National University of Science and Technology POLITEHNICA BUCHAREST
Faculty of Biotechnical Systems Engineering

Doctoral School of Biotechnical Systems Engineering

Field of Environmental Engineering

DOCTORAL THESIS

PHYTOREMEDIATION APPLIED IN WATER TREATMENT

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CONTENS

PERSPECTIVE

WORD BEFORE

The PhD thesis entitled "Phytoremediation applied in water treatment" aims at biosorption of wastewater pollutants using the plant Typha angustifolia and the algae Sargassum fusiforme and Enteromorpha prolifera.

The PhD thesis is structured in 6 chapters, developed in 145 pages, contains 85 figures and graphs, 19 tables and a bibliography of 343 references.

The PhD thesis presents a synthesis of the theoretical and experimental research carried out by the author with the application of plants and algae in the field of industrial water treatment.

In chapter I of the PhD thesis entitled "Objectives of the PhD thesis. Importance of the theme" are presented the proposed objectives and also achieved based on the experimental research and the importance of the theme chosen to develop it in the present PhD thesis during the doctoral training.

Chapter II of the PhD thesis entitled "Literature review on heavy metal water treatment" consists of 5 sub-chapters and presents the current state of scientific research on heavy metal water treatment. This chapter is based on a comprehensive literature review on methods for the removal of heavy metal ions from wastewater and the application of phytoremediation in water treatment. These sub-chapters bring into focus the crucial environmental issue of heavy metal polluted waters, the types of heavy metals present in wastewater, their sources, conventional methods such as adsorption, chemical, electrical and photocatalytic treatments used in heavy metal removal, and the effects that heavy metals have on the human body and the environment.

Chapter III of the PhD thesis entitled "Water purification by phytoremediation" includes phytoremediation techniques consisting of phytoextraction (or phytoaccumulation), phytofiltration, phytostabilisation, phytovolatilisation and phytodegradation.

Chapter IV entitled "Experimental research methodology" details how the experimental research presented in the PhD thesis was carried out.

In Chapter V, "Experimental Research", of the PhD thesis we have included the experimental research carried out during the doctoral internship. The results of the researches for the biosorption of Cu2+, Pb2+, Ni2+ and Mn2+ ions from wastewater by testing the plant Typha angustifolia and the algae Sargassum fusiforme and Enteromorfa prolifera are detailed.

Chapter VI presents the own contributions made in the PhD thesis and its general conclusions, by applying phytoremediation for water purification purposes, as well as future perspectives, and is entitled: "Original contributions from the PhD thesis. Final conclusions. Perspectives".

The list of published articles, the conferences I attended and the references consulted for the construction of the PhD thesis are presented at the end.

The experimental data from the research were used for the publication of 4 articles in national and international journals. The most important of these articles was published in the journal Biology with an impact factor of 5.168.

CHAPTER 1. OBJECTIVES OF THE DOCTORAL THESIS.

1.1. IMPORTANCE OF THE TOPIC.

1.2. Objectives of the PhD thesis

Scince the removal of heavy metals from wastewater by conventional methods is carried out with low treatment efficiency leading to environmental pollution, the research direction chosen for the PhD thesis was to find an effective unconventional alternative, phytoremediation, using plants and algae.

In support of this aim of the PhD thesis the following objectives were achieved:
I) Study the ability of plants (*Typha angustifolia*) to remove heavy metal ions (Cu, Pb, Ni, Mn) from wastewater;
II) To study the capacity of algae (*Sargassum fusiforme* and *Enteromorpha prolifera*) to remove heavy metal ions (Cu, Pb, Ni, Mn) from wastewater;

The main objective of the PhD thesis is water treatment by phytoremediation process using the plant Typha angustifolia and the algae *Sargassum fusiforme*, *Enteromorpha prolifera*. The aim is to reduce the level of pollution in water and improve water quality in an environmentally friendly and sustainable way**.**

1.3. Importance of the theme

The industrial sources from which the wastewater originates release many pollutants. The industries from which wastewater is discharged into rivers or lakes are: paint industry (Fig. 1.1. (a)), fertiliser industry (Fig. 1.1. (b)), steel industry (Fig. 1.1. (c)), mining industry (Fig. 1.1. (d)), textile industry (Fig. 1.1. (e)), leather industry (Fig. 1.1. (f)) etc.

If ingested, heavy metals have harmful effects on human health and cause diseases such as Wilson's disease - copper poisoning (Figure 1.2. (a)), Burton line - lead poisoning (Figure 1.2. (b)), various allergies - nickel poisoning (Figure 1.2. (c)), chromium poisoning (Figure 1.2. (d)), neurodegenerative disorder - manganese poisoning (Figure 1.2. (e)), pink disease/Acrodynia - mercury poisoning (Figure 1.2. (f)), keratosis - arsenic poisoning (Figure 1.2. (g)), Itai-itai disease - cadmium poisoning (Figure 1.2. (h)).

Fig. 1.1. Wastewater sources from different industries: (a) paint industry, (b) fertilizer industry, (c) steel industry, (d) mining, (e) textile industry, (f) leather industry [1].

Fig. 1.2. Human health effects of heavy metal ingestion: (a) Wilson's disease, (b) Burton's line, (c) and (d) various allergies, (e) neurodegenerative disorder, (f) pink disease/Acrodynia, (g) keratosis, (h) Itai-itai disease [1].

Water treatment is very important because human health and the environment must be protected. Water treatment refers to the process of removing pollutants from wastewater to prevent pollution of the environment, including human health. In the context of the circular economy, water treatment has a positive influence on the following areas: human health, environmental protection, water supply, economic protection, regulatory compliance. The use of plants for water treatment is a natural and effective method. This process is known as phytoremediation and involves using plants to retain and metabolise toxic and polluting substances in wastewater. Some reasons why the use of plants for water treatment is important are: natural and environmentally friendly method, cost reduction, removal of pollutants from water.

CHAPTER 2. LITERATURE REVIEW ON HEAVY METAL WATER TREATMENT

2.1. Introduction

Water polluted with heavy metals is a major environmental problem in the world. Whether these heavy metals are found in rivers, streams, ponds or ditches, they affect human health. Phytoremediation has been considered cost-effective and environmentally friendly technology for removing heavy metals from the environment such as soil, surface water surfaces including groundwater [10, 12].

2.2. Heavy metals

In terms of their role in biological systems, heavy metals are classified as essential and non-essential. Essential heavy metals are those that are required by living organisms in minute quantities for vital physiological and biochemical functions. Examples of essential heavy metals are Fe, Mn, Cu, Zn and Ni [28, 29]. Non-essential heavy metals are those that are not required by living organisms for any physiological and biochemical function. Examples of non-essential heavy metals are Cd, Pb, As, Hg and Cr [30 - 36]. Heavy metal concentrations exceeding threshold limits have negative health effects as they interfere with the normal functioning of living systems.

2.3. Sources of heavy metal water pollution

Today, environmental pollution by heavy metals is a major global concern. This is caused by rapid industrialisation, in particular metal smelting and plating, battery manufacturing, mining activities, oil refining, tanneries, paint manufacturing, pesticides, printing and photographic industries. Due to untreated or partial treatment before discharge into the environment, ions of common heavy metals, especially Zn, Hg, Cu, Cd, Pb and Cr, are detected in industrial wastewater [46].

2.5. Methods for the removal of heavy metals from waste water

Recent studies have focused on a particular method of heavy metal ion removal, such as electrocoagulation (EC), adsorption using synthetic and natural adsorbents, advanced oxidation processes, membranes, etc. These studies looked at the advantages and disadvantages of a specific method for water treatment, including the removal of heavy metals. It is essential to choose the best method taking into account the treatment yield, added chemicals, initial concentration of heavy metal ions in the water, optimal pH value and other operating conditions [37]. Treatment methods are classified into adsorption-based, membrane, chemical, electrical and photocatalytic treatments. Research in the literature is selected based on the availability of operating conditions and the performance of operating and operational parameters for each method [37].

2.5.1. Adsorption

The adsorption mechanism is defined by the physicochemical properties of the adsorbent and the heavy metals as well as the operating conditions (e.g. temperature, amount of adsorbent material, pH value, adsorption time and initial concentration of metal ions). In general, heavy metal ions can be adsorbed on the adsorbent surface as shown in Fig. 2. Different types of adsorbent nanomaterials have been developed for wastewater treatment, as follows: carbon-based adsorbents, chitosan-based adsorbents, minerals, magnetic adsorbents, biosorbents.

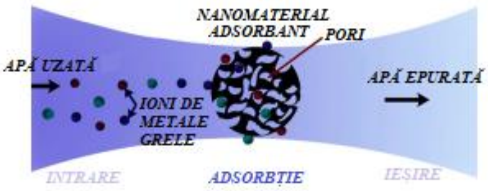

Fig. 2.1. Adsorption process.

2.5.2. Membrane filtration and separation

Over time, technological progress in membrane development has led to an increase in the use of membranes for filtration and extraction of heavy metal ions from wastewater [98].

2.5.2.1.Ultrafiltration

Ultrafiltration (UF) is used at low transmembrane operating pressure. Because the pores of the UF membrane can be larger than the heavy metal ions, additives can be bound to the metal ions to increase the size of the metal ions. Therefore, micelle-enhanced ultrafiltration (MEUF) and polymer-enhanced ultrafiltration (PEUF) are proposed [37].

2.5.2.2. Nanofiltration

Nanofiltration (NF) is used to concentrate constituents whose molecular weight is >1000 Da and to remove solutions with dimensions of 0.0005-0.007 μm and molecular weights >200 Da [107]. Thus, the operating range of NF lies between UF and reverse osmosis (RO) processes [102]. NF membranes are composed of thin-layer polymer composites with multiple layers of negatively charged chemical groups. NF membranes containing CeO_2/Ce_7O_{12} and PES were synthesized by phase inversion and used to extract Fe^{3+}, Al^{3+}, Co^{2+}, Cd^{2+}, Cu^{2+} and humic acid from wastewater and achieved an extraction efficiency between 94 and 98% [108].

2.5.2.3. Microfiltration

Microfiltration (MF) uses a microporous membrane to remove micron-sized particles (bacteria, viruses, protozoa, pollutants, etc.) from a solvent/fluid/solution. The MF process is also a low-pressure membrane process whose membrane pores are in the range of 0.1-10 μm. Some of the MF membranes are made of silica, ceramic, zirconium, PVC, polysulfone, PTFE, polypropylene, PVDF, polyamide, polycarbonate, cellulose acetate, cellulose esters or composite materials. However, application of the MF system can be found in the removal of particles from rinse water in the semiconductor industry, beer and wine sterilization, clarification of other juices and cider, and water treatment [107]. Depending on the application, the MF process is available in two main configurations: cross-flow and dead-end [37].

2.5.2.4.Reverse osmosisv

RO is a pressure-based separation process that uses a semi-permeable membrane (pore size 0.5-1.5 nm) to allow only small molecules to pass through. The RO process reverses the normal osmosis process by applying a pressure (20-70 bar) > the osmotic pressure of the feed solution. The molecular size of the blocked solutions is usually in the range 0.00025-0.003 μm. The RO process could extract 95-99% of inorganic salts and charged organics. The RO separation process has been used to extract heavy metal ions including Ni^{2+}, Cr^{6+} and Cu^{2+} from electroplating wastewater with a removal efficiency of >98.7562.

2.5.2.5. Direct osmosis

Direct osmosis (FO) is an osmosis process that requires a membrane to balance selectivity and permeate water flux [98]. In FO, a semi-permeable membrane separates a feed solution from the extraction solution. The FO process is also environmentally friendly, easy to clean and low fouling; therefore, it is widely used in water treatment [110].

2.5.2.6. Electrodialysis

Electrodialysis (ED) is used to separate ions at the expense of electrical potential difference. ED uses a series of cation exchange membranes (CEMs) and anion exchange membranes (AEMs) arranged alternately in parallel [98]. In the ED process, anions pass through the AEM, while cations pass through the CEM. In such a case, the treated (dilute) flow is produced from half of the ED stack channels, while the concentrated flow is expelled from the other half. ED offers high water recovery without phase change, reaction or chemical involvement [112] and can operate over a wide range of pH values. However, ED also exhibits a high degree of membrane fouling, high membrane cost and electrical potential demand [37]. Alte metode bazate pe membrane

Membrane and liquid membrane separation is also used for water treatment. Membrane separation exists in four configurations: direct contact, air gap, sweep gas and vacuum membrane separation. It has been reported that the membrane separation process achieves over 96% removal of Ca^{2+}, Mg^{2+}, $Fe^{3+}$and F^{2+} [113] and over 99% for As^{3+} and As^{5+} [114].

On the other hand, the liquid membrane is made up of a liquid phase or a thin-layer organic phase, which acts as a barrier between two aqueous phases. The membrane in liquid form on the support has achieved a removal efficiency of 89% for Zn^{2+}, C^{2+}, C^{2+} and Fe^{3+} [116].

2.5.3. Separation using chemicals

Chemical methods of removing heavy metals from wastewater have been used since early times. In this section, chemical-based methods including precipitation, coagulation-flocculation and flotation will be discussed. Precipitarea

Chemical precipitation (so-called coagulation precipitation) is widely used in industry and is considered one of the most efficient methods. It changes the shape of metal ions dissolved in solid particles to facilitate their sedimentation. The reagent precipitates the metal ions by changing the pH, the electro-oxidizing potential or by co-precipitation [117]. It is usually followed by sediment removal [37].

