in the middle of the ring.

Distinct zones with sparse or without vegetation has been observed around some shrubs in chaparrals. This includes the observation of the inhibition of adjoining plants by *Juglans nigra* (black walnut).

The choice of measurement parameter for the demonstration of allelopathy must also be considered. In several bioassays, seed germination and seedling development is measured after the exposure to alleged allelochemicals because seed and seedlings development is generally considered to be the most susceptible stages . Unless plants are in contact with allelochemicals at their sensitive stages allelopathic effects will not be observed. This should always be taken into account in the design of experiments aimed at demonstration of allelopathy.

If major morphological changes are not apparent within the experimental period after the plants have been exposed to allelochemicals, the effects may be overlooked. Sometimes seed germination is not inhibited but the process may be delayed,cotyledon and root size diminished or radicle or seedling development abnormal e.g. in form of twisted growth or in form of adverse effects on their metabolism . The effect on population size may be apparent only after a relatively long period of time when some of the seedlings in a population are inhibited.

In the search for potential herbicides derived from plants, the purpose is to demonstrate allelopathic activity of an isolated compound and to determine the activity range of the resultant allelochemical herbicide with respect to necessary dose and target weeds (e.g. Macias 1995, Macias et al. 1997, 1998). In such experiments, the effect on selected sensitive species such as lettuce and tomato may be important in the process, but the obtained effects must not be confused with the demonstration of allelopathy as an important ecological mechanism.

To demonstrate whether allelopathy offers the most reasonable explanation of an observed pattern, a series of experiments must typically be carried out and may include both laboratory and field tests. The design of each experiment will depend on the actual/ precise purpose of the investigation and on the characteristics of the donor and afflicted plants and on habitat. Some of the approaches and factors affecting the sensitivity of the tests used as part of the allelopathic research are described below.

The bioassay conditions influence the effect concentration and thereby the results of the bioassay. For example in seed germination tests, test species, light conditions, osmotic potential and interactions between these factors strongly influence the result. Also, solution volumes and seed number can influence the result of seed germination bioassays .

Factors such as seed size, seed dormancy and the length of the after-ripening period to which the seed has been subjected can influence on the concentration of allelopathic compound necessary to produce an effect on seed germination . The natural variation in seed germination may also in some cases pose some challenges to the design of experiments due to a low and inconsistent germination of relevant test species.

Seeds, from species such as lettuce, tomato and cress, which germinate readily, are often used in various germination tests. Such test species can be practical and useful for fractionation, isolation, and purification of the most important allelopathic compounds. However, to be able to relate the results to natural conditions species actual involved in the system must be evaluated.

The density dependent test, as suggested by Weidenhamer et al. 1989, Thijs et al. 1994, implicates that the density of the donor species is kept constant while the density of the receiver species is increased. As phytotoxic effects are assumed to be density-dependent, maximum size of receiver plant will occur at an intermediate density, with reduced size at both low density (the result of phytotoxicity) and at high density (due to intense resource competition). Density-dependent tests has both been carried out as a Petri-dish radicle elongation assays and as greenhouse and field experiments with whole plants.

Competition experiments have been used to compare the competitive ability of genotypes with alleged difference in allelopathic activity toward a target species. An enhanced release of the allelochemical would expectedly result in a better competitive ability of this genotype towards a target species compared to the genotype with a lower release.

The performance of the two genotypes must be analysed both as absolute yield and as relative yield total, where the relative yield of a genotype in a mixture is the ratio between its yield in that mixture and its yield in a pure stand.

The ratio between the donor species and the target species can be varied in competition experiments.

The effect of density on the allelopathic effects is thereby considered.

Experiments have been set up to determine if allelochemicals are present in soil samples in active concentrations, so associated plant species are influenced. Soil samples can be collected from the rhizosphere of the alleged allelopathic plant and seeds of test species can thereafter be placed in that soil to germinate. Germination percentage, speed of germination and plant development can then be compared to controls. Soils samples from adjacent fields or from sites in the same fields where the alleged allelopathic plant is not present can be used as controls.

Amendment of plant material to soil to test the allelopathic effect has often been carried out. However, the enhanced concentration of organic material may result in enhanced microbial activity, which may result in depletion of some nutrients. Thereby the effect caused by allelopathic toxicity can not be separated from the effect of microbial activity. To avoid that any growth response after the addition of plant material are caused by nitrogen and phosphorus depletion in the soil due to enhanced microbial activity and not caused by allelopathic toxicity, fertilizers has been added in some experiments.

1.4. Effects and selectivity of allelochemicals

Several of the allelochemicals identified as phytotoxic compounds are also involved in insect resistance. This indicates that some allelopathic interactions are part of a general plant defence.

The visible effects of allelopathy frequently observed as inhibited or delayed seed germination or reduced seedling growth, are secondary expressions of primary effects on metabolic processes. Analogous to the marketed herbicides, the diversity of structure among allelochemicals suggests that they have no common mode of action. The primary molecular target whereby an allelochemical interferes with physiology and growth is, with a few exceptions, unknown. Similarities within certain groups exists and will probably be further exploited as the functioning of allelochemicals. In some cases or as part of the set-up, sterilisation of such soils for experimental purposes could also be considered.

The recovery of some allelopathic compounds (phenolics) has been compared between soils infested with a suspected allelopathic plant and non-infested soils. The quantitative increase in the allelopathic pool of soil owing to an allelopathic plant has been determined. The phytotoxicity of the soils can then be compared.

Such an experimental design can demonstrate whether or not a plant has the potential of releasing allelopathic compounds into the rhizosphere and to affect the growth of other plant species. Still, as previously discussed, the importance of allelopathic interactions in ecosystems can neither be determined by the actual nor by net changes in the concentration of allelochemicals in soil. Activated carbon has been used to detoxify allelochemicals – either directly on the soil surface, incorporated into the soil, with plant extracts or in hydrophonic culture. Anticipating that the activated carbon totally absorbs all the allelochemicals and does not influence other factors of significance, the effect of allelochemicals can be estimated by comparison to controls without activated carbon.

Some of the reports are based only on results of in vitro bioassays. It remains to be answered whether the processes are also affected *in vivo* and how many processes are inhibited in addition.

Several allelochemicals have been shown to possess a broad activity spectrum. *In vitro* experiments with more than 70 alkaloids indicate that most alkaloids are toxic or inhibitory to more than one group of organisms including plant seedlings, bacteria, insects and mammals.

The authors conclude that alkaloids can beconsidered as “multipurpose” defence substances due to their wide activity range.

The broad biological activity of gramine could be explained by its effects on energy metabolism as it inhibits photo-phosphorylation, Pi-ATP exchange reaction, proton gradient and enhances electron transport in thylakoid membranes. Further, it was suggested, that compounds that affect one, or several, basic molecular targets are more likely to affect a wide range of organisms than compounds that affect targets specific to one organism.

Similarly, argue that the understanding of the role of phenolic compounds produced by a plant with allelopathic activity, on the establishment of other plant species, requires an ecosystem approach. This is because the phenolic compounds are also likely to have biological toxicity towards other organisms such as microflora and soil animals.

The ecological roles of terpenoids have been reviewed by Langheim (1994). Terpenoids are in contrast to phenolic and alkaloids, not commonly identified as allelochemicals in temperate agricultural crops), but occur abundantly in particularly conifers, composites, mints and euphorbias . The review illustrated that terpenoids produced by a plant may contribute to:

1)seed germination inhibition

2) defence against generalist and specialist herbivores,

3) defence against insect vectored fungi and
pathogenic fungi,

4) attraction of pollinators and

5) inhibition of soil bacteria.

Numerous individual mono-terpenoids have been demonstrated to have multiple effects. Although multiple effects may appear to be broad spectrum, the effect may be quite specific in terms of dosage levels on different populations of organisms in different communities. This way, the dosage factor can create a degree of specificity.

The allelochemicals released from a crop may affect non target species, both in fields and in natural habitats if the allelopathic plant is spread. In most agricultural research experiments, the effects of allelochemicals or allelopathic plants has been tested on weedy species or cultivated plants that may be affected by the allelopathic crops due to cultivation practices (e.g. rotational practices) or on species that are valuable as test species in seed germination tests due to their synchronic germination. The results of both laboratory and field experiments has indicated a selective response of different plant species to allelochemicals. Despite some studies have indicated that dicotyledons were more sensitive than monocotyledons to allelochemicals released from grasses, the response of plant species to allelochemicals cannot be predicted until the exact mode of action of the allelochemicals is known. Seed characteristics such as seed size and seed coat permeability may influence the uptake and effects of allelochemicals in seeds. In seed germination assays it was shown that species with small seeds were more inhibited than larger seeded species at a given concentration of allelochemicals.