2.5.3.2. Coagulation and flocculation

Coagulation is the destabilization of colloids by neutralizing the forces holding them apart, while flocculation is the agglomeration of destabilized particles [130]. Flocculation binds particles forming large agglomerations using a flocculant such as polyaluminium chloride (PAC), polyferric sulfate (PFS), polyacrylamide (PAM) and other macromolecular flocculants [131].

Typical heavy metals removed by this method include Cu^{2+}, Pb^{2+} and Ni^{2+}. Other metals, such as As^{2+}, Se^{2+}, Cr^{2+}, Sb^{3+}, Sb^{5+}, Ag^{2+}, could also be removed efficiently [37].

2.5.3.3. Flotation

Flotation is used to remove various metal ions. Dissolved air flotation, ionic flotation and precipitation flotation have been extensively studied.

In general, flotation processes have advantages such as fast operation, compact process and moderate cost [37].Separare utilizând metode electrochimice

In this section, different electrochemical methods (i.e. electrochemical reduction (ER), electrocoagulation (EC), electroflotation (EF) and electrooxidation (EO)) as well as the ion exchange method are discussed.

2.5.4.1. Electrochemical treatment

In an electrochemical system, oxidation takes place at the anode (positive side), where electrons are transferred to the cathode (negative side), and where the reduction process takes place.

In the ER method, also known as electroplating and electroplating, the targeted atoms or molecules are deposited on the cathode surface. Carbon or sulphur mixture based cathodes with different proportions under acidic conditions are suitable for the removal of Hg^{2+} Cd^{2+}, Pb^{2+} and Cu^{2+} from wastewater [140]. Energy consumption is a barrier that should be solved to use the method in industrial applications.

In the EC method, mainly steel (iron) or aluminium electrodes are used, which are non-toxic and reliable [142]. The mechanism of the EC method is sequential as: dissolution of anodic metal cations, formation of hydroxocomplexes (coagulants, equation), aggregate stability and phase separation, and precipitation and flotation.

The EF mechanism is mainly based on performing water electrolysis on insoluble electrodes, while the flotation effect is introduced to facilitate the treatment process [146]. Therefore, hybridization between EF, membrane and CE has been a promising approach to improve the overall performance of heavy metal removal system [150].

The mechanism of removal of compounds from wastewater using EO is direct and indirect. The direct mechanism is simple. The performance of indirect chlorine oxidation depends on the NaCl concentration and is independent of the current strength [151]. The pollutants exchange electrons directly with the anode surface, and the polymer layer forms on the anode surface, which leads to electrode deactivation and degradation of the efficiency.

Highly effective anode materials are expensive. Therefore, there is an urgent need to find efficient anode materials with high efficiency in dilute solutions [37].

2.5.4.2. Ion exchange treatment

The ion exchange method is a reversible chemical reaction used to replace unwanted metal ions with harmless and environmentally friendly ions [153]. A heavy metal ion is removed from a wastewater solution by attaching it to an immobile solid particle as a replacement for the cation of the solid particle. The ion exchange method can remove target heavy metal ions (some or all) such as Pb^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, V^{4+}, V^{5+}, Cr^{3+}, Cr^{4+}, Cu^{2+} and Zn^{2+} from wastewater [153].

2.5.5. Separation based on photocatalysis

The photocatalytic process has been reported to be a simple water treatment process using light and semiconductors such as titanium dioxide (TiO2) [5]. Three key steps are undergone in this process: photogeneration of charged carriers, separation and diffusion of charged carriers to the photocatalyst surface, and redox reaction on the photocatalyst surface [157]. Real soil wash wastewater effluents were purified using a dual solar photocatalytic process in the open air with a flat plate collector for the removal of 93.50% Cu^{2+}, 99.60% Fe^{3+} and 99.40% Zn^{2+} [158].

CHAPTER 3. WATER TREATMENT BY PHYTOREMEDIATION

Phytoremediation is an environmentally friendly solution to the problem of heavy metal pollution. Phytoremediation basically refers to the use of plants and associated soil microorganisms to reduce the concentrations or toxic effects of pollutants in the environment [164]. It can be used to remove heavy metals as well as organic pollutants (such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls and pesticides). The term "phytoremediation" is a combination of two words: the Greek phyto (meaning plant) and the Latin remedium (meaning to correct or remove an evil). Green plants have an enormous capacity to absorb pollutants from the environment and detoxify them by various mechanisms [72].

Phytoremediation approaches involve different plant-based technologies with different modes of action and mechanisms [201]. Figure 3.5. shows a schematic representation of the phytoremediation mechanism.

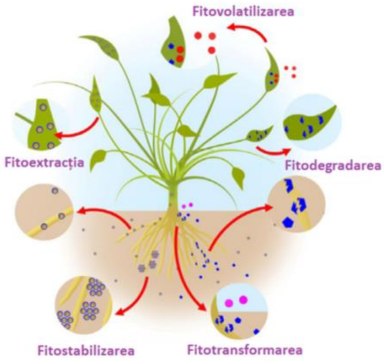

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Fig. 3.5. Reprezentarea schematică a abordărilor de fitoremediere.

3.1. Phytoextraction

Phytoextraction (Fig. 3.1.) (also known as phytoaccumulation, phytoabsorption or phytosecretion) is the uptake of pollutants from soil or water by plant roots and their translocation to and accumulation in aboveground biomass, i.e. shoots [178 - 180]. Translocation of metals into shoots is a crucial biochemical process and is desirable in an efficient phytoextraction, as harvesting root biomass is generally not feasible [181, 182].

3.2. Phytofiltration

Phytofiltration is the removal of pollutants from surface water or polluted wastewater using plants [183]. Phytofiltration can be rhizofiltration (using plant roots), blastofiltration (using seedlings) or caulofiltration (using shoots removed from plants; Latin caulis = shoot) [184]. In phytofiltration, pollutants are absorbed or adsorbed and thus their movement to groundwater is minimised [72].

3.3. Phytostabilisation

Phytostabilisation (Fig. 3.2.) or phytoremediation is the use of certain plants to stabilise pollutants in polluted soils [185]. This technique is used to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration into groundwater or their entry into the food chain [186]. Plants can immobilise heavy metals in soils by biosorption by roots, by precipitation, by complexation or by reducing the valence of metals in the rhizosphere [52, 179, 187, 188]. However, phytostabilization is not a permanent solution because heavy metals remain in the soil; only their movement is limited.

3.4. Phytovolatilisation

Phytovolatilisation (Fig. 3.3.) is the uptake of pollutants from the soil by plants, their transformation into volatile form and subsequent release into the atmosphere. This technique can be used for organic pollutants and for some heavy metals such as Hg and Se. However, its use is limited by the fact that it does not completely remove the pollutant; it is just transferred from one segment (soil) to another (atmosphere) from where it can be redeposited. Phytovolatilisation is the most controversial of the phytoremediation technologies [80].

3.5. Phytodegradation

Phytodegradation (Fig. 3.4.) is the degradation of organic pollutants by plants using enzymes such as dehalogenase and oxygenase; it does not depend on rhizosphere microorganisms [191]. Plants can accumulate organic xenobiotics from polluted environments and detoxify them through their metabolic activities. From this point of view, green plants can be considered the 'green liver' of the biosphere. Recently, scientists have shown interest in studying the phytodegradation of various organic pollutants, including synthetic herbicides and insecticides.

3.6. Rhizodegradation

Rhizodegradation refers to the breakdown of organic pollutants in soil by microorganisms in the rhizosphere [183]. The main reason for the increased degradation of pollutants in the rhizosphere is probably the increased number and metabolic activities of microorganisms. In addition to secreting organic substrates to facilitate the growth and activity of rhizospheric microorganisms, plants also release certain enzymes capable of degrading organic pollutants in soils [194, 195].

3.7. Phytodesalination

Phytodesalination is a recently reported and emerging technique [196]. Phytodesalination refers to the use of halophytic plants to remove salts from salt-affected soils to enable them to support normal plant growth [197, 198]. According to one estimate, two halophytes, Suaeda maritima and Sesuvium portulacastrum, could remove 504 and 474 kg of sodium chloride from 1 ha of saline soil in a 4-month period, respectively.

Phytoremediation uses aquatic and/or terrestrial plants to remove heavy metals through metabolism-dependent biosorption and bioaccumulation processes in roots, stems, shoots and leaves [204]. Many researchers have found that floating plants such as water hyacinth (Echhornia crassipes) (Fig. 3.6.(a)) has demonstrated the ability to accumulate many heavy metals such as As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Zn [208], water lettuce (Pistia stratiotes) (Fig. 3.6.(b)), water lynx (Lemna minor) (Fig. 3.6. (c)), floating moss (Salvania cucullata) (Fig. 3.6. (d)) from sewage.

Fig. 3.6. Heavy metal tolerant plant species: (a) water hyacinth (Echhornia crassipes), (b) water lettuce (Pistia stratiotes), (c) water lynx (Lemna minor), (d) floating moss (Salvania cucullata) [1].

3.8. Phytoremediation mechanisms using aquatic accumulator plants

In general, the accumulation of heavy metals in plants involves the uptake of metals into plant tissue and the release of absorbed metals back into the external environment. In aquatic ecosystems, adsorption of heavy metals onto sediments occurs. Free-floating plants take up metals from the water through their roots. Despite uptake, metals may be released back into the aquatic environment and into the soil from plant tissue. In addition, metals could be released into the air in gaseous form from leaf surfaces [213].

Biosorbent plants can survive unaffected by large numbers of metals retained in their aerial tissues due to their ability to biodegrade and biotransform metals into non-toxic forms. In contrast, there are plants that limit the uptake of metals into their plant biomass due to the presence of barriers [215]. However, an exception is hyperaccumulators, which can absorb and tolerate concentrations of thousands of ppm of metals in their tissues. The reason for this is that hyperaccumulators possess several detoxification mechanisms to prevent metal toxicity, such as metal storage in vacuoles, metal chelation and metal efflux [216].

It has been shown that there are two different mechanisms of heavy metal uptake in plants, namely root uptake and foliar uptake [217]. Regarding root uptake, plant roots absorb heavy metals into the apoplast while absorbing water. This in turn becomes a transport medium for heavy metals to move into the cell wall from the external environment via diffusion or mass flow, where absorption actively takes place. The total concentration of metal uptake could be bound to anions in the cell wall, transported apoplastically and into cells [215]. The distribution of absorbed metals between these three sites is based on the metal types, species and genotype of the plants [213]. Because water hyacinths have dense, fibrous root systems, aerobic bacteria are well established in these aquatic environments. These bacteria collect nutrients and inorganic pollutants that serve as food for plant nutrition [217]. In addition to root uptake, foliar uptake could also occur in plants, where passive uptake of heavy metals occurs through stomatal cells and cuticle cracks on plant leaves [218]. A higher density of stomatal cells stimulates a higher ion uptake capacity, as most of the uptake process is initiated in the ectoderm. In aquatic macrophytes, the usual metal transport mechanism is rhizosphere, in which metal is immobilized and accumulated in plant roots [219]. Roots exude within the rhizosphere, allowing metal adsorption onto plant root surfaces [213].

3.8.2. Bioconcentration, translocation and distribution of metals

The two important parameters for assessing the retention of heavy metals by aquatic plants are the concentration factor (CF) and the bioconcentration factor (BCF). CF is an indicator that assesses the total accumulation of metals by plants through uptake and adsorption, while BCF is an index that accounts for the uptake of metals by plants from the external environment [220]. BCF values greater than 1000 are commonly considered as a sign of high phytoremediation potential [221].

In addition, it has been found that most metals tend to bind to cell walls during their transport [215]. Findings showed that there was about 75-90% uptake of metals by plant roots, while only 10-25% was subsequently translocated to shoots. For example, Cd distribution was lower in the upper parts of the plants, following the descending order: dense fibrous roots > storage roots > stems > leaves. It was also found that more Pb accumulated in water hyacinth roots than in leaves [222]. In addition, a higher accumulation of Pb in water hyacinth roots than in stems and leaves was reported [223].

The translocation factor (TF) is the ratio of the concentration of metal ions accumulated in the plant shoot to that in the plant root. Ideally, a hyperaccumulating plant should have a TF value greater than one [221]. A TF value greater than one indicates that heavy metals absorbed by the plant have been efficiently translocated to the aerial parts of the plant [225]. In contrast, a TF value less than one implies that heavy metals tend to accumulate and deposit in the roots of the plant.

Another study reported the distribution of metals in plant shoots and roots, indicated by the root-to-shoot (R/S) ratio. The R/S ratio implies the concentration of metal accumulated in the plant root relative to the shoots. For example, about 80% of Cr, Cu, Fe and Ni accumulated in plant roots with an R/S ratio equal to or greater than 6, while Fe has an R/S ratio greater than 17 [214]. Specifically, plant roots are the final destination of absorbed metals, as roots can concentrate a higher amount of metal ions than their shoots.

3.8.3.. Phytotoxicity of heavy metals in plants

Undesirable effects on plant growth and development have been observed due to the accumulation of toxic metals in roots, stems and leaves. Bioactive metals can be classified into two groups: redox active metals and redox non-reactive metals. Redox active metals, such as Cr, Cu, Mn and Fe, could directly disrupt plant cell homeostasis, break DNA strands, defragment proteins or cell membranes, destroy photosynthetic pigments and cause cell death. Conversely, redox non-reactive metals could impose oxidative stress on plants [227].

De exemplu, s-a raportat că expunerea jacintului de apă (Fig. 3.6. (a)) la ioni de Cr în concentrații de 10,0 și 20,0 mg/L ar putea duce la îngălbenirea frunzelor plantelor, cloroză și exfolierea rădăcinilor [219]. În plus, s-a constatat că, conținutul de clorofilă, proteine și zahăr din plante se reduce odată cu creșterea concentrației de metal și a timpului de expunere. Aceștia au constatat, de asemenea, că Cr a demonstrat un grad mai mare de toxicitate a metalului în comparație cu Zn.

3.9. Plant mechanisms for heavy metal tolerance and detoxification

Relevant components of homeostatic networks for metal detoxification include ion transporters, metallochaperones and ligands that act together to ensure metal uptake, transport to different cell types and delivery into cells. Membrane proteins can transport different metals across cell membranes playing a central role in each step of the influx-efflux, translocation from roots to shoots. The function of several transporters is the import, transport, mobilization and export of essential metals into the vacuole membrane, tonoplast or chloroplast envelope [230-232].

3.9.1. ZIP Family

The ZIP family contains Zn^{2+}-transporting ZRT-IRT-like proteins (Fig. 3.7.) and is involved in several homeostatic processes, including uptake and translocation from root to shoot [233, 234].

Zinc transporter proteins are membrane transport proteins of the solute carrier family that control the membrane transport of zinc and regulate its intracellular and cytoplasmic concentrations[235].

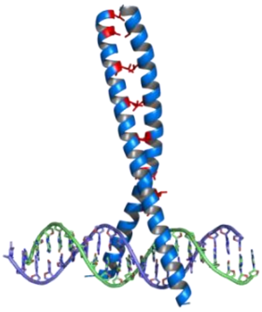

Fig. 3.7. ZIP proteins [236].

3.9.2. NRAMP Family

Natural resistance-associated macrophage proteins (NRAMPs, Fig. 3.8.) also known as metal ion (Mn^{2+}) transporters are a family of metal transport proteins found in all areas of life [237]. They transport a variety of transition metals, such as cadmium or manganese, using an alternative access mechanism characteristic of secondary transporters [238].

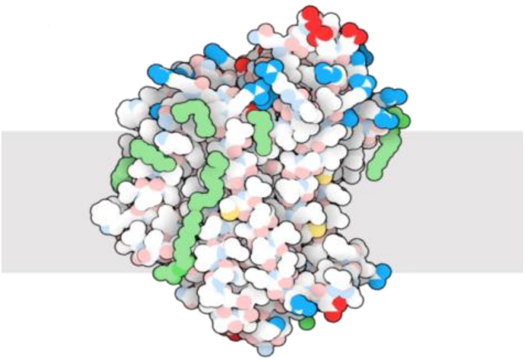

Fig. 3.8. NRAMP family of proteins [236].

3.9.3. HMA proteins

The HMA proteins, comprising a relatively large number of heavy metal transport proteins, are shown in Fig. They contribute to the pumping of cations from the cytoplasm via ATP hydrolysis[241].

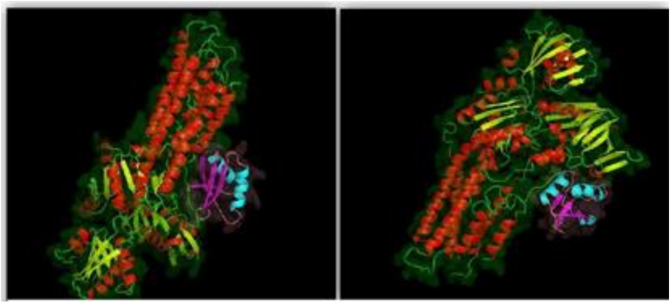

Fig. 3.9. HMA protein [241].

a) HMA1 is located in the chloroplast envelope (plastids, photosynthesizing organelles) with an important role in detoxifying excess Zn^{2+} [240];

b) HMA3 is involved in the detoxification of Zn^{2+}, Cd^{2+}, Co^{2+} and Pb^{2+} through their uptake into vacuoles;

c) HMA4, a plasma membrane transporter, plays a role in Zn^{2+} removal from the cytoplasm and xylem loading/unloading (it is one of the two transporter tissues in vascular plants, the other being phloem [242, 243].

3.9.4. CDF proteins

The CDF family (cation diffusion difusers), another group of transporters that tightly regulates metal homeostasis, ensures adequate metal supply to tissues. Its members are involved in the translocation of metals to internal compartments and extracellular space [231].

Based on early phylogenetic analysis, the CDF family has been divided into three major groups according to the specificity of metal ions: carriers of Mn^{2+},carriers of Fe^{2+}/Zn^{2+};carriers of Zn^{2+} and other metal ions, but not Fe^{2+} or Mn^{2+}[245].

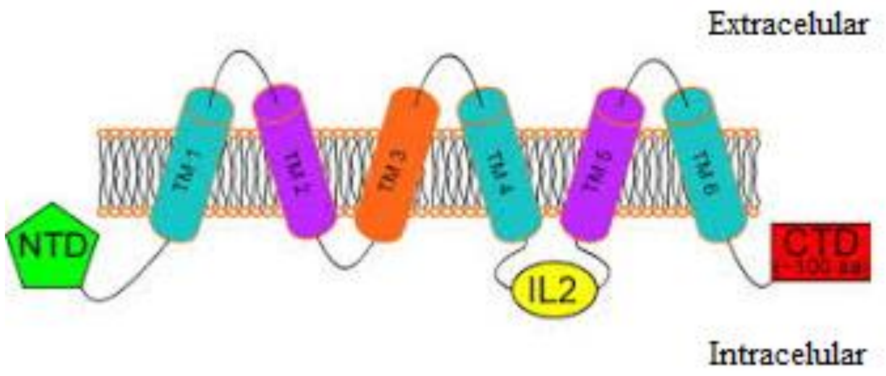

Fig. 3.10. CDF ptoteins [245].

Several MTPs (metal-tolerant proteins) have been described in a variety of plant species. The schematic representation of a cell and the different roles of MTPs in cellular metal homeostasis in each plant species are shown in Fig. 3.11.

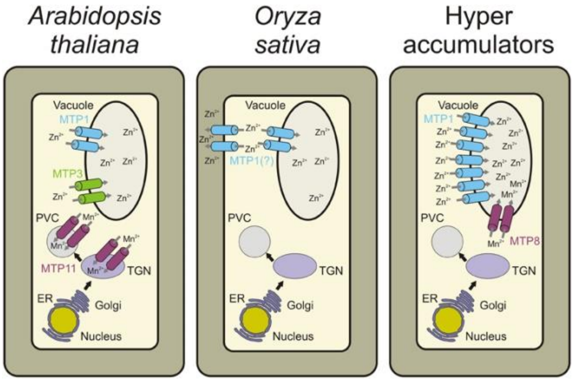

Fig. 3.11. Schematic representation of a cell and the different roles of MTP in cellular metal homeostasis in each plant species [241].

- In Arabidopsis thaliana (gascarita), MTP1(whole plant) and MTP3(root epidermis) are vacuolar Zn transporters;

- In Oryza sativa (rice), MTP1 is described as a transporter of Zn2+ in the plasma membrane of the onion epidermal cell or localized in the tonoplast when found in yeast and Arabidopsis [248].

3.10. Effective absorption mechanism factors

There are several factors that influence the absorption of heavy metals. Plant species, environmental properties, root zone, vegetative uptake, addition of chelating agent are presented as factors of uptake mechanisms [182, 255]. These are shown schematically in Figure 3.12 .

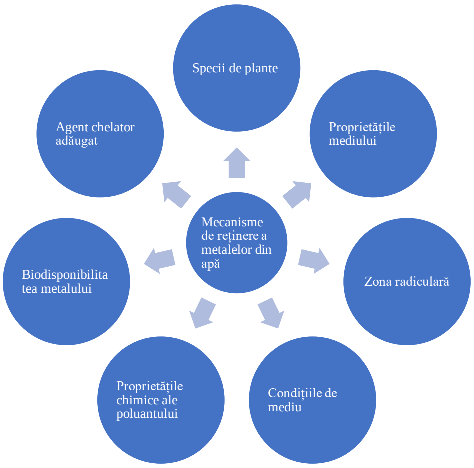

Fig. 3.12. Factors influencing the absorption of heavy metals.

3.10.1. Plant species

The success of phytoremediation depends on the properties of the selected plant species. Several common, hyperaccumulating plants that are applied for phytoremediation are Typha latifolia [256], Echhornia crassipe [209, 257], Pistia stratiotes [258], Scirpus tabernaemontani [259], Arabis paniculata Franch [260]..

3.10.2. Environmental properties

Environmental properties are very important for the uptake of heavy metals by plants, especially pH, chelating agents, fertilizers. pH, e.g. soil containing organic matter and phosphorus affect the amount of Pb uptake by plants. In addition, the addition of fertilizers helps plants to adapt to the new environment they use for phytoremediation [60].

3.10.3. Root zone

The root zone is of particular interest in phytoremediation. It can absorb pollutants from soil or water and metabolise them within the plant tissue. This process is called translocation. Pollutants in the root are translocated to other parts of the plant through the plasma membrane [256, 261].

3.10.4. Vegetative uptake

Environmental conditions affect the vegetative uptake of plants. The uptake of heavy metals depends on the bioavailability of heavy metals in wastewater as well as on the interaction of other elements and substances in the water.

3.10.5. Addition of chelating agent

By adding the chelating agent, micronutrients cause plants to absorb heavy metals more quickly and also have less expensive remediation times. EDTA was found to be used to grow plants for 2 weeks. Plants could improve their translocation of heavy metals into plant tissues as well as overall phytoextraction performance.

CONCLUSIONS

Selecting the most appropriate technique for removing heavy metal ions from wastewater depends on many key factors, including the cost of the operation, initial metal ion concentration, environmental impact, pH values, added chemicals, removal efficiency and economic feasibility. These methods are classified into adsorption processes (using different adsorbents, e.g. carbon-based, carbon compounds, mineral, magnetic, biosorbents), membrane processes (e.g. ultrafiltration, nanofiltration, microfiltration, reverse osmosis, direct osmosis and electrodialysis), chemical processes (i.e. chemical precipitation, coagulation-flocculation and flotation), electrical processes (i.e. electrochemical treatments (reduction, EC, EF and advanced oxidation) and ion exchange) and photocatalysis.

Phytoremediation is an environmentally friendly and responsible technology with good public acceptance. It is a relatively new technology and is mainly in the research stage. Its research is highly interdisciplinary in nature and requires basic knowledge in soil chemistry, plant biology, soil ecology and microbiology, as well as environmental engineering. Fortunately, interdisciplinary studies and research are appreciated and highly encouraged in the broad-minded scientific communities around the world and it is fully expected that the integration of scientific disciplines will be extremely fruitful. Research is ongoing to select native plants for phytoremediation of the targeted heavy metals and to evaluate the effect of different parameters on phytoremediation efficiency. In addition, research is being carried out to genetically modify suitable plants for better phytoremediation of heavy metals and other xenobiotics.

CHAPTER 4. EXPERIMENTAL RESEARCH METHODOLOGY

The experimental research conducted and presented in this PhD thesis has been developed through the following steps:

- Use of the plant (*Typha angustifolia*) for the absorption of copper, manganese, nickel and lead ions from wastewater;

- Use of algae (*Sargassum fusiforme* and *Enteromorpha prolifera*) to absorb copper, manganese, nickel and lead ions from wastewater;

At the basis of my PhD thesis is the study of the literature, which provided me with all the necessary information for the application of phytoremediation for the treatment of water containing heavy metal ions..

The experimental research considered the influence of the number of plants used, the concentrations of heavy metal ions and the treatment time required for water treatment. The research methodology, the work plan of the experiments, as well as the scientific experiments themselves were carried out at the Doctoral School of Biotechnical Systems Engineering of POLITEHNICA University of Bucharest.

CHAPTER 5. EXPERIMENTAL RESEARCH

5.1. Use of *Typha angustifolia* for water treatment

For the experimental investigations presented in this chapter the plant *Typha angustifolia* (Fig. 5.1.) was studied, which has the following components: roots, rhizome (underground stem), stem, narrow lanceolate leaves, female and male flowers (spikelets).

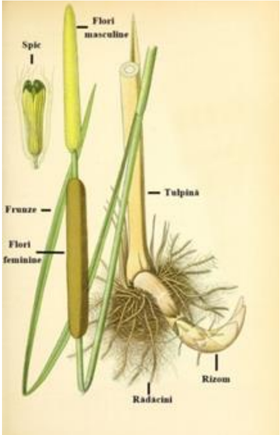

Fig. 5.1 *Typha angustifolia* plant. [1].

The research presented in this chapter consists of testing the *Typha angustifolia* plant in the phytoremediation of wastewater containing the heavy metals copper, manganese, nickel and lead.

5.1.1. Theoretical part

Typha are emerging, rooted monocotyledonous macrophytes with a wide distribution [263], comprising 49 recorded species and hybrids. Typha grow in a variety of aquatic and semi-aquatic habitats, including wetlands, lake shores, pond margins, coastal estuaries, roadside ditches, and agricultural drainage and irrigation canals [263, 264]. Typha has 208 entries in the ECOTOX database (https://cfpub.epa.gov/ecotox/) as of February 2021, indicating that it has been successfully used in toxicity testing to generate data for risk assessment [267].

Typha seeds (Fig. 5.2.) may show intraspecific variability in germination rate. In one study inflorescences were hand pollinated and resulted in average seed germination rates after seven days of 86% in T. angustifolia, 66% in T. latifolia and 78% in T. glauca [288].