The hydroxamic acids are often mentioned and examined as allelochemicals released from intact plants of cereal crops such as wheat and rye . They are present in leaves, stems and roots of cereal plants. Their potential in the control of pest and diseases of the crops has often been discussed . The role of hydroxamic acids in crop resistance to aphids, fungi and bacteria is well documented.

For instance, constitutive levels of hydroxamic acids have been shown to deter aphid feeding and decrease aphid survival and reproduction . In wheat and barley seedlings, inverse correlation have been found between concentrations of hydroxamic acids or gramine, respectively, and population growth rate of two aphids species,

Metopolophium dirhodum and *Schizaphis graminum*, on the plants . Even though the performance of these aphid species on the wheat plants was negatively affected by hydroxamic acid, the wheat seedlings with the higher content of hydroxamic acids were more damaged by aphids. This was explained by the feeding behaviour of the aphids as at least *S. graminum* probed for a longer time and thereby made more damage on the seedlings with higher contents of hydroxamic acids . Hence the plant content of compounds that can be released as allelochemicals and also function as insect deterring agents can both be advantageous and disadvantageous for the success of the plant.

1.5. Conclusions to Chapter 1

In the phytoremediation technology, allelopathic interaction is considered to be the influential aspect that may define the efficiency and success of plants introduction in a phytocenosis of the investigated territory and consequently, the entire process of recovery.

The demonstration of allelopathy as an ecological significant mechanism comprises several challenges. Especially, the interactions with abiotic and biotic factors are considered to play an important role in the expression of allelopathy. A holistic approach where the experimental designs are adapted to the species and the ecosystem and on molecular level is a fast growing research area. Exact information about the allelochemical, or allelochemicals, responsible for the allelopathic effects must be sought when assessing the effects of plants with enhanced allelopathic traits.

The following sites or processes are known targets for allelochemicals: cell division, production of plant hormones and their balance, membrane stability and permeability, germination of pollen, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, amino acid syntheses, nitrogen fixation, specific enzyme activities and conduction tissue.

To develop the efficient and successful strategy of such decontamination technology as phytoremediation, it is crucial to understand a mechanism by which the indigenous species may affect the introduced phytoremediation plants and to define a possible rate of plants suppression and inhibition.

CHAPTER 2
MATERIALS AND METHODS OF PLANTS ALLELOPATHYC ACTIVITY EVALUATION

2.1. Method of determination of allelopathic activity of plants

The objects of the investigation were such species:*Amaranthus retroflexus L., Cirsium arvense L.,Barbarea vulgaris R. Br.* and *Artemisia absinthium L.*(i.e. indigenous species of investigated territory), (Fig. 2.1., 2.2.).

The selected potential phytoremediation plants were *Amaranthus caudatus L.* and *Amaranthus paniculatus L.* (Fig. 2.3., 2.4.).

The current research work provides an estimationof an allelopathic activity of the water- soluble extracts exhausted from the mentioned indigenous species andits influence on the investigated phytoremediation plants. The influence of extracts on biotester was investigated in publications. In this investigation, *Lactuca sativa L.* was used as a target species and as an additional control sample which purpose was to define correlation between influenceson phytoremediation plants and biotester, and to compare the obtained results.

Bioassay and seed germination test are considered to be the appropriate methods of analysis due to their efficiency, their high responsivity to affect various chemicals, and their short-time experiments.

The first stage consisted in preparing the water solubleextracts from indigenous species. The plants with radicals were selected in a period of florescence on the selected territory. Initially, the selected material was dried, then reduced to fine particles with further pounding. The extracts were prepared by taking 20 g of crushed plants material and adding 100 ml of distilled water. The extraction went on during 24 hours.

The second stage of the investigation for esaw bioassay using seeds of phytoremediation plants and *L. sativa* (control sample). The process of seeds

1 2

Fig. 2.1. I *Artemisia absinthium L.*(1), *Cirsium arvense L.*(2).

germination is known as the most vulnerable phase of plant development therefore minimal resistance to such negative factors as allelochemicals action may be observed.

Bioassay has been performed according to the accepted methodology on filter paper in the Petri's dishes [28]. The prepared extracts in amount of 5 ml served as a medium. The seeds of biotester *L. sativa 'Kucheriawy'*, *A. caudatus 'Karmin'* and *'Helios'*,*A. paniculatus 'Zhaivir'* were used. Four replications (25 seeds in one) were made for each sample and the concentration of extracts remained the same.

Lactuca sativa L. - annual vegetable plant with increased sensitivity to soil contamination with heavy metals, as well as gas pollution emissions of vehicles. This phyto-indicator is characterized by quick pro seed plant and almost one hundred percent germination, which is significantly reduced present in the presence of contaminants. In addition, the shoots and roots of this plant under contaminants undergo significant morphological changes (growth retardation and curvature of shoots, reduction of root length and mass, and also the number and mass of seeds).

Lactuca sativa L. as a bio-indicator is also convenient in that the action of stressors can be studied simultaneously on a large number of plants with a small area workplace (Petri dish, cuvette, pan, etc.). Attractive as well very short experiment times. Watercress seeds germinate already on the tree tiy - the fourth day, and

The experiments have been carried out under the standard conditions with a temperature of 22±10C without penetration of light. The incubation period was equal to 120 hours. Potable water served as a control medium.

A screening-test was fulfilled immediately after incubation and gave the ability to define the reactionof plants on allelochemicals presented in the investigated extracts. The screening-test provided measuring of the following parameters: the germination percentage, the plumules length and the radicals length. The morphological changes of these edlings structure grown in the prepared media may confirm suppressing and depressing affects.

The germination percentage is defined in accordance to the expression:

$$G_{\%} = \frac{\sum G}{N} \times 100, \quad (2.1)$$

where *G*% is the germination percentage; *G* is the number of the germinated seeds; *N* is the number of seeds.

for most questions of the experiment you can get response within 10-15 days.

2.2. Characteristics of indigenous species of investigated territory

Cirsium arvense L.(Fig. 2.1.)

Cirsium arvense is a carbon fixation plant.The plants tend to thrive in areas where sunlight intensity is moderate, temperatures are moderate, and ground water is plentiful. Creeping thistle is a herbaceous perennial plant growing up to 150 cm, forming extensive clonal colonies from thickened roots that send up numerous erect shoots during the growing season. The leaves form a rosette, from which the stem from 30 to 200 cm in height then grows; at the top of the branch branches.

The plant is usually dioecious , well propagated vegetatively . It happens that it forms colonies only of persons of the same sex. Then the plant blooms, but the seeds do not give. Flowering time is from June to October.

Field plowing through the vegetative propagation can fill the entire field, displacing cultivated plants. In nature it is also found on meadows, in bushes , along roads in lowlands and in mountains.

A strong rod root can penetrate 2 to 3 meters, sometimes with roots of 5-6 meters in length. At about a depth of 35 cm from the main root parallel to the surface depart the tuber thickened roots that store nutrients.

Artemisia absinthium L. .(Fig. 2.1.). Wormwood (*Artemisia absinthium L.*) field or real (Latin Artemisia absinthium) is a perennial herbaceous root-sprout plant of the Astrov family.

In total, there are about 400 species of wormwood (approximately 170 of them can be found in Ukraine).

Wormwood belongs to the category of the most ancient medicinal plants, since it contains a large number of useful substances. As medicinal raw materials, the flower-bearing tops and roots of the plant collected during flowering are usually used.

Wormwood is widespread almost throughout Eurasia, in addition, it can be found in North America and the north of the African continent.

1 2

Fig. 2.2. *Barbarea vulgaris R. B.* (1), *Amaranthus retroflexus L.,* (2)

Barbarea vulgaris R. B.(Fig. 2.2.)

Common colza clogs crops of perennial grasses and winter crops, vegetable gardens, orchards, less often crops of spring grain and row crops. It grows especially abundantly in poorly cultivated steam fields on clay soils. Protective measures: low mowing of the weed during mass flowering in crops of perennial grasses, in the fall - small plowing, pre-sowing harrowing and cultivation. In the early phases of growth, common colza is sensitive to most herbicides. Due to the substances contained in the seeds, the plant can be dangerous for cattle, horses and poultry.

Amaranthus retroflexus L., .(Fig. 2.2.)

The amaranth is tailed, or the Shiritsa is thrown back, Sugar-beet (lat. Amaránthus retrofléxus) is an annual herbaceous plant, a species of the genus Shiritsa (Amaranthus) of the Amaranth family.

Widespread, very aggressive weed, one of the first to appear in wastelands and abandoned farmland. It prefers humus, water-permeable, rich in nutrients, and especially nitrogen, soils. The North American species, widely spread over all continents and is now cosmopolitan.

In Ukraine in recent years studies have been conducted on the search for plants suitable for phytoremediation purposes .

2.3. Characteristics of plants used in phytoremediation technologies

Successful phytoremediation require plants that are capable of producing high biomass, while accumulating large amounts of heavy metals. Vegetables of the family *Amaranthaceae* have been found to absorb Pb from the soil .