Fig. 5.2. Cattail seeds [1].

Once germinated, shoots from T. latifolia seeds show less variation in their length compared to radicle roots within 4-7 days of placing cleaned seeds in water [296, 297].

To assess the effects of pollutants on macrophytes, a variety of morphological and physiological parameters were used, the most common being growth (e.g. length and number of shoots or roots), biomass (e.g. fresh or dry weight above or below ground) and pigment content (e.g. chlorophyll or carotenoids) [299].

5.1.2. Experimental part

The experimental research carried out using *Typha angustifolia* plants is based on the use of a treatment system (Fig. 5.3.) consisting of the following components: (1) container, (2) waste water, (3) *Typha angustifolia* plant, (4) stand, (5) mechanical agitator.

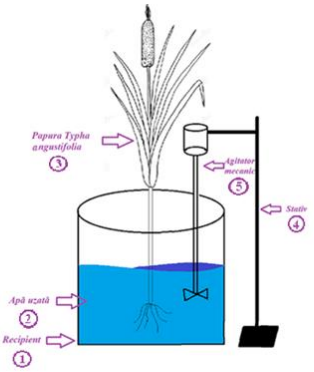

Fig. 5.3. Treatment system consisting of: (1) container, (2) waste water, (3) *Typha angustifolia* plant, (4) stand, (5) mechanical agitator.

During the experiments, the concentration of heavy metal ions in the wastewater was analysed for a clearly defined period of time (0-10 hours) and the standard treatment value was calculated for each analysis. The influence of operational parameters on the efficiency of the treatment process was studied.

The harvesting of *Typha angustifolia* plants (narrow-leaved papyrus) (Fig. 5.4, 5.5), was carried out with the help of a picker/plough, from water drainage ditches located on plots of arable land in the Municipality of Oarja, Arges County (Fig. 5.6.).

Fig. 5.4. Harvesting *Typha angustifolia* plants in the commune of Oarja.

Fig. 5.5. Oarja Commune, Arges County [1].

The pappus specimens used in the research had all the component parts: root, rhizome (creeping, creeping, thick, gnarled), simple, unbranched stem (2.10 m high, 2 cm thick, cylindrical, upright, filled with pith), usually with leaves (2 cm long, linear, fleshy, vaginate), arranged biseriad. The unisexual flowers, located at the tip of the stem, are grouped into a male spike (at the top) and female spike (at the base).

In order to observe the amount of plant needed to remove metal ions from water, in the experimental research of the PhD thesis we used 1, 3 and 5 plants *Typha angustifolia*.

Probele de apă uzată prelevate au fost pregătite în prealabil (Fig. 5.7.) și concentrațiile ionilor metalici au fost determinate cu ajutorul fotometrului PhotoLab S12. Concentrațiile obținute au fost utilizate pentru a determina eficiențele de epurare, utilizând următoarea formulă de calcul:

$$\eta(\%) = \frac{C_i - C_f}{C_i} * 100 \tag{14}$$

unde: η is the treatment efficiency [%];

ci represents the initial concentration of metal ions [mg/L];

cf is the final concentration of metal ions [mg/L].

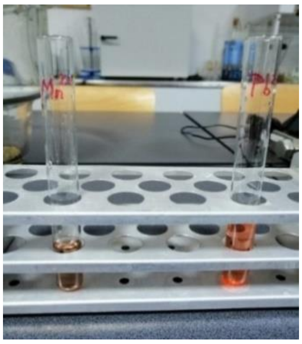

Fig. 5.6. Preparation of samples for measurement of $Pb^{2+}$and Mn^{2+} concentrations.

5.1.2.1. Use of *Typha angustifolia* for the removal of lead ions from waste water

We used 1, 3 and 5 strains of *Typha angustifolia* respectively to remove a concentration of 0.65 mg/L Pb(II) from a wastewater volume of 2 L. The time required for treatment was 6 hours. The influence of the number of *Typha angustifolia* strains on the yield in the water treatment process was studied. For high efficiency, we homogenized the wastewater throughout the experiment using a mechanical stirrer, as shown in Figures 5.8., 5.9. and 5.10. Samples were taken at 4, 6 and 8 hours after the start of the experiments using 1 (Fig. 5.11.), 3 (Fig. 5.12.) and 5 plants of *Typha angustifolia* (Fig. 5.13).

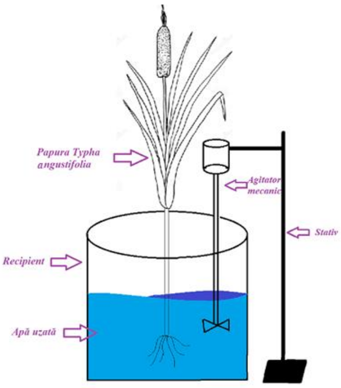

Fig. 5.7. Description of the treatment system in the thesis containing a strain of Typha. angustifolia for the removal of metal ions from wastewater

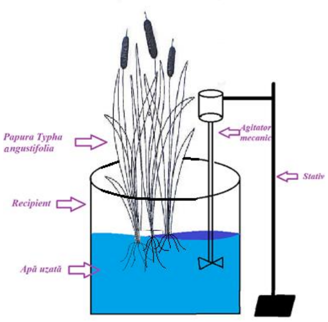

Fig. 5.8. Description of the treatment system in the thesis containing three strains of *Typha angustifolia* for the removal of metal ions from wastewater.

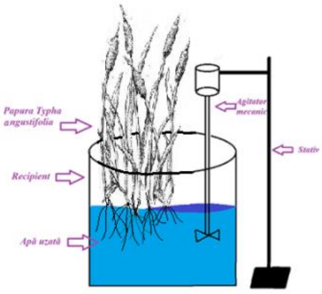

Fig. 5.9. Description of the treatment system in the thesis containing five strains of *Typha angustifolia* for the removal of metal ions from wastewater.

Fig. 5.10. Samples taken during the use of *Typha angustifolia* plant in the study on wastewater containing Pb^{2+} ions.

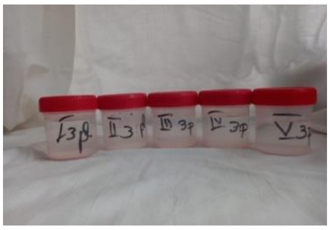

Fig. 5.11. Samples taken during the use of three *Typha angustifolia* plants in the study on wastewater containing Pb^{2+} ions.

Fig. 5.12. Samples taken during the use of five *Typha angustifolia* plants in the study on wastewater containing Pb^{2+} ions.

In fig. 5.13. the lead ion concentrations determined in relation to contact time are plotted and in Fig. 5.14. plot the calculated treatment efficiencies versus contact time using 1, 3 and 5 *Typha angustifolia* plants.

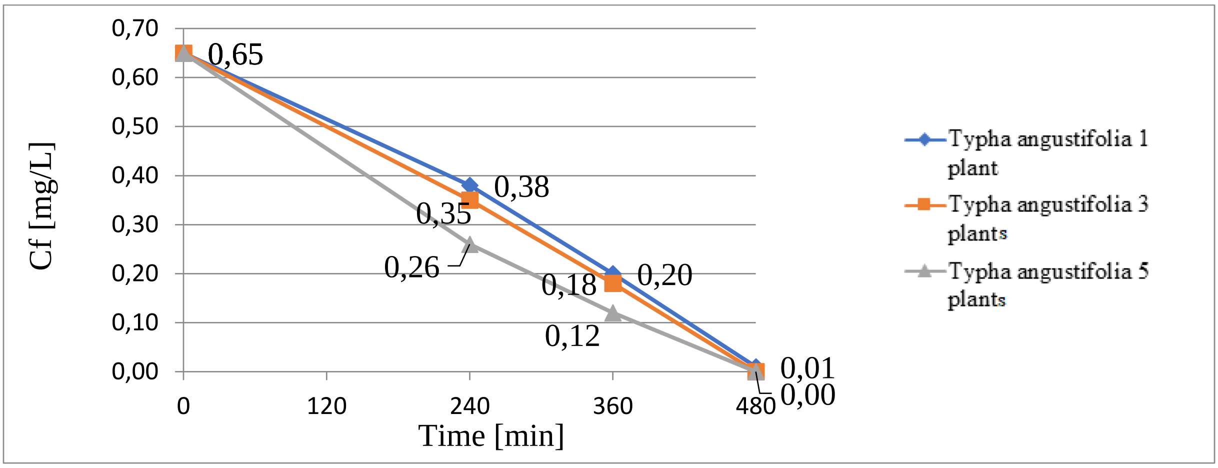

Fig. 5.13. Variation of Pb(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 0.65mg/L.

Figure 5.13. shows that the concentrations of lead ions in wastewater reached values of 0.01 mg/L using 1 *Typha angustifolia* plant and 0.00 mg/L using 3 and 5 *Typha angustifolia* plants. Concentrations gradually decreased until complete removal of the water treatment. When using 5 *Typha angustifolia* plants the concentration of lead ions in the wastewater decreased faster than when using 1 plant or 3 *Typha angustifolia* plants..

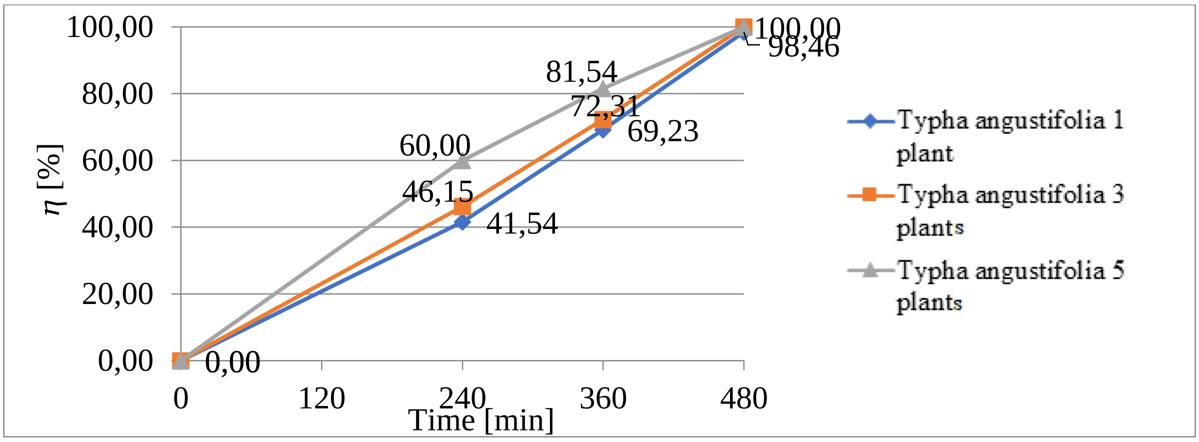

Fig. 5.14. Variation of treatment yield over time for wastewater containing Pb(II) ions, Ci =0.65mg/L.

In fig. 5.14. shows that using 3 and 5 *Typha angustifolia* plants gave 100% yields for the removal of lead ions from wastewater. Even when only one *Typha angustifolia* plant was used, the treatment yield was high, i.e. 98.46%. It is observed that the treatment process is faster using 5 plants. Considering these results we can state that *Typha angustifolia* plants are very effective for the removal of lead ions from wastewater.

5.1.2.2. Use of Typha angustifolia for the removal of copper ions from waste water

In these experiments we tested one strain, 3 strains and 5 strains of *Typha angustifolia* to remove copper concentrations of 0.60, 1.10 and 2.00 mg/L from wastewater. The maximum contact time was 40 hours and we sampled every 8 hours. The homogenization of the wastewater in contact with the plants was carried out with the mechanical stirrer. Experiments were conducted at room temperature.

Figures 5.15., 5.17., 5.19. show graphs showing the variation of copper ion concentrations in wastewater as a function of treatment time, starting from initial copper ion concentrations of 0.60, 1.10 and 2.00 mg/L. Figures 5.16., 5.18., 5.20. show the treatment efficiencies recorded during the experiments using 1, 3 and 5 *Typha angustifolia* plants.

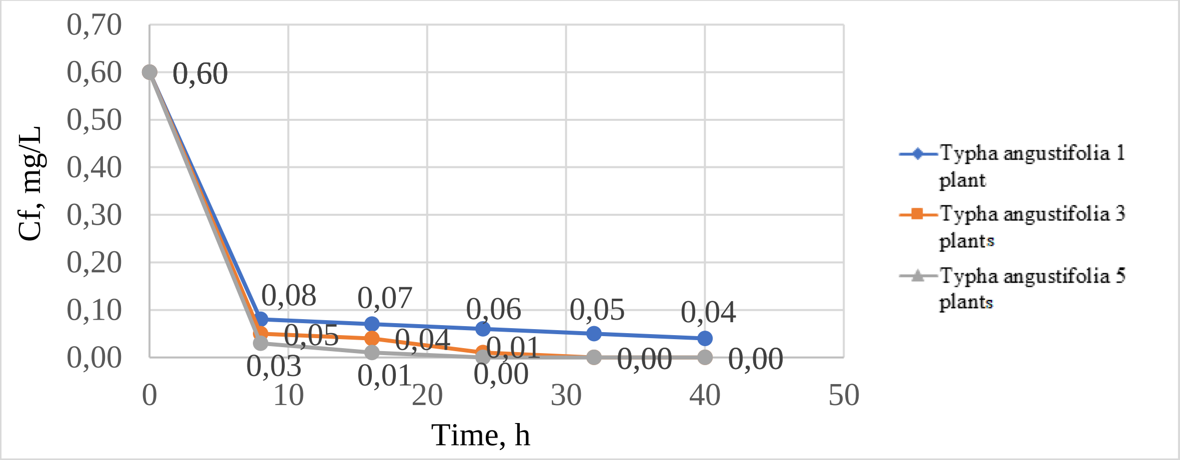

Fig. 5.1.5. Variation of Cu(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 0.60 mg/L.

Figure 5.15. shows that 5 *Typha angustifolia* plants completely removed copper ions at a concentration of 0.60 mg/L from wastewater even after 24 hours of contact time. Also 3 Typha angustifolia plants completely removed copper ions from water, but after a treatment time of 32 hours. Using a single *Typha angustifolia* plant resulted in a minimum concentration of 0.04 mg/L which could not be removed.

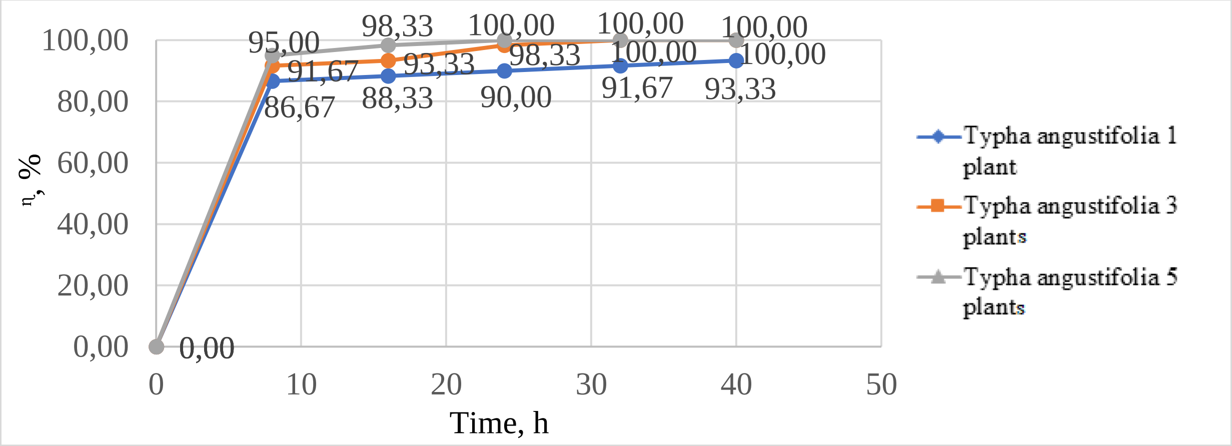

Fig. 5.1.6. Variation of treatment yield over time for wastewater containing Cu(II) ions, Ci = 0.60 mg/L.

According to the calculations made taking into account the values of copper ions concentrations in water during the treatment process using *Typha angustifolia*, treatment efficiencies of 93.33% were obtained using 1 plant and 100.00% using 3 and 5 *Typha angustifolia* plants. It can be seen in Figure 5.16. that the treatment process was fast in the first 8 hours and then the efficiencies steadily increased to maximum treatment efficiencies.

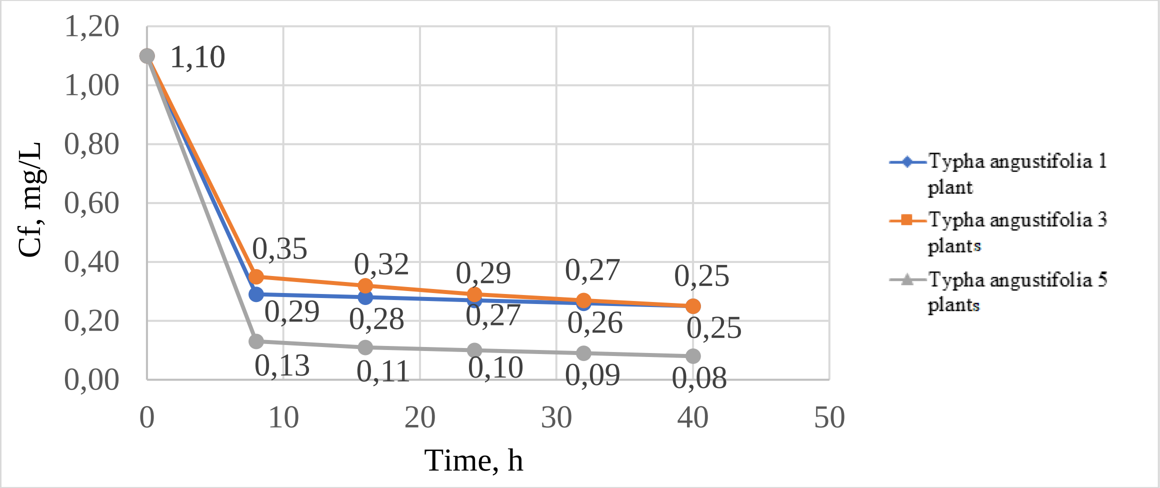

Fig. 5.17. Variation of Cu(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 1.10mg/L.

The copper ion concentration of 1.10 mg/L was no longer completely removed from the wastewater using *Typha angustifolia* plants, but only a concentration of 0.85 mg/L of the original concentration when 1 or 3 plants were used and 1.02 mg/L of the original concentration when 5 plants were used. The final concentrations of copper ions in the wastewater reached values of 0.25 and 0.08 mg/L respectively (Figure 5.17.).

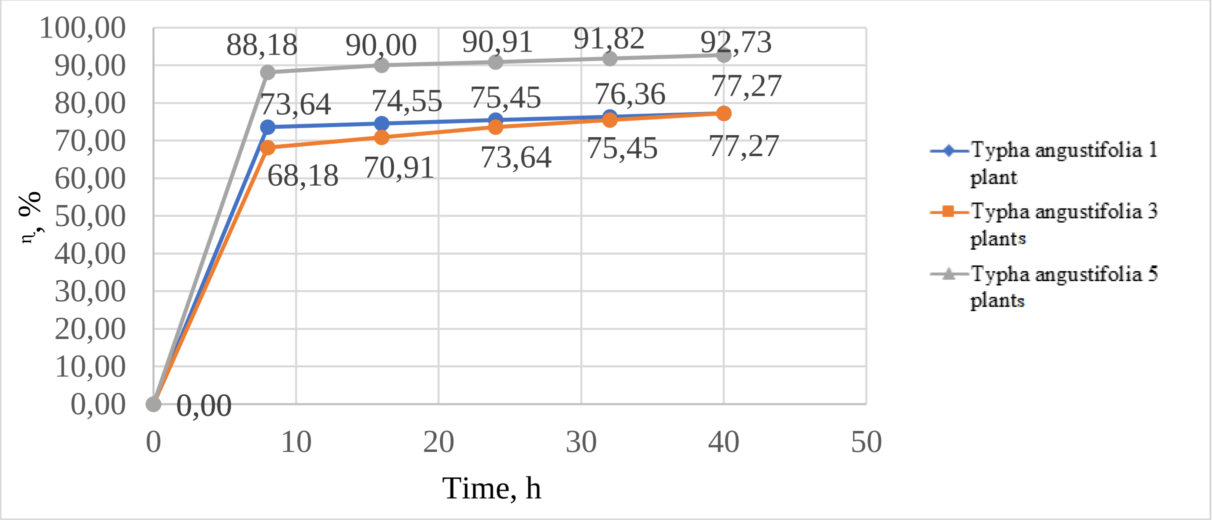

Fig. 5.18. Variation of treatment yield over time for wastewater containing Cu(II) ions, Ci = 1.10 mg/L.

Figure 5.18. shows that for the removal of an initial copper ion concentration of 1.10 mg/L, the treatment efficiencies increased rapidly in the first 8 hours of contact time reaching values of 68.18; 73.64 and 88.18 % using 1, 3 and 5 *Typha angustifolia* plants. However, by the end of the experiment, efficiencies did not reach 100.00%. The highest treatment efficiency obtained in this experiment was 92.73%.

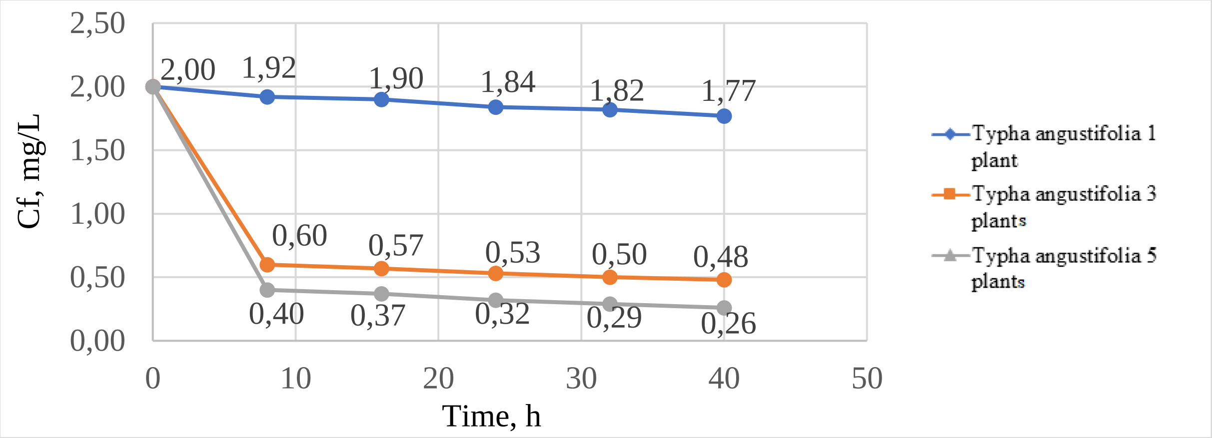

Fig. 5.19. Variation of Cu(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 2.00 mg/L.

By increasing the initial concentration of copper ions to 2.00 mg/L, the process was not as effective using a single *Typha angustifolia* plant. The concentration of copper ions decreased steadily and only reached the final concentration of 1.77 mg/L. It can be seen that when using 3 and 5 plants the final concentrations reached values of 0.48 and 0.26 mg/L (Figure 5.19.).

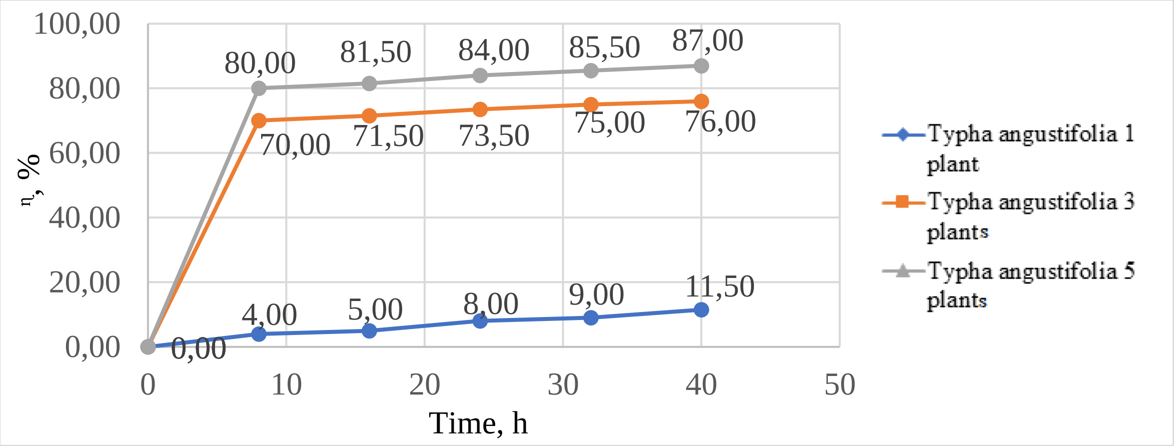

Fig. 5.20. Variation of treatment yield over time for wastewater containing Cu(II) ions, Ci = 2.00 mg/L**.**

According to the calculations made considering the values of copper ion concentrations in the water during the treatment process using *Typha angustifolia*, treatment yields of 11.50, 76.00 and 87.00 % were obtained using 1, 3 and 5 plants at an initial copper ion concentration of 2.00 mg/L. It can be seen in Figure 5.20. that the treatment process was fast in the first 8 hours using 3 and 5 plants.

5.1.2.3. Use of *Typha angustifolia* for the removal of nickel ions from waste water

The nickel ion concentrations studied in these experiments were 0.90; 1.35 and 2.56 mg/L and we used 1, 3 and 5 strains of *Typha angustifolia* in the water treatment process. The results of these experimental investigations were framed in Table 5.4. Samples were taken at 8, 16, 24, 32 and 40 hours contact time.

Figures 5.21., 5.23., 5.25. show graphs showing the variation of nickel ion concentrations in wastewater as a function of the time required for treatment, starting from initial nickel ion concentrations of 0.90, 1.35 and 2.56 mg/L. Figures 5.24., 5.24., 5.26. show the treatment efficiencies recorded during the experiments using 1, 3 and 5 *Typha angustifolia* plants.