The above mentioned facts contributed to the decision to conduct studies, the aim of which was to determine whether the investigated species of ornamental plants, i.e. *Amaranthus caudatus L*. and *Amaranthus paniculatus L*.

Amaranthus caudatus (Fig. 2.3.), is a popular edible plant with high nutritional value.

This plant was chosen because it was widely cultivated all year round and can attain maturity within a number of days.

Fig. 2.3. I *Amaranthus caudatus L*.

Fig. 2.4. *Amaranthus paniculatus L*.

Amaranthus paniculatus L. (cruentus) (Fig. 2.4.) is a flowering plant species that yields the nutritious staple amaranth grain. It is one of three Amaranthus species cultivated as grain source, the other two being *Amaranthus hypochondriacus* and *Amaranthus caudatus*. In Mexico, it is called huautli and in English it has several common names, including blood amaranth, red amaranth, purple amaranth,[1] prince's feather, and Mexican grain amaranth.

The intensively developing civilization, next to numerous benefi ts, is also connected with contamination of the environment with heavy metals. The presence of these metals in polluted soil results in their uptake by plants, animal organisms and humans, causing many adverse effects. Soil contaminated with heavy metals becomes a source contaminating in turn all elements of the food chain. Thus for many years effective but cheap methods of purifi cation of soil contaminated with heavy metals have been searched for.

2.4. Conclusions to Chapter 2

An increasing number of studies is being conducted worldwide on the dynamically developing field of bioremediation, such as phytoremediation, in which living organisms, e.g. biopreparations from autochthonous bacteria and fungi and plants [12], are used to purify the contaminated environment, including also soil.
Plants accumulating very high concentrations of heavy metals in their organs, capable of growing under extremely adverse conditions, toxic for other species, are referred to as hyperaccumulators .

Many species of plants considered to be hyperaccumulators are known worldwide, while in the Ukraine climate there are few tested species potentially suitable for phytoremediation. Successful phytoremediation require plants that are capable of producing high biomass, while accumulating large amounts of heavy metals. Vegetables of the family *Amaranthaceae* have been found to absorb Pb from the soil .

The above mentioned facts contributed to the decision to conduct studies, the aim of which was to determine whether the investigated species of ornamental plants, i.e.

Amaranthus caudatus L. and *Amaranthus paniculatus L*.

CHAPTER 3

EVALUATION OF ALLELOPATHIC ACTIVITY OF PLANTS WHEN USED IN PHYTOREMEDIATION TECHNOLOGIES

3.1. Consideration of allelopathic interactions between plants in the development of effective phytoremediation technologies

For the first time, allelopathic interactions were indicated and described as a secretion of phytotoxic compounds by plants, algae and microorganisms (e.g. actinomycetes, fungi etc.) in works of Hans Molisch in 1937 [17]. Further researches led to the following directions: modes of allelopathic compounds influence on plants development; identification of allelochemicals released by plants; modeling of allelopathic compounds influence on the biochemical and physiological processes of an organism-target; allelopathic interactions on ecosystem levels; allelopathy using in the biotechnology, agriculture and other industrial sectors.

The process of allelopathic compounds discharging into the environment, then the chemistry and interactions, and finally their characteristics and biological functions were analyzed in a great deal of scientific publications [6, 7, 9, 11]. The majority of the allelopathic chemicals is represented by hydrophilic compounds, among which are: phenolics, terpenoids, alkaloids, flavonoids and glycosides etc. [6, 7, 8, 10, 14, 15].

The mechanism of allelochemicals releasing bythe exudation of radicals, the factors affecting thisprocess and the exudates impact on nutrients andsoil microflora were firstly reviewed in the work of Rovira and then in the further publications of Inderjit, Chou, Blum [12, 15, 18, 19]. Chou described allelopathic chemicals and educed more than 20 phenolic compounds as the radical exudates and decomposed crop residues [15]. The significant role of allelopathy as chemical interactions between species in a process of adaptation and communities' organization was also investigated in a set ofscientific publications [15, 16].

With most of the scientists assuming his position, Einhellig proposed that in consequence of the diversity of allelopathic compounds, their manifestation of phytotoxicity may be caused by a common mechanism starting with cellular disruption rather than the specific modes of action [9]. The several possible modes of influence for allelochemicals were declared using the example of ferulic acid [10], phenolic compounds [9] terpenoids [8], alkaloids [6], and BOA [11, 14]. Thus, the subsequent actions after penetration ofallelochemicals as phytotoxic compounds into a plant-target are supposed to be different. In the scientific literature, frequently observed modifications are followings: changing of the membrane permeability; affecting of mitosis, elongation and creation of ultra structures of cells; suppressing of photosynthesis, protein biosynthesis, metabolism of organic acids and lipids, minerals and water uptake; inhibiting of enzymes activity and respiration process [4, 6, 9, 10].

The influence of allelochemicals (i.e. alkaloids) on DNA and RNA synthesis was established in the work of Wink and Latz-Bruning [6].

In reason of their influence on the biological and environment availability, on the chemical stability and toxicity of allelochemicals, the external factors may define the effectiveness of an allelochemical activity.

The main factors are the followings: composition and structure of the soil, temperature, photoperiod, and interactions with other resealed allelopathic compounds. It is presumed that the appearing of a lack of nutrients and a water limitation may also influence on the allelochemicals production and activity. In some cases, the activation and intensification of allelochemicals production by plant were observed; in others it was found an augmentation of the toxicity of these compounds [20].

Thus, the majority of the earlier researches in the sphere of allelopathy focused on the elucidation of allelopathic mechanisms. At present, there is a continuous scientific interest for the allelopathic effects application to promote sustainable agriculture and forestry, and to increase the efficiency of technologies based on plant species introduction. In the mentioned spheres, the research connects mainly with examination of interactions and effects in systems: weed plant–crop plant; crop plant–weed plant, and crop plant–crop plant [20, 21, 22, 23, 24].

Phytoremediation is a technology based on plants introduction into the contaminated environment, consequently the non–indigenous species might interact with the native plants. Mutual influences may reveal manifestations of the allopathic activity of both species. These influences can be noticed by the direct secretion of allelochemicals (in the case of "in situ"), or by the ongoing presence of allelochemicals in the excavated soil in a form of products of plants residues decay (in the case of "in vitro"). Thus, investigation of allelopathic activity of weed species, being the quite widespread on the industrial and urbanized contaminate territories, has become actual environmental issues.

From agricultural point of view, the allelopathic activity of weeds and its potentiality is under continuous scientists' attention, and the results of their investigation are represented in a set of publications [20, 21, 23, 24, 25].

Acroptilon repens (L.) DC. was amongd the first investigated weed species. This plant was defined to cause inhibition of other species (i.e. *Panicum miliaceum* L., Medicago *sativa* L., and *Echinochloa crus-galli* (L.) P.Beauv.) sharing the same habitat [26]. The allelopathic potential of *Pedicularis kansuensis* was also examined. The research were conducted to evaluate this species influence on seed germination and seedling growth of two native species of grasses *Poa pratensis* and *Elymus nutans* Griseb. [24].

The allelopathic effect of such weeds, as: *Melilotus indicus* (L.) All.*, Cynodon dactylon* (L.) Pers., *Chenopodium album* L.*, Convolvulus arvensis* L., *Coronopus didymus* (L.) Sm.*, Lathyrus aphaca* L.*, Vicia hirsuta* (L.) Gray*, Rumex acetosella* L. etc., on *Phalaris minor* Retz. Was investigated in work of Om and his co-authors. Such species as *C. album, M. indicus, C. arvensis* were considered as able to manifest strong inhibiting effects causing suppression of 100 % germination over control [21].

The allelochemicals which were extracted from leaves of *Lantana camara* Linn. and their effects on *Bidens pilosa* Linn. (i.e. chlorophyll and protein contents, and amount of carbohydrate) were examined in the publication of Sisodia and Siddiqui [25].

The allelopathic activity of *Croton bonplandianum* Baill. and its influence on seed germination and rate of seedlings development of both weed plants (*Vicia sativa* L.*, Melilotus albus* Medik.*,* and *Medicago hispida* Gaertn) and crop plants (i.e. *Brassica rapa* L.*, Brassica oleracea var. botrytis* L. and *Triticum aestivum* L.) was also reviewed [27].

Such ruderal weed species, as: *Amaranthus retroflexus* L.*, Cirsium arvense* (L.) Scop.*, Barbarea vulgaris* W.T.Aiton and *Artemisia absinthium* L. which are considered as quite widespread in soils of urbanized territory and were investigated in present research work.