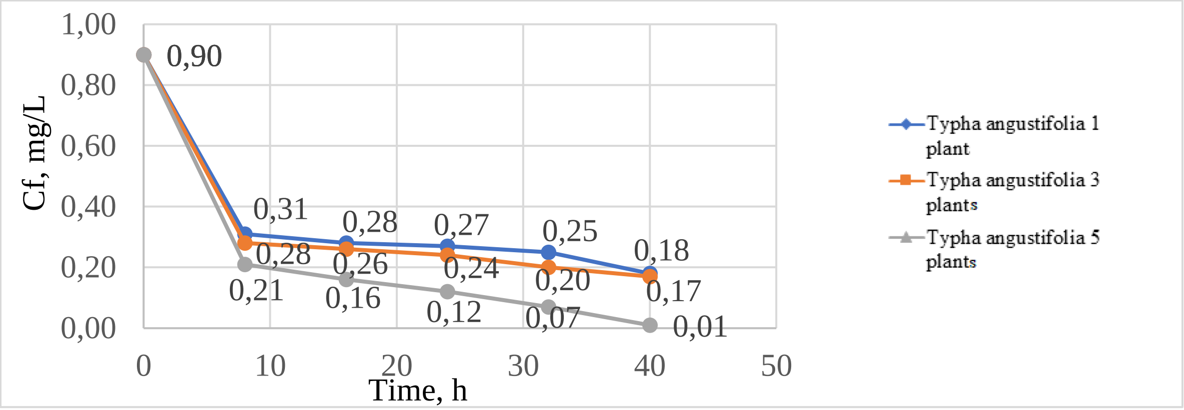

Fig. 5.21. Variation of Ni(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 0.90 mg/L.

Figure 5.21. shows that 5 *Typha angustifolia* plants almost completely removed nickel ions at a concentration of 0.90 mg/L from wastewater in 40 hours of contact time. Using 3 plants and one *Typha angustifolia* plant the equilibrium concentrations of 0.17 and 0.18 mg/L were reached.

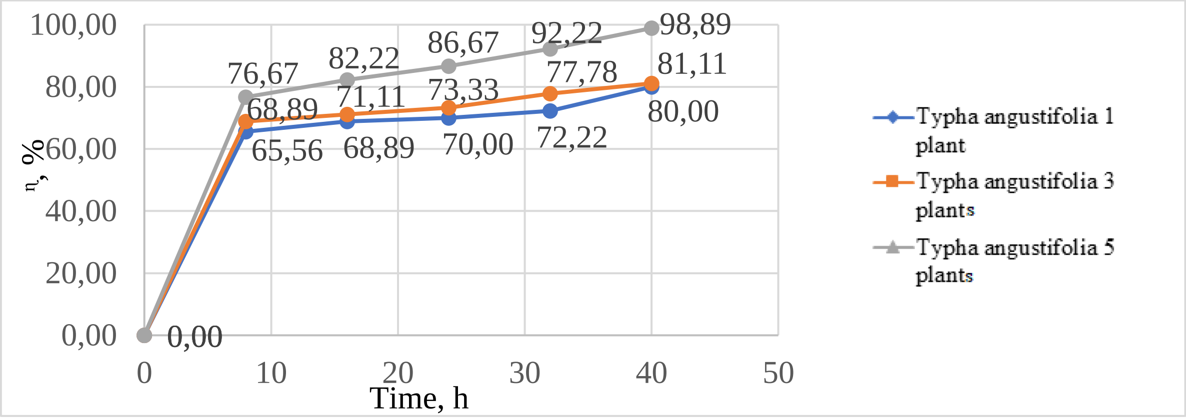

Fig. 5.22. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci = 0.90 mg/L.

According to calculations made considering the values of nickel ions concentrations in water during the treatment process using *Typha angustifolia*, treatment yields of 80.00; 81.11 and 98.89 % were obtained using 1, 3 and 5 Typha angustifolia plants. It can be seen in Figure 5.23. that the treatment process was fast in the first 8 hours reaching efficiencies of 65.56; 68.89 and 76.67 %, and then the efficiencies steadily increased.

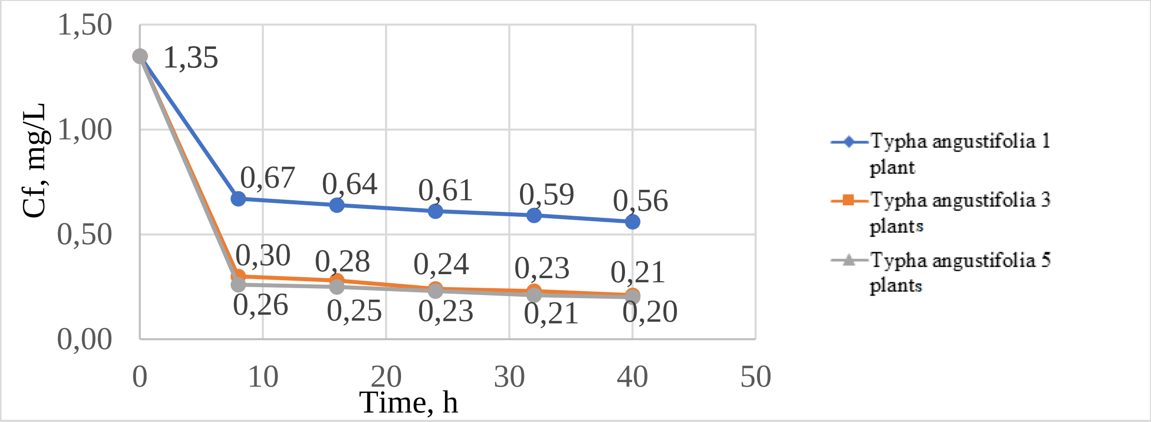

Fig. 5.23. Variation of Ni(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 1.35 mg/L.

The nickel ion concentration of 1.35 mg/L was not completely removed from the wastewater using *Typha angustifolia* plants, but only concentrations of 1.15; 1.14 and 0.79 mg/L of the original concentration when 1, 3 and 5 plants were used. The final concentrations of nickel ions in the wastewater reached values of 0.20; 0.21 and 0.56 mg/L respectively (Figure 5.23.).

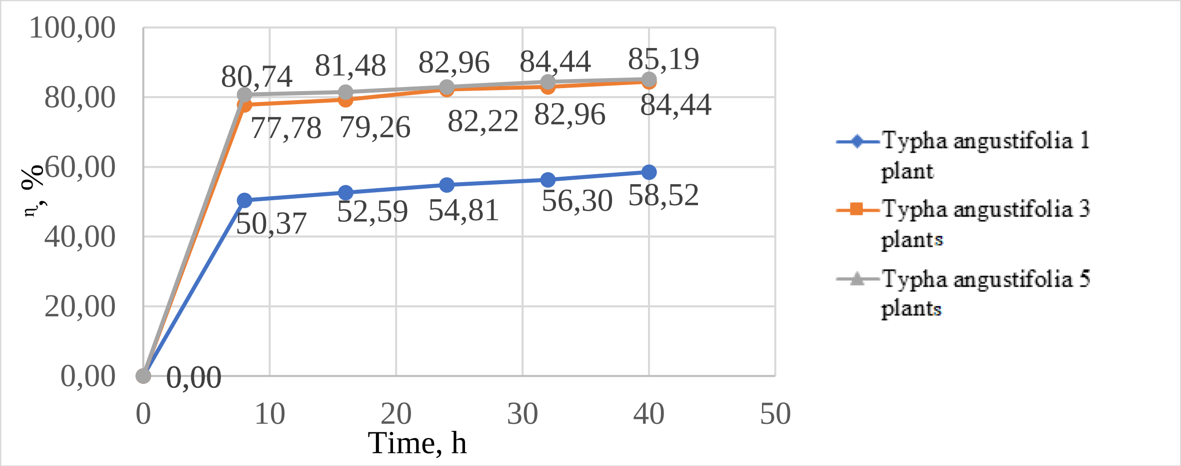

Fig. 5.24. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci = 1.35 mg/L.

Figure 5.24. shows that for the removal of an initial nickel ion concentration of 1.35 mg/L, the treatment efficiencies increased rapidly in the first 8 hours of contact time reaching values of 50.37; 77.78 and 80.74 % using 1, 3 and 5 *Typha angustifolia* plants. However, by the end of the experiment, efficiencies did not reach 100.00%. The highest treatment efficiency achieved in this experiment was 85.19%.

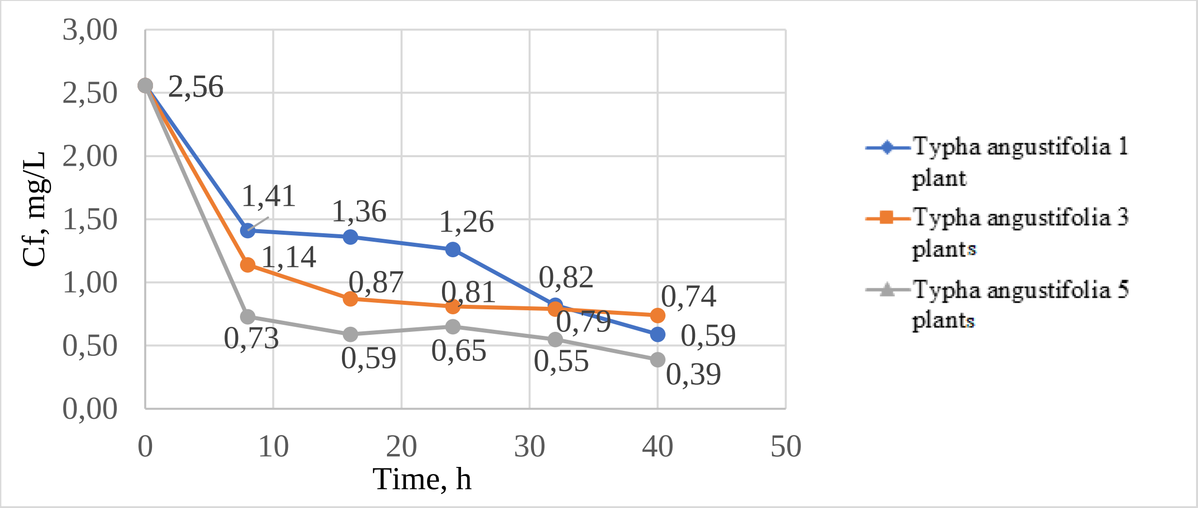

Fig. 5.25. Variation of Ni(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 2.56 mg/L.

By increasing the initial nickel ion concentration to 2.56 mg/L, final concentrations of 0.39, 0.59 and 0.74 mg/L were obtained using 1, 3 and 5 *Typha angustifolia* plants. (Figure 5.25.).

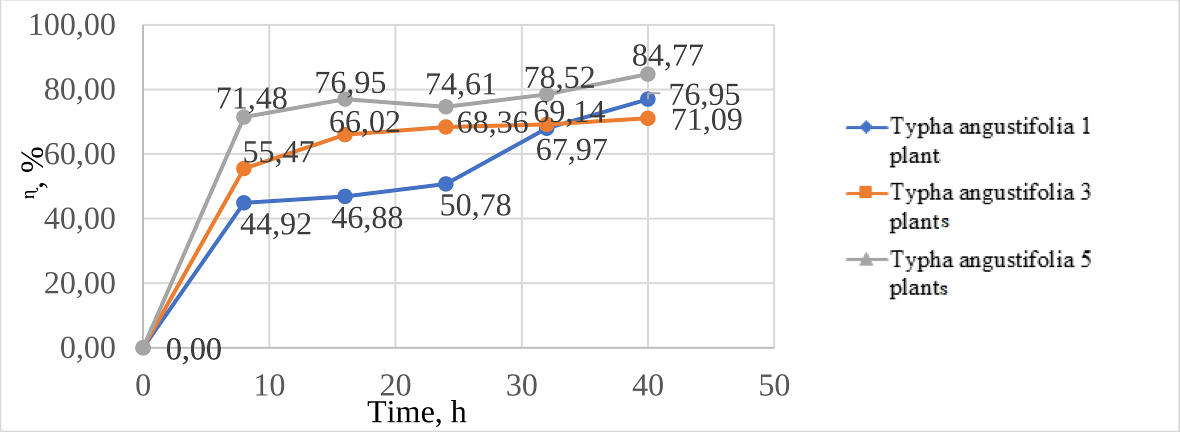

Fig. 5.26. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci = 2.56 mg/L.

According to the calculations made considering the values of nickel ions concentrations in water during the treatment process using *Typha angustifolia*, treatment efficiencies of 76.95%; 71.09% and 84.77% were obtained using 1, 3 and 5 plants at an initial copper ion concentration of 2.56 mg/L (Figure 5.26.).5.1.2.4. Utilizarea plantei *Typha angustifolia* în vederea îndepărtării ionilor de mangan din apele uzate

For these experiments, a volume of wastewater of 2.5 l was used, with initial manganese ion concentrations of 2.00, 1.74 and 1.64 mg/L. The ability of one strain and 3 strains of *Typha angustifolia* to remove manganese concentrations of 2.00 and 1.74 mg/L and 5 strains of *Typha angustifolia* to remove manganese concentrations of 1.64 mg/L from wastewater was tested. The maximum contact time was 40 hours and we sampled every 8 hours (Fig. 5.27., 5.28., 5.29.). The homogenization of the wastewater in contact with the plants was carried out with mechanical stirring system.

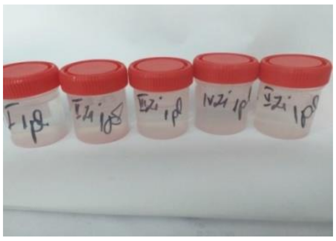

Fig. 5.27. Samples taken when using *Typha angustifolia* plant in the study on wastewater containing Mn^{2+} ions.

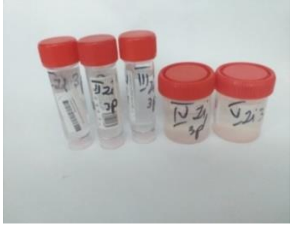

Fig. 5.28. Samples taken using three *Typha angustifolia* plants in the study on wastewater containing Mn^{2+} ions.