3.2. Effects of mutual allelopathic activity of endogenous plants and phytoremediants

Experimental studies of the effect of water-soluble extracts from local plant species on the amaranth species that are supposed to be used for phytoremediation – *Amaranthus caudatus* and *Amaranthus paniculatus*, as well as biotest L. sativa s , conducted on the basis of the allelopathy department of the M.M. Grishko National Botanical Garden, showed the results presented in tables 3.1-3.3.

A comparative analysis of the germination test allows us to establish the fact that plants of the species *Barbarea vulgaris* had a significant effect on the development of seedlings. At the same time, strong inhibition of seed germination was observed in the promising phytoremedian *Amaranthus caudatus*.

As the studies showed, the number of developed seedlings under the influence of the corresponding extract decreased by 44% compared with the control. At the same time, the test object *Lactuca sativa* showed the result of 60% of germinated seedlings. Our studies showed that among all species *Amaranthus paniculatus* had the highest germination rate, which was 65% of the number of seeds used in the experiment (see Table 3.1.). This indicates a significant resistance of this species to the influence of the allelopathic effect of endogenous plant species.

Water-soluble extracts of *Artemisia absinthium* and *Cirsium arvense*, according to the studies and the results obtained, was more affected by *Amaranthus caudatus*, Karmin and *Lactuca sativa* . It should also be noted the inhibition of germination of 44% of plant seeds in the case of *Artemisia absinthium*. Plant extract *Cirsium arvense*, caused a 54% decrease in seed development rate. Summarizing the data presented in table 3. 1, we can conclude that promising phytoremediants of the species *Amaranthus paniculatus* and *Amaranthus caudatus* of the Helios cultivar were found as the least affected species.

Table 3.1

Germination rate of the investigated species

Type of plant	Amount of germinated seeds, [amount/%]			
	A. caudatus 'Karmin'	*A. caudatus 'Helios'*	*A. paniculatus 'Zhaivir'*	*L. sativa*
A. retroflexus	20/80	22/88	22/88	20/80
C. arvense	16/64	15/72	19/76	16/64
B. vulgaris	14/56	14/56	16/64	15/60
A. absinthium	14/56	16/64	18/72	14/56
Control	24/96	24/96	25/100	25/100

In our studies, it was shown that the obtained extract of *Artemisia absinthium* suppressed the seed germination of perspective phytoremediants by 28 and 36%, respectively, while the extract of *Cirsium arvense* suppressed 24% of the seeds of *Amaranthus caudatus* Helios and 28% of *Amaranthus paniculatus*. At the same time, *Amaranthus retroflexus* caused a relatively small suppression of seed germination: 12% *Amaranthus paniculatus* and 20% *Amaranthus caudatus* of the Helios 'cultivar. Moreover, *Amaranthus caudatus* cultivars 'Karmin' and *Lactuca sativa* were evaluated as undeveloped (see Table 3.1). It should be noted that a decrease in the length of roots and plumules can also be considered as a result of suppressing the activity of growth processes by the allelochemicals present in the prepared extracts. The obtained results of the measurement of roots are presented in table 3.2.

The next effect we obtained in experimental studies was that the extract of *Artemisia absinthium* caused a significant decrease in the length of the roots. Seedlings of *Amaranthus caudatus* cultivar ‘of the Karmin’ showed the largest decrease in the length of roots - 52% (2.3 ± 0.03 cm) compared with the length of the control sample (4.7 ± 0.03 cm). For other plant species studied, the inhibition of roots was in the range of 46–50% (the length of *Amaranthus caudatus* Helios roots was 2.3 ± 0.02 cm,

Amaranthus paniculatus - 2.7 ± 0.04 cm and L. sativa - 3.1 ± 0.01 cm).

Table 3.2

Inhibition influence of extracts on the radicals length of phytoremediation plants

Type of plant	Average value of the radicals length, [cm]			
	A. caudatus *‘Karmin’*	*A. caudatus* *‘Helios’*	*A. paniculatus* *‘Zhaivir’*	*L. sativa*
A. retroflexus	3.8 ±0.01	3.6 ±0.01	4.4 ±0.02	4.8 ±0.01
C. arvense	3.3 ±0.02	3.2 ±0.02	4.0 ±0.03	3.3 ± 0.03
B. vulgaris	2.5 ±0.03	2.5 ±0.04	3.6 ±0.04	3.9 ±0.04
A. absinthium	2.3 ±0.03	2.3 ±0.02	2.7 ±0.04	3.1 ±0.01
Control	4.7 ±0.03	4.5 ±0.02	5.1 ±0.01	5.9 ±0.02

The *Barbarea vulgaris* extract studied by us reduced the length of the roots by 47% for *Amaranthus caudatus* of the Karmin cultivar, which has a length of 2.5 ± 0.03 cm. The length of the root *Amaranthus caudatus* "Helios" was reduced by 45%, 2 cm shorter than the control sample (4.5 ± 0.02 cm). At the same time, it should be noted that the degree of inhibition of the roots *Amaranthus paniculatus* and L. sativa was respectively 30% (1.5 cm less compared to the control) and 35% (2 cm less).

Our studies showed that the most vulnerable to the action of *Cirsium arvense* was the plant biotester *Lactuca sativa* , in which the decrease in root length was 41% (3.3 ± 0.03 cm, length of the control sample 5.9 ± 0.02 cm) . *Cirsium arvense* caused a decrease in root development by 39% in *Amaranthus caudatus* of the Carmine variety (3.3 ± 0.02 cm, control sample 4.7 ± 0.03 cm) and 29% in *Amaranthus caudatus* of the Helios variety (3.2 ± 0.02 cm, control sample - 4.5 ± 0.02 cm). According to our data, *Amaranthus paniculatus* turned out to be the species with the greatest influence of *Cirsium arvense* extract (reduced by 22%).

In the case of *Amaranthus retroflexus*, the suppression of root length was the smallest, and the values were in the range of 14–20% (see Table 3.2).

The results of measuring the length of the plumulee obtained by us in experimental studies are presented in table 3.3.

Endogenous plant species *Amaranthus retroflexus* and *Artemisia absinthium* had a relatively weak effect on the development of all the studied plumulee species. A decrease in length was found, corresponding to 15-18% for the extract of *Amaranthus retroflexus* and 18-22% for *Artemisia absinthium*. The level of resistance, the smallest of those obtained in these experiments, to the effects of the studied extracts was demonstrated by the *Lactuca sativa* biotester. *Amaranthus retroflexus* caused a relatively small decrease in the length of plumules by 21%, and *Artemisia absinthium* reduced the upper part of plants by 30% compared to control samples.

It should be noted that both varieties of *Amaranthus caudatus* were the most vulnerable to the action of *Cirsium arvense* extract. In our studies, the length of the roots *Amaranthus caudatus* of the Karmin variety decreased by 46%, amounting to 2.3 ± 0.05 cm, while at the same time, the control sample was 4.1 ± 0.02 cm long. We can state that the average plumule length of the promising phytoremedian *Amaranthus caudatus* of the Karmin variety grown in the extract was 3.1 ± 0.03 cm, while the length of the plumules in the control replication reached 4.0 ± 0.01 cm. Thus , *Cirsium arvense* caused a reduction in the upper part of this species by 40%.

As for the inhibition effect of *Barbarea vulgaris*, it should be noted as a decrease in the length of the plumule *Amaranthus caudatus* of the 'Karmin' variety by 49% (the average length is 2.1 ± 0.03 cm), and L. sativa by 46% (t. E. The length of the plumule was 1.9 ± 0.03 cm).

Table 3.3

Inhibition influence of extracts on the plumules length of phytoremediation plants

Type of plant	Average value of the plumule length, [cm]			
	A. caudatus 'Karmin'	*A. audatus 'Helios'*	*A. paniculatus 'Zhaivir'*	*L. sativa*
A. retroflexus	3.4 ±0.01	3.3 ±0.02	3.6 ±0.02	2.8 ±0.01
C. arvense	2.3 ±0.05	2.4 ±0.04	3.1 ±0.03	2.8 ±0.01
B. vulgaris	2.1 ±0.03	2.5 ±0.02	2.3 ±0.02	1.9 ±0.03
A. absinthium	3.3 ±0.01	3.2 ±0.03	3.5 ±0.01	2.4 ±0.02
Control	4.1 ±0.02	4.0 ±0.01	4.2 ±0.01	3.5 ±0.02

B. vulgaris extract was specified as being able to suppress development of upper part of *Amaranthus caudatus* 'Helios' as well as *Amaranthus paniculatus*. Our experimental studies showed a decrease in the length of plumuleules in both types of promising phytoremediants by 39% (on average, the length was 1.5-1.9 cm less than in the control replicates).

Our review of scientific publications showed that some authors noted a decrease in the root and length of the plumulee as a result of secondary effects [8]. At the same time, morphological defects the structures of seedlings, primarily their roots, are considered

the main signs of changes caused by inhibitory compounds [8].
The results of our screening test regarding morphological changes obtained by us are presented in Figures 3.1-3.3.

Analyzing the data obtained, it should be noted that the extract of *Amaranthus retroflexus* had a negligible effect on the studied species. It showed root endings, withering in 4% of varieties of *Amaranthus caudatus*. At the same time, in the case of *Amaranthus paniculatus* and *Lactuca sativa*, we did not observe morphological damage. In our studies, it was shown that the *Cirsium arvense* extract caused suppression of both roots and plumules. On the one hand, we found that wilting of roots is a specific feature for 8% *Amaranthus caudatus* of the cultivar “Helios” and for 4% *Amaranthus paniculatus*. On the other hand, root twisting was observed in 4% *Amaranthus caudatus* of the cultivar “Karmin” and in 8% *Lactuca sativa*. It can be noted that the negative effect of the extract of *Cirsium arvense* also demonstrated the decay of plumules, which was characteristic of all species. Thus, we can conclude that *Amaranthus caudatus* was identified as the most vulnerable (see Fig. 3.1).

As for the *Barbarea vulgaris* extract studied by us, it mainly influenced the development of plumules, suppressing it and causing wilting of seedlings. This phenomenon appeared in 16% of plants of the species *Amaranthus caudatus* of the cultivar Karmin, *Amaranthus paniculatus* of the cultivar Zhaivir, as well as in L. sativa. Regarding the twisting of roots, it was observed in 12% of plants of the species *Amaranthus caudatus* cultivar 'Karmin', in 8% of the species *Amaranthus caudatus* cultivar 'Helios' and in 4% of the biotester *Lactuca sativa*, while the roots faded in 4% *Amaranthus caudatus* cultivar 'Helios' and for 8% L. sativa (see Fig. 3. 2).
The most vulnerable species were *Amaranthus caudatus* cultivars ‘Karmin’ and *Lactuca sativa*, and the total number of damaged plants in each replication studied by us was up to 28%.
Plant extract of the species *Artemisia absinthium* caused depression of the root system in the studied species of promising phytoremediants.

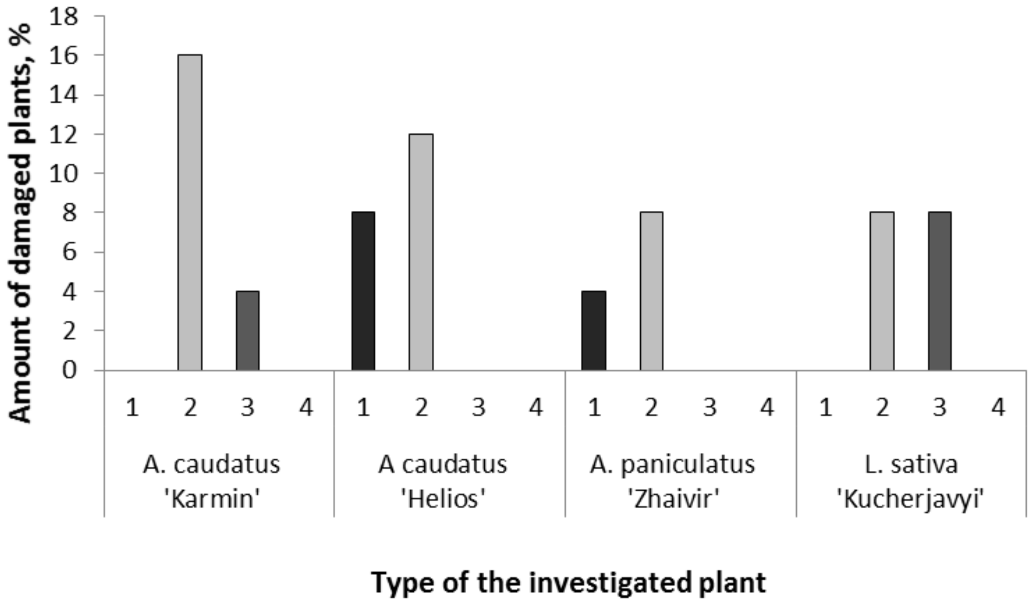

Fig. 3. 1. Morphological changes of seedlings structure grown in the water extract of C. arvense:1 – withering of radicals; 2 – withering of plumules; 3 – twisting of radicals; 4 – others

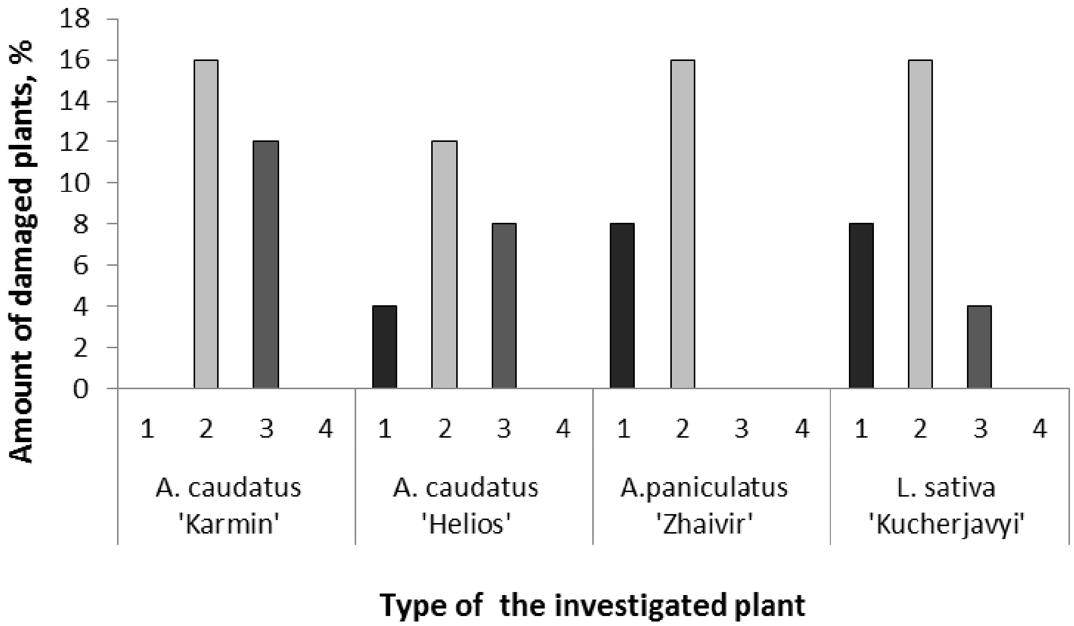

Fig. 3.2. Morphological changes of seedlings structure grown in the water extract of B. vulgaris:1 – withering of radicals; 2 – withering of plumules; 3 – twisting of radicals; 4 – others

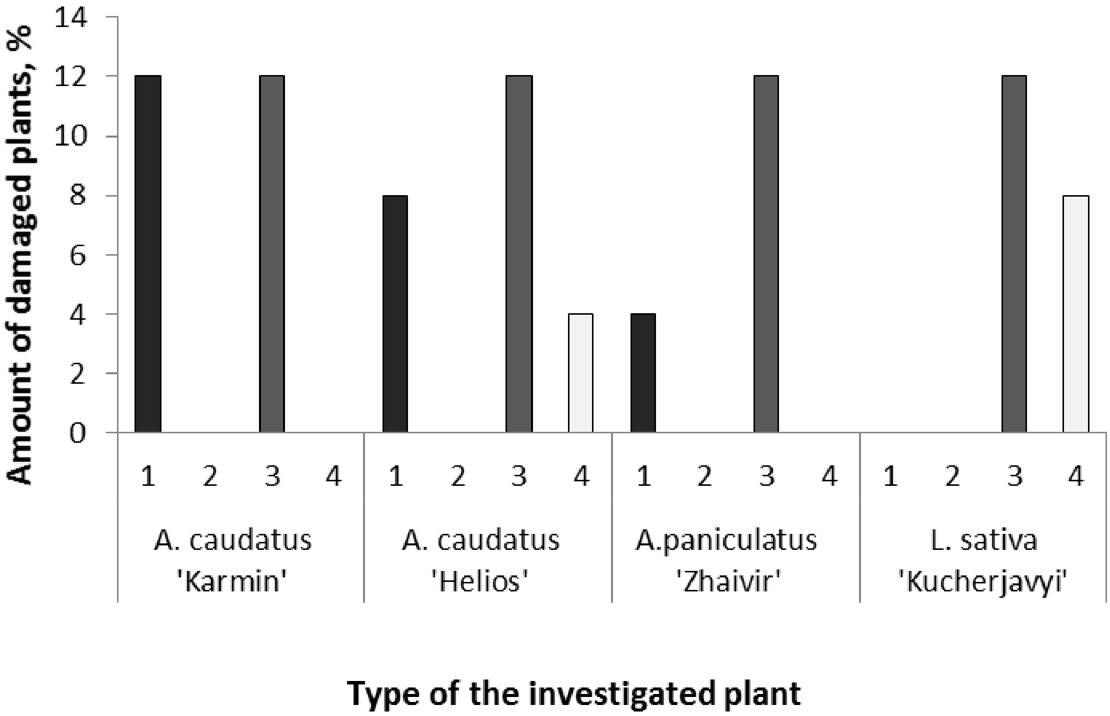

Fig. 3. 3. Morphological changes of seedlings structure grown in the water extract of A. absinthium:1 – withering of radicals;2 – withering of plumules;3 – twisting of radicals;4 – others

In our experimental studies, it was shown that plants less resistant to the extract were obtained from both varieties of *Amaranthus caudatus* (24% of damaged seedlings), while *Amaranthus paniculatus* was defined as less vulnerable (up to 16%). It should be noted that the number of seedlings with swirling roots in each case was 12% (see Fig. 3.3).