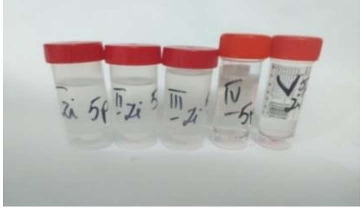

Fig. 5.29. Samples taken using five *Typha angustifolia* plants in the study on wastewater containing Mn^{2+} ions.

Figures 5.30., 5.32., 5.34. plot the determined manganese ion concentrations versus contact time for removal of initial manganese ion concentrations of 2.00; 1.74 and 1.64 mg/L, and Figures 5.31., 5.33., 5.35. The calculated treatment efficiencies versus contact time for the removal of initial manganese ion concentrations of 2.00, 1.74 and 1.64 mg/L, using 1, 3 *Typha angustifolia* plants for the first two initial manganese ion concentrations and 5 plants for the third initial manganese ion concentration of 1.64 mg/L, are plotted.

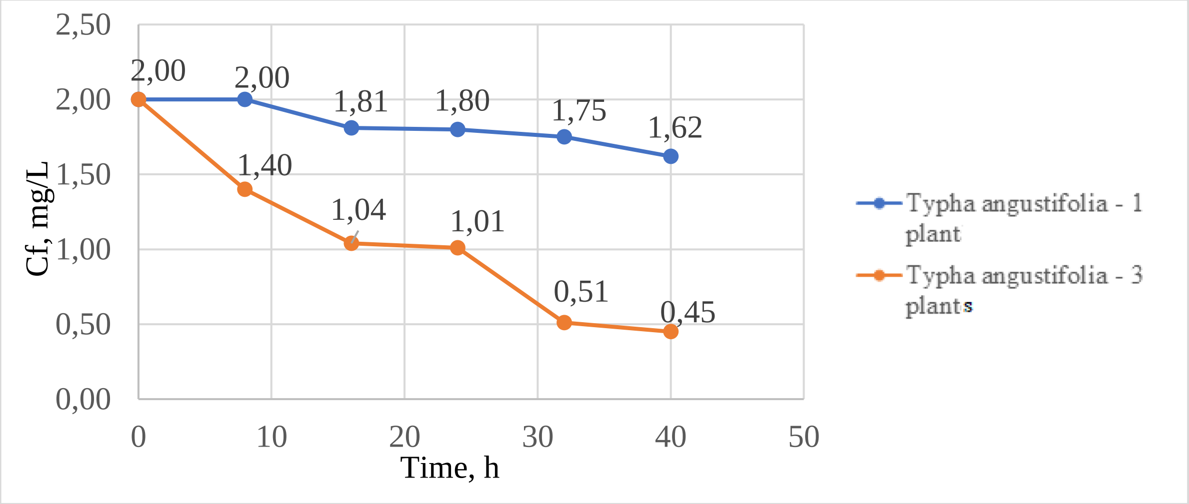

Fig. 5.30. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 2 mg/L.

Figure 5.31. shows that the use of a single *Typha angustifolia* plant is not sufficient to remove a manganese concentration of 2.00 mg/L from wastewater, the equilibrium concentration being only 1.62 mg/L Mn(II). Using a number of 3 *Typha angustifolia* plants the concentration of manganese ions in the wastewater began to decrease rapidly to a final concentration of 0.45 mg/L. Furthermore, after 8 hours of contact, 3 *Typha angustifolia* plants removed more manganese ion concentration than was removed after 40 hours of contact with a single *Typha angustifolia* plant.

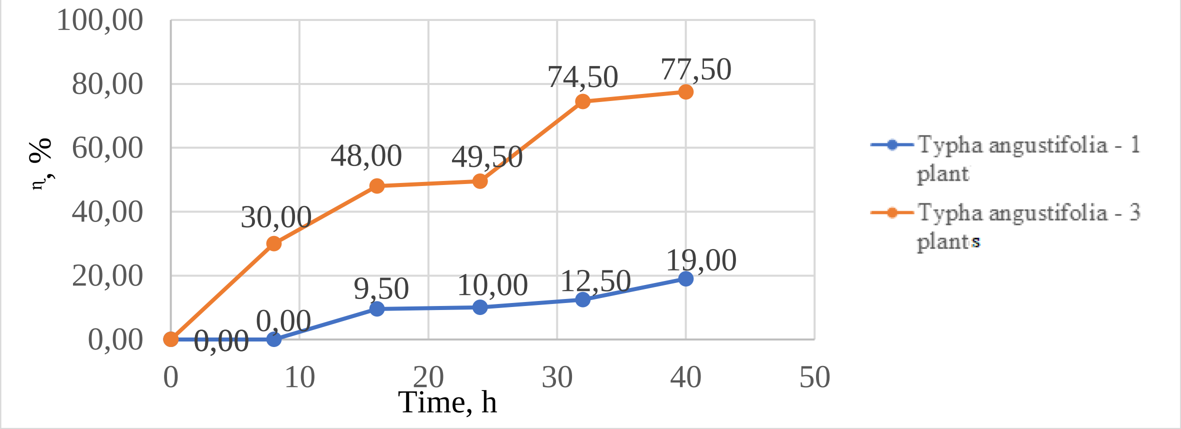

Fig. 5.31. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci = 2 mg/L.

The Mn(II) ion retention capacity using *Typha angustifolia* plants increases with increasing contact time (8, 16, 24, 32, 40 hours) of the plant with wastewater. However, it is observed that the treatment process is faster using more *Typha angustifolia plants*. The yield obtained after 40 hours of contact was 77.50%, as shown in the graph in Figure 5.3.1.

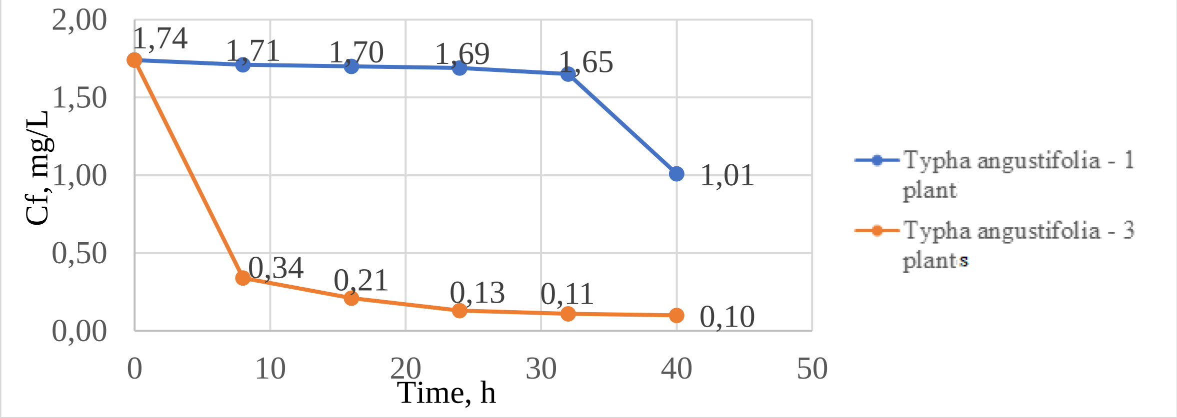

Fig. 5.32. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 1.74 mg/L.

Also, a single *Typha angustifolia* plant could not remove a concentration of 1.74 mg/L of manganese ions from wastewater, the equilibrium concentration being only 1.01 mg/L Mn(II). Using a number of 3 *Typha angustifolia* plants the concentration of manganese ions in the wastewater started to decrease rapidly to the final concentration of 0.10 mg/L. Furthermore, after 8 hours of contact, 3 *Typha angustifolia* plants removed more manganese ion concentration (in 8 hours the concentration of 0.34 mg/L was reached) than was removed after 40 hours of contact with a single *Typha angustifolia* plant, reaching the final concentration of 1.01 mg/L (Figure 5.32.).

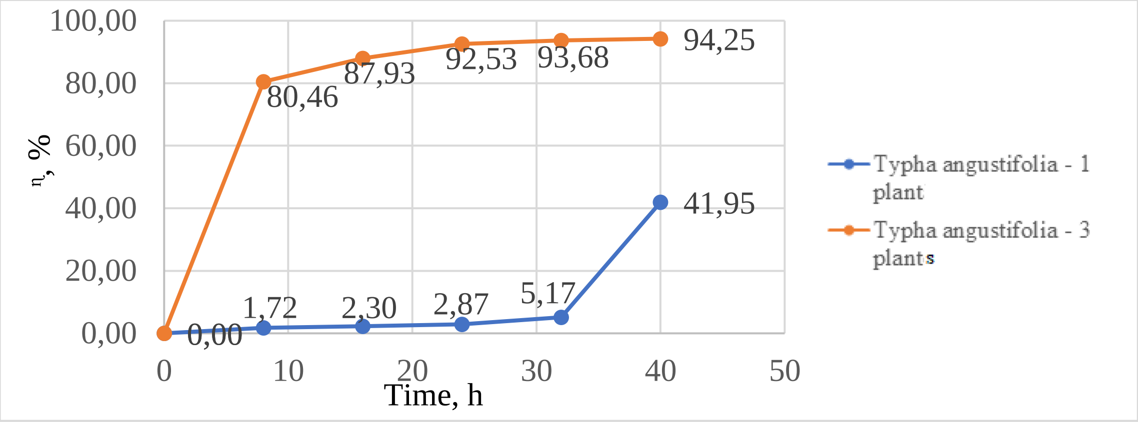

Fig. 5.33. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci = 1.74 mg/L.

Figure 5.33. shows that the treatment process is faster and more efficient using 3 *Typha angustifolia* plants. The treatment efficiency reached the value of 41.95 % when one plant was used and when 3 plants were used the treatment efficiency in 40 hours of contact time was double, 94.25 %, for the removal of manganese ions from wastewater.

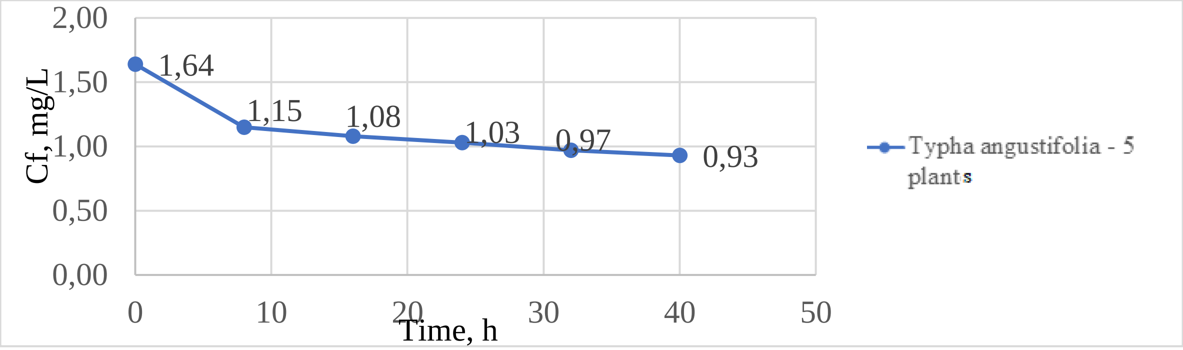

Fig. 5.34. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 1.64 mg/L.

Figure 5.34. shows that the concentration of manganese ions in wastewater steadily decreases using 5 *Typha angustifolia* plants, but after 40 hours of contact time the final concentration reached only 0.93 mg/L.

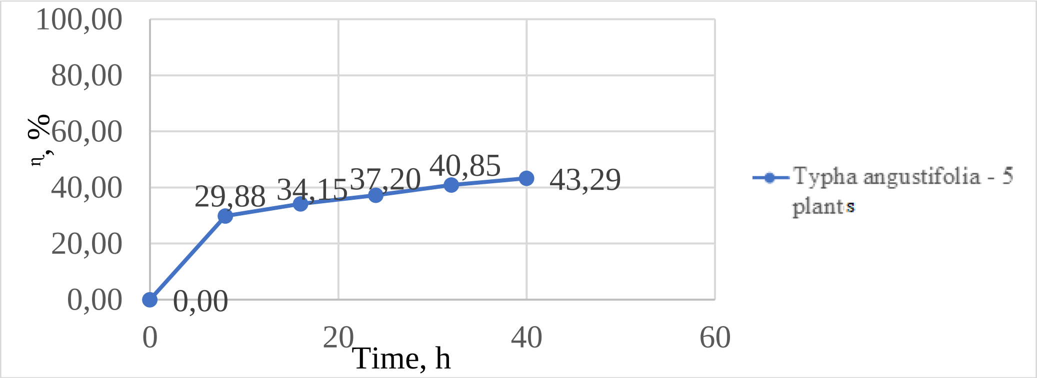

Fig. 5.35. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci = 1.64 mg/L.

Taking into account the concentrations plotted in Figure 5.35., the treatment yields over the whole period of the experiment were calculated. It can be seen that the treatment efficiency reached a value of only 43.29 % when trying to remove a concentration of 1.64 mg/L Mn(II) from the wastewater using 5 *Typha angustifolia* plants.

Compared to previous experiments, it is observed that this is a rather small value. The explanation could be that the plants differ from one to another and there is a strong possibility that the plants used in this experiment were already charged with other ions from the environment from which these plants were taken.