The specific nature of the root decay was determined for 12% *Amaranthus caudatus* “Karmin”, for 8% *Amaranthus caudatus* “Helios”, and for 4% *Amaranthus paniculatus*. While seedlings of *Lactuca sativa* (8%) and *Amaranthus caudatus* "Helios" (4%) were found with undeveloped plumules (see figure 3. 3).

We found that the number of damaged seedlings in each investigated case of morphological modification did not exceed 16% of the initial number of seeds.

At the same time, no morphological changes were observed in the control samples. The results of our experimental data showed that extracts of endogenous plants of the species *Cirsium arvense* and *Barbarea vulgaris*. inhibited the highest growth of the L. sativa biotester. In some cases, the length of the plumules was reduced by 49% (for the species *Amaranthus caudatus* cultivar 'Karmin'), comparing with the length of the control sample.

As for extracts of the species *Artemisia absinthium* and *Barbarea vulgaris*:, they can also cause a significant suppression of the development of the root system. At the same time, we observed a maximum decrease in roots in *Amaranthus caudatus* species of the Karmin variety. It was 52% in the case of extract of *Artemisia absinthium* and 47% in the case of *Barbarea vulgaris*.

According to the analysis of scientific literature, it can be noted that *Amaranthus retroflexus* was believed to have a negligible effect on the development of the species, and the level of suppression was in the range of 15-21%.

According to the results of our germination test, in general *Amaranthus paniculatus* was identified as the least vulnerable species to the negative influence of all the studied extracts. But in the case of the influence of *Barbarea vulgaris* extracts, the degree of depression of this promising plant for phytoremediation was the highest and amounted to 36%. At the same time, the least amount of undeveloped seeds was observed in the case of the influence of extracts of *Amaranthus retroflexus* (12%).

3.3. Phytoremediation strategy using hyperaccumulative plants

Phytoremediation is a potential remediation strategy that can be used to decontaminate soils contaminated with inorganic pollutants. Research related to this relatively new technology needs to be promoted and emphasized and expanded in developing countries since it is low cost. In situ, solar driven technology makes use of vascular plants to accumulate and translocate metals from roots to shoots. Harvesting the plant shoots can permanently remove these contaminants from the soil.

Phytoremediation does not have the destructive impact on soil fertility and structure that some more vigorous conventional technologies have such as acid extraction and soil washing. This technology can be applied “in situ” to remediate shallow soil, ground water and surface water bodies. Also, phytoremediation has been perceived to be a more environmentally-friendly “green” and lowtech alternative to more active and intrusive remedial methods. The broader importance of protecting soils and improved management for the services they provide are currently receiving considerable attention from policy-makers. Soils provide fundamental ecosystem services, with extensive economic, ecological, and sociological influences on the wellbeing of the human society. Metal-contaminated soils provide a significant but previously neglected component of the global soil resource. There is much scope to optimize the utilization of this resource for improved services.

Phytoremediation does have real applications, but it is vital that it emerges as a realistic technology and in the right context. It has been tested successfully in many places around the world for many different contaminants.

Bioavailability of metals is the primary factor responsible for the uptake of metals. In soils, metals exist as a variety of chemical forms in a dynamic equilibrium governed by the physical, chemical and biological processes of the soil. Bioavailability of soil pollutants, a primary basis of remediation efficacy, refers to a fraction of the total pollutant mass in the soil and sediment available to plants. Uptake of metals by plants involves root interception of metal ions, entry of metal ions into roots and their translocation to the shoot through mass flow and diffusion. Plants have evolved highly specific mechanisms to take up, translocate, and store these nutrients. For example, metal movement across biological membranes is mediated by proteins with transport functions. In addition, sensitive mechanisms maintain intracellular concentration of metal ions within the physiological range. In general, the uptake mechanism is selective and plants preferentially acquired some ions over others. Ion uptake selectivity depends upon the structure and properties of membrane transporters. These characteristics allow transporters to recognize, bind and mediate the trans-membrane transport of specific ions.

Two basic strategies of metal phytoextraction have been suggested, continuous or natural phytoextraction and induced, enhanced, or chemically assisted phytoextraction

(Salt et al., 1998). After the plants have been allowed to grow for some time, they were harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of ash will be less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment. In some cases, it is possible to recycle the metals through a process known as phytomining, though;

this is usually reserved for use with precious metals. Metals such as Ni, Zn, and Cu are the best candidates for removal by phytoextraction because the majority of the approximately 400 known plants that absorb unusually large amounts of metals have a high affinity for accumulating these metals. Plants that absorb Pb and Cr are currently being studied and tested. According to report, in the presence of vegetation, the exchangeable form of Cd was partly removed by plant uptake that accompanied with the intake of nutrition (

Hyperaccumulator plants do not only accumulate high levels of essential micronutrients, but can also absorb significant amounts of non-essential metals such as Cd. The mechanism of Cd accumulation has not been elucidated. It is possible that the uptake of this metal in roots is through a system involved in the transport of another essential divalent micronutrient, possibly Zn2+. Cd is a chemical analogue of the latter, and plants may not be able to differentiate between the two ions.

Once the metal is bioavailable to the plant, the entry of metal ions inside the plant, either through symplast (intercellular) or apoplast (extracellular), depends on the type of metal and the plant species. The apoplast continuum of root epidermis and cortex is readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without crossing the membrane.

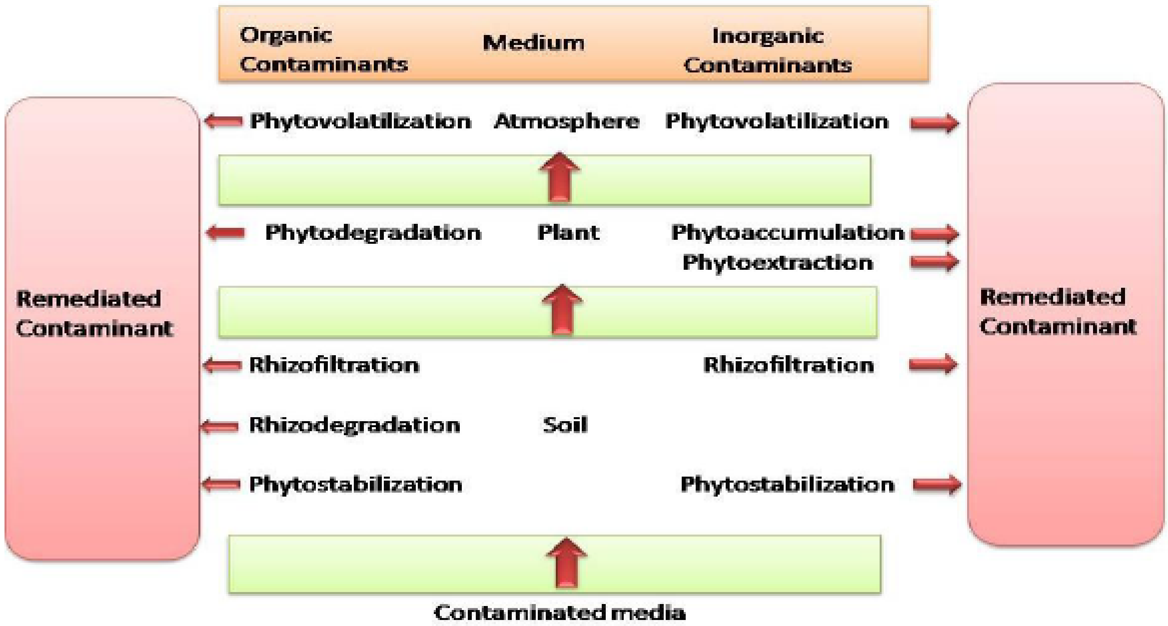

Fig. 3.4. Phytoremediation technology strategy

The cell walls of the endodermal layer act as a barrier for apoplastic diffusion into the vascular system. Apoplastic transport is limited by high cation exchange capacity (CEC) of the cell wall. In the symplastic transport, metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of the metal ions. Most metal ions enter plant cells by an energy-dependent process through specific or generic metal-ion carriers or channels. On entry into the roots, metal ions can either be stored in the root or forwarded to the shoot, primarily, through the xylem. The rate of metal translocation to the shoot may depend on metal concentration in the root. A phytochelatin (PC)-mediated metal binding in the xylem sap as a possible mechanism for metal translocation has been proposed.

Nutrients destined for the developing cereal grain encounter several restricting barriers on their path towards their final storage sites in the grain. In order to identify transporters and chelating agents that may be involved in transport and deposition of Zn in the barley grain, expression profiles have been generated of four different tissue types; the transfer cells, the aleurone layer, the endosperm, and the embryo (Tauris et al

., 2009). Low molecular weight chelators such as citrate and free histidine as in Alyssum lesbiacum were associated with this process. Other chelating compounds like malate, citrate, and histidine may also have a role in the metal-ion-mobility in plants. Membrane transport systems are likely to play a central role in the translocation process. For cleaning and curing of the polluted sites, plants utilize several methods.

3.4. Conclusions to chapter 3

Analyzing the obtained results, it is possible to assume *B. vulgaris* and *A. absinthium* had the strongest negative influence on germination process due to the higher level of inhibition – up to 44 % of undeveloped seeds in one series.

The observed defects of species structure may be considered to be appeared as a result of the negative action of allelochemicals presented in the prepared extracts. The processed results led to defining major tendencies. Firstly, the extract of *A. retroflexus* had the lower suppressing influence on the investigated species. While the effect of *C. arvense* revealed in inhibition of both radicals and plumules development. The extract of *B. vulgaris* mostly influenced on plumules by reducing their development (up to 16 % of affected seedlings in each replication).

A. absinthium suppressed radicals growth by twisting 12 % of all spouts, and additionally, the radicals withering was observed for all cultivars of *Amaranthus*.

By summarizing the results of this investigation concerning morphological changes and suppression rate, it is possible to appreciate *A. caudatus* as the most vulnerable, and *A. paniculatus* as the most influence-resistant plants.

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CHAPTER 4
LABOUR PRECAUTION

4.1. Hazardous and harmful production factors when working in the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine

In accordance with the Labor Law "On Occupational Safety" [1], STATE SANITARY STANDARDS AND RULES "Hygienic classification of labor according to indicators of harmfulness and danger of factors of the production environment, severity and intensity of the work process" [2], Regulations on the Development of Guidelines for Labor Precaution approved by the Order of Labor Precaution Supervisory Committee of Ministry of Labor and Social Policy of Ukraine as of January 29, 1998, No. 9, dangerous and harmful factors in their action are divided into the following groups: physical, chemical, biological, psychophysical.

The main physical factors that affect a lab worker at the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine include:

- increased or lowered temperature, humidity and air mobility, which according to “Sanitary standards of the microclimate of industrial premises” [3] are the main parameters of the microclimate of the working room;
- insufficient or excessive illumination of the work area;
- chemical substances.

It should also be noted that glass laboratory glassware has a number of disadvantages in terms of safety, including fragility and low resistance to sharp temperature fluctuations. The majority of accidents in the breach of the rules for the handling of glass belong to the category of micro-injuries (after which you can continue to work) and minor injuries (loss of working capacity for one or more days). First of all, these are cuts of the hands when breaking the glassware, parts, appliances, etc., as well as the burns of the hands, with careless handling of the heated to high temperature glass parts. Particularly dangerous cuts are made to the fragments of the cookware contaminated with chemicals, since in such cases toxic substances can enter the bloodstream directly.

In the case of gross violations of the rules of work with the glass, serious injuries to the hand brush, including damage to the tendons, associated with a longer disability and related to injuries of moderate severity, are possible.

Severe injuries (requiring long-term treatment) and injuries leading to disability can be caused by the glass breaking into the eyes. The danger of this type of injury arises in the absence of personal protective equipment (goggles, mask) and other protective equipment (protective screens) in the process of working with vacuum devices and in all cases where the tearing of the glass equipment is possible.

In addition to injuries during the breakage of glass apparatus and utensils, other types of accidents and accidents are also possible - fires, explosions (in the discharge of combustible liquids, oxidizing agents, etc.), poisoning and burns (when toxic or caustic substances are released into the atmosphere or on the skin).

Room climate is the main factor that determines working conditions. The main parameters of meteorological conditions - temperature, humidity, air velocity affect the heat transfer and the general condition of the human body.

The sources of high temperature in the laboratory are a drying cabinet, a radiator, as well as a large number of computers. The heat from all these sources causes a slight increase in indoor air temperature.

Considering the mechanisms of influence of meteorological factors of the production environment (temperature, humidity, air velocity) on humans, we mean that the human body seeks to maintain the relative dynamic stability of its functions under various meteorological conditions. This constancy provides first of all one of the most important physiological mechanisms - the mechanism of thermoregulation. It is observed at a certain ratio of thermal formation (chemical thermoregulation) and heat transfer (physical thermoregulation).

It is known that excess humidity has a negative effect on the mechanism of thermoregulation of the body. Particularly harmful is the humidity of the air, which exceeds 70-75% at temperatures of 30 ° C and more. The upper limit of a person's thermal equilibrium at rest is an air temperature of 30-31 ° C at a relative humidity of 85% or 40 ° C at a relative humidity of 30%.

According to the results of research, a person is able-bodied and normally feels comfortable if the ambient temperature does not go beyond 18–20 ° C, relative humidity - 40 - 60%, air velocity – 0.1– 0.2 m / s [3,4], (table 4.1).

High temperature weakens the body, causes sluggishness, and low - inhibits movement, which when working with the equipment of the laboratory causes an increased risk of injury. Due to heat and humidity, body overheating, even heat stroke, can occur.

Table 4.1

Optimal and permissible microclimate parameters values for category Ib premises

Season	**Microclimate parameters**	**Optimal value**	**Permissible value**	**Actual value**
Cold	Room temperature	21-23 ^oC	20-24 ^oC	15-22 ^oC
	Relative humidity	60-40 %	Up to 75 %	55%
	Room's air velocity	0.1 m/s	<0.1 m/s	–
Warm	Room temperature	22-24 ^oC	21-28 ^oC	22-27 ^oC
	Relative humidity	60-40 %	60 if t=27 ^oC	60%
	Room's air velocity	0.2 m/s	0.1-0.3 m/s	–

About 90% of all information received by a person is from the organs of vision. Therefore, one of the main conditions of industrial sanitation is the factor that determines the favorable working conditions - the rational illumination of the work area [5,6]. The organs of vision are adversely affected by both insufficient and excessive illumination. In poor light, which is often the case indoors, the eyes of the worker are very tense, thus deteriorating vision. Excessive illumination results in blinding, which is characterized by sharp eye irritation. Visual perception is worsening.

Insufficient and irrational illumination leads to eye fatigue, central nervous system disorders, decreased mental and physical performance, and in some cases may be the cause of injury (about 5% of injuries account for the proportion of irrational and insufficient illumination).

With regard to chemical factors, highly toxic and carcinogenic chemical compounds are not used in laboratory studies of soil quality. According to the mode of action, the chemicals used in the experiments can be attributed to the irritants, and their path of entry into the human body occurs mainly through the skin and mucous membranes.

No biologically hazardous agents are used in the laboratory, but the soil under study may be biologically contaminated, a factor that must also be taken into account during the experiments [7].

Psychophysiologically dangerous production factors that affect a lab worker are manifested through mental overload, monotonous work, and overvoltage of the visual analyzer, as a result of prolonged computer work or a spectrophotometer [5].

4.2. Technical and organizational measures for reducing the level of influence of hazardous and harmful production factors when working in the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine

Safety precautions when handling glassware and appliances having glass components in construction. In the laboratory, chemical analysis and glassware are widely used in analyzes. To avoid the destruction of glassware, it is first checked with a polariscope.

When assembling glass equipment, rubber plugs and tubes are selected by the size of the glass, and the hands are protected by a towel or cloth to avoid cuts when the devices are destroyed. It is not recommended to close heated glass vessels with ground stoppers until cooled.

Wash chemical dishes in rooms that have sinks, sinks and equipment for storing and drying them. Do not allow to throw or drain concentrated acids and alkalis, chromium, odorless substances and other reagents into the sink. They merge into special containers for further disposal and to eliminate the risk of burns.

When disassembling the equipment, care must be taken when touching hot glassware and heaters. Hot flasks are placed on sheet asbestos.

Utensils that contain strong acids, alkalis, or other toxic substances are released and neutralized, and only then can they be washed.

Laboratory microclimate analysis is carried out on the basis of "Sanitary standards of the microclimate of industrial premises", which establish such microclimate parameters as temperature, humidity and air mobility depending on the type of work and the period of the year [4].

Work carried out in the l Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine can be classified as Category 1a because it is performed sitting and requires no physical effort. Energy consumption of the human body in this type of work is up to 120 kcal / h.

The source of the thermal radiation is a central heating radiator consisting of seven sections, a drying cabinet and a computer.

Rational lighting must meet a number of requirements:

- Indoor natural lighting must be provided in the form of side illumination. When performing the work of high accuracy, the illumination coefficient must be at least 1.5%, while the average accuracy of visual work is at least 1.0%;
- artificial lighting in the premises of the laboratory should be carried out in the form of a combined lighting system using fluorescent light sources in general lighting;
- the value of illumination in artificial lighting with fluorescent lamps must be in the horizontal plane not less than 300 lux - for the system of general lighting;
- emergency lighting must be provided in the laboratory premises for the continuation of work and other purposes;
- the light sources in relation to the workplace should be positioned in such a way as to prevent direct light from entering the eyes;
- the fluctuation of the illumination of the fluorescent lamps used shall not exceed 10%. In natural light, it follows between natural light and screen glow. Means can be

used - films with metallic coating or adjustable blinds.

The laboratory uses a general uniform lighting system. As a light source, low-pressure fluorescent lamps LB 40 in the amount of 20 pieces, placed in ten lamps, are arranged on the ceiling in two rows.

Each workplace is also equipped with a local lighting source to perform possible work related to high precision operations.

Chemical substances. A chemical is a substance that, when contacted with a human body, in the event of a breach of safety requirements, may cause occupational injuries, occupational diseases or abnormalities in health.

Chemicals can enter the body in three ways: through the respiratory system in the form of vapors and gases; through the digestive system most often from the surface of contaminated hands; through the skin and mucous membranes.

In order to protect against the harmful effects of chemicals, it is necessary to observe safety rules in chemical laboratories. Effective ventilation, which is equipped by all chemical laboratories, is an effective protection of humans from harmful impurities and substances in the air.

Personal protective equipment is used as an additional preventive measure.

To avoid or reduce the harmful effects of chemicals on the body of a researcher, the following safety measures should be clearly followed:

1) conduct work briefing at the workplace with the contractors before commencing work, and, in the case of particularly difficult work with high risk, first prepare an appropriate outfit;

2) observe the complete tightness of the systems;

3) to carry out systematic supervision of the operation of ventilation systems;

4) work should be carried out only in special clothing and special footwear with obligatory use of personal protective equipment (for protection against acids use work clothes made of acid-resistant fabrics such as SHV-30 and SVH-1, from alkalis - cotton, linen fabrics and rubber gloves, and for eye protection - special glasses, etc.);

5) Provide all workplaces with the required amount of water and neutralizing agents.

Overalls, work shoes and personal protective equipment must fully protect the person from the harmful effects of toxic substances.

4.3. Providing of fire and explosion safety at work in the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine

General requirements for fire and explosion safety systems are regulated in accordance with State Standard ДСТУ Б В.1.1-36:2016 «Definition of Category of Premices, Buildings and External Facilities According to Explosion and Fire Hazard»[8].

Considered premises can be classified in category "B" on explosion - fire hazard, the work area of the room according to PUE belongs to the class P-IIa on fire danger, ie it is a room in which are solid and fibrous combustible substances (door frames, doors, furniture, etc.).

There are devices (spectrometers, thermostats, autoclaves, etc.) in the laboratory, so a fire can result in large material losses. Consequently, work to create conditions in which the likelihood of a fire is reduced is essential.

Possible causes of fire may be:

- short circuit wiring;
- smoking in unauthorized places, use of household electric heating devices;
- self-ignition of computers.

When working in the laboratory, the possibility of evacuating people in the event of a fire is limited. Based on this, the calculation is based on the assumption that it is impossible to evacuate people quickly (1 - 2 minutes). In this approach, the object considered to be a fire hazard is the probability of a fire in which Op <10 - 6.

In this regard, the following measures should be envisaged:

- careful isolation of all conductive conductors to workplaces;
- periodic inspection and verification of insulation;
- strict compliance with fire safety standards in the workplace;

- the calculation of the probability of self-ignition of computers.

Thus, when carrying out preventive work every 5000 hours, which is approximately equal to 7 months of operation (checking the parameters of the protection elements, the quality of the connecting cords, etc.), the system is fireproof.

Organizational and technological measures (smoking ban, briefing) are conducted.

In the event of a fire, it is possible to safely evacuate people through escape routes. There is an evacuation plan in the room. The minimum evacuation time and the maximum distance of workplaces from evacuation exits is in accordance with DBN B.1.1-7: 2016. The required number of evacuation exits, the width of passageways and the degree of fire resistance of the building also meets the requirements of [9,10]. The premises of the laboratory are:

- OUB-3 fire extinguisher - 1 pc .;
- OP-1 "Moment" fire extinguisher - 1pc.

The number of fire extinguishers complies with the requirements of ISO3941-87, which provides for the mandatory presence of two fire extinguishers per 100 m2 of floor space for rooms.

According to the fire resistance of the laboratory premises refers to the II degree of fire resistance, mechanical structures in the room, the walls are made of non-combustible materials [9]. Workplaces to perform work in a sitting position, organized in accordance with DBN B.1.1-7: 2016. The height of the desk is chosen equal to 0.8 m.

In the event of a fire on the staircase on the premises installed fire shield, equipped with fire equipment and fire extinguisher brand OU-5 in accordance with the requirements of IS0 3941 77 (fire extinguisher carbon dioxide, manual) for extinguishing fires of various materials and installations under chemical conditions. 10 extinguisher for extinguishing solid materials. According to DBN B.1.1-7: 2016 «Fire safety of construction objects. General requirements" 12.4.009-83 [9] the fire shield includes:

- asbestos;
- sandbox;
- fire equipment.

In addition, the stairwells have a water supply system with internal fire cocks. An internal telephone is used to communicate with the fire department. The workplace fulfills all fire safety requirements in accordance with the requirements of CODE OF CIVIL DEFENSE OF UKRAINE (para.33) [Electronic resource] / Verkhovna Rada (BB) information [10].

4.4. Conclusions to chapter 4

This section of the thesis defines harmful and dangerous factors when working in the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine.

The definition of occupational safety as a system of normative documents, socio-economic, organizational-technical, sanitary-hygienic and medical-preventive measures and methods aimed at preserving the health and efficiency of people in the process of work is given. The purpose of park protection is to ensure safe and harmless working conditions for each worker.

It is determined that the legislative framework in the field of labor protection is represented by normative documents, which provide for the formation of a system of measures, which, in turn, are aimed at ensuring safe working conditions. One of the main documents is the Law of Ukraine “On Occupational Safety”, the Law of Ukraine “On Health Care”, the Law of Ukraine “On Fire Safety”, etc.

Particular attention is paid to fire and explosion safety measures.

In the Allelopathy laboratory of the National Botanical Garden named after MM Grishko of the National Academy of Sciences of Ukraine we must conduct to the following rules:

not to come into contact with hazardous and harmful production factors;

to observe technical and organizational measures to reduce the level of exposure to hazardous and harmful production factors when working in the laboratory;

observe fire and explosion safety when working in a laboratory.

Therefore, in order to prevent dangerous situations when working in a laboratory, it is first of all to provide all premises with competent technical maintenance and means of fire extinguishing in accordance with established norms.

CONCLUSIONS

1. To develop the efficient and successful strategy of such decontamination technology as phytoremediation, it iscrucial to understand a mechanism by which the indigenous species may affect the introduced phytoremediation plants and to define a possible rate of plants suppression and inhibition. In particular, our research has been focused on knowing the allelopathic effects of indigenous species (i.e. *C. arvense, A. absinthium, A. retroflexus,* and *B. vulgaris*) over introduced phytoremediation plants (i.e. *A. paniculatus and A. caudatus).*

2. Analyzing the obtained results, it is possible to assume *B. vulgaris* and *A. absinthium* had the strongest negative influence on germination process due to the higher level of inhibition – up to 44 % of undeveloped seeds in one series.

3. The observed defects of species structure may be considered to be appeared as a result of the negative action of allelochemicals presented in the prepared extracts. The processed results led to defining major tendencies. Firstly, the extract of *A. retroflexus* had the lower suppressing influence on the investigated species. While the effect of *C. arvense* revealed in inhibition of both radicals and plumules development. The extract of *B. vulgaris* mostly influenced on plumules by reducing their development (up to 16 % of affected seedlings in each replication).

4. *A. absinthium* suppressed radicals growth by twisting 12 % of all spouts, and additionally, the radicals withering was observed for all cultivars of *Amaranthus*.

5. By summarizing the results of this investigation concerning morphological changes and suppression rate, it is possible to appreciate A. caudatus as the most vulnerable, and A. paniculatus as the most influence-resistant plants.

The identified features should be taken into account when developing effective soil phytoremediation technology with the use of these plant species as phytoremediants.

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