5.1.2.5. Use of *Typha angustifolia* plant for simultaneous removal of copper and nickel ions from wastewater

To observe the number of plants required to remove metal ions from water, 1, 3 and 5 *Typha angustifolia* plants were used in the experimental investigations. The initial concentrations of copper and nickel ions in the wastewater were 1.14 and 0.74 mg/L, respectively.

The maximum time required to treat wastewater containing copper and nickel ions was 40 hours. Variations of metal ion concentrations and treatment efficiency as a function of time are shown in Figures 5.36., 5.37. and in Figures 5.38., 5.39. respectively..

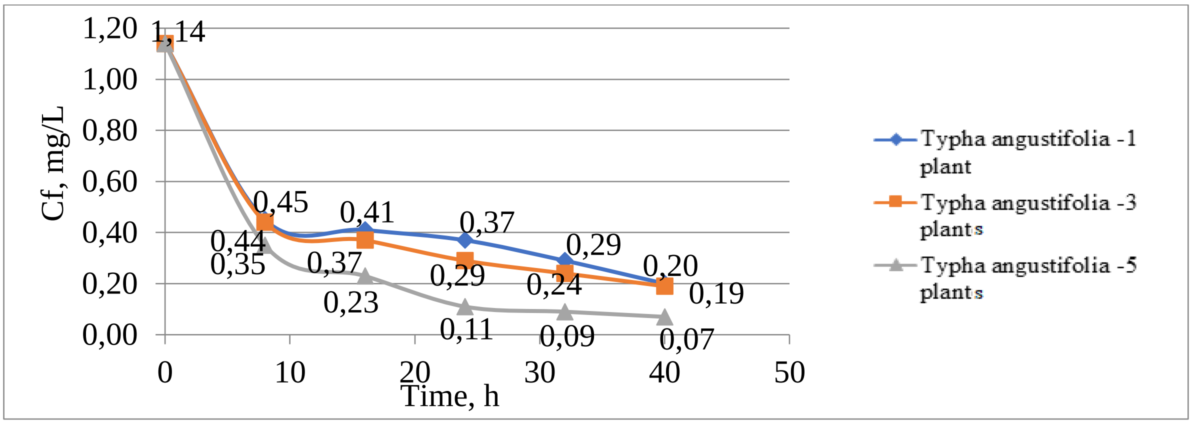

Fig. 5.36. Variation of Cu(II) ions in wastewater over time in a treatment system containing *Typha angustifolia* for Ci = 1.14 mg/L.

In the case of removal of copper ions from wastewater using *Typha angustifolia*, a rapid decrease in metal ion concentrations is observed within the first 8 hours of contact. Thus, copper ion concentrations reached concentrations of 0.45; 0.44 and 0.35 mg/L using 1, 3 and 5 *Typha angustifolia* plants in 480 minutes. The final concentrations of copper ions in wastewater were 0.20; 0.19 and 0.07 using 1, 3 and 5 *Typha angustifolia plants* (Fig. 5.36.).

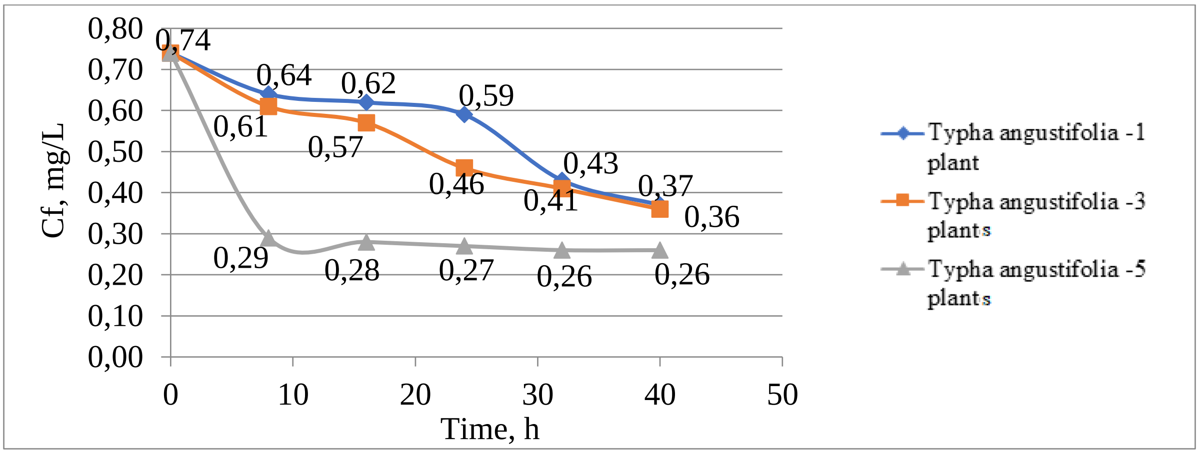

Fig. 5.37. Variation of Ni(II) ions in wastewater over time in treatment system containing *Typha angustifolia* for Ci = 0.74mg/L.

In the case of removal of nickel ions from wastewater using the *Typha angustifolia* plant, a rapid decrease in the concentration of metal ions is observed in the first 8 hours of contact time only when using five plants, the concentration reaching 0.29 mg/L. Then, the nickel ion concentration stabilized, finally reaching a concentration of 0.26 mg/L. When one and three *Typha angustifolia* plants were used, the nickel ion concentrations in the wastewater gradually decreased to concentrations of 0.37 and 0.36 mg/L over a period of 40 hours, respectively (Fig. 5.37.).

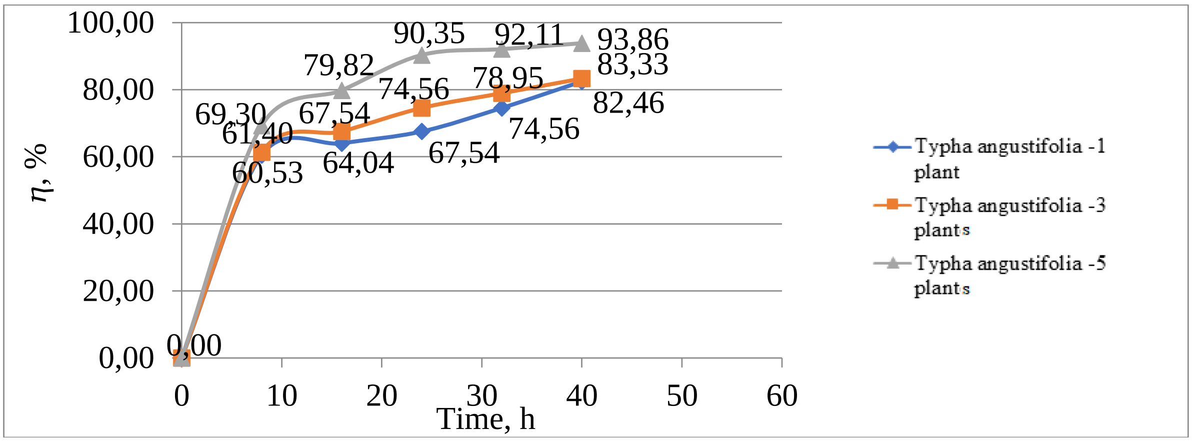

Fig. 5.38. Variation of treatment yield over time for wastewater containing Cu(II) ions, Ci = 1.14 mg/L.

The removal efficiency of copper ions from wastewater increased rapidly, reaching yields of 60.53; 61.40 and 69.30% in the first 8 hours of contact time using 1, 3 and 5 *Typha angustifolia* plants. At the end of the phytoremediation process, treatment yields reached values of 82.46, 83.33 and 93.86% (Fig. 5.38.). The rapid uptake in the first hours of contact may be due to the existence of free uptake sites in the plant. The process started to slow down slightly as more absorption sites were occupied by metal ions.

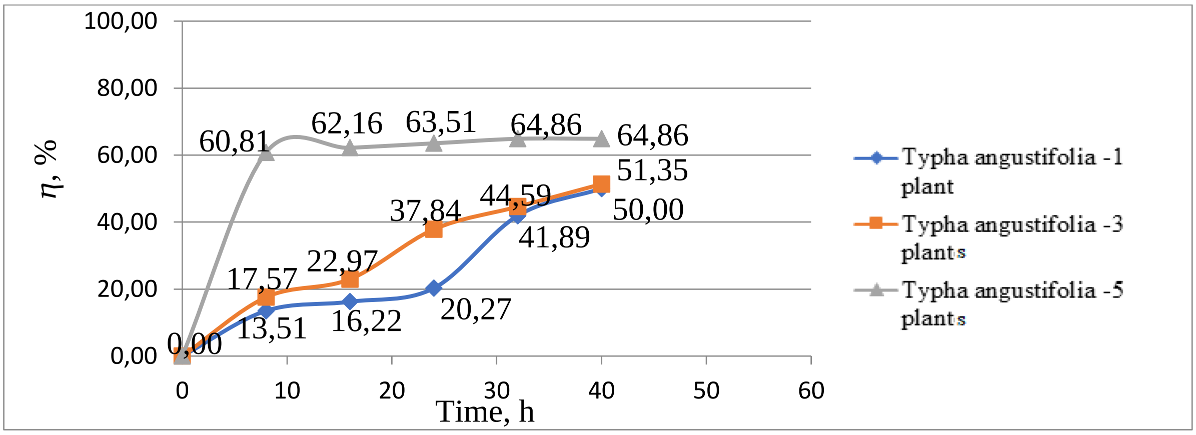

Fig. 5.39. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci = 0.74mg/L.

The removal efficiency of nickel ions from wastewater reached percentages of 50.00; 51.35 and 64.86 respectively using 1, 3 and 5 *Typha angustifolia* plants (Fig. 5.39.). Using 5 plants, a rapid increase in treatment efficiency was observed in the first 8 hours, with the treatment yield slightly exceeding 60%.

Figure 5.40. shows graphically the differences in treatment efficiencies for the removal of copper and nickel ions from wastewater.

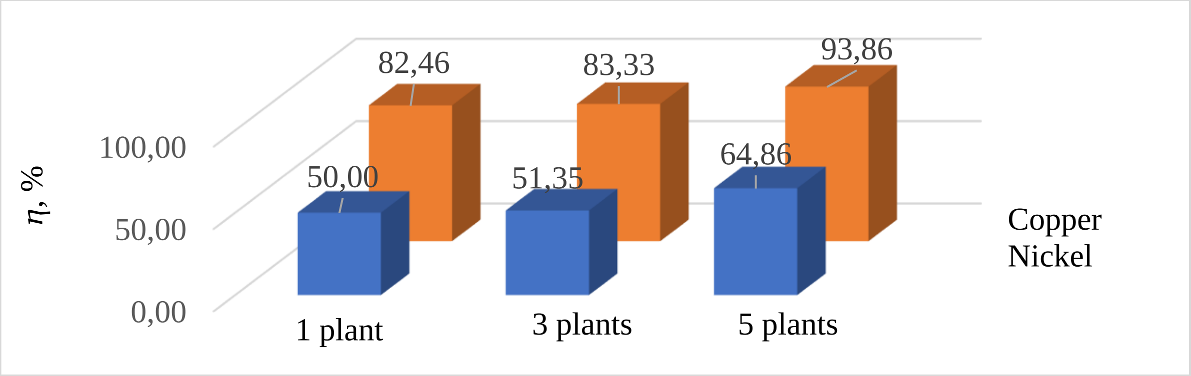

Fig. 5.40. Differences in removal efficiencies of copper and nickel ions from wastewater.

From Figure 5.40. it can be seen that *Typha angustifolia* was much more receptive to copper ions and less receptive to nickel ions. The differences between the treatment efficiencies, observing the two cases, were about 30%. Nickel ions were removed 64.86% from the wastewater and copper ions were removed 93.86% when 5 *Typha angustifolia* plants were used. When using a single plant, the removal percentages are about 15% lower than when using 5 *Typha angustifolia* plants.

5.1.3. Conclusions

Typha angustifolia is a plant that can be used in phytoremediation to remove metal ions from wastewater. It is very effective and does not require high costs for water treatment.

Typha angustifolia was found to have an efficiency of 19% and 77.5%, respectively, in capturing Mn(II) ions, this capacity varying according to the number of plants applied in the experiment, the growing season, the season and the type of pollutant (wastewater composition).

The results show a gradual decrease in metal concentration throughout the 40 h period, suggesting that *Typha angustifolia* has reached maximum saturation, although yields at 1 and 5 strains respectively did not exceed 42% unlike 3 plant strains where the yield was 94.25%.

From the experiments that were conducted for the removal of two pollutants at the same time from wastewater, it was observed that *Typha angustifolia* plants were more efficient in the removal of copper ions than in the removal of nickel ions from wastewater, with the highest removal efficiency reaching 93.86%. In contrast, a 64.86% was obtained when the removal of nickel ions from wastewater was investigated. The required treatment time was 40 hours. In the case of removal of copper ions from wastewater, the process was fast in the first 480 minutes of contact time.

5.2. Algae used for water treatment

5.2.1. Theoretical part

Many treatment methods have been studied and applied for the removal of metal ions from wastewater, such as coagulation/flocculation, ion exchange, photocatalysis, flotation, electro-remediation, solvent extraction, biological sludge and others [306, 307]. The use of biomass sources as absorbent materials for water treatment supports circular economy systems and environmental sustainability [310 - 312]. Algae have been studied using simulated wastewater and showed high water treatment efficiencies by removing heavy metal ions [313].

The biological mechanisms (Fig. 5.41.) for the removal of heavy metals by algae are classified into three main mechanisms:

(i) extracellular precipitation/accumulation of heavy metals;

(ii) complexation or cellular uptake;

(iii) intracellular uptake [316].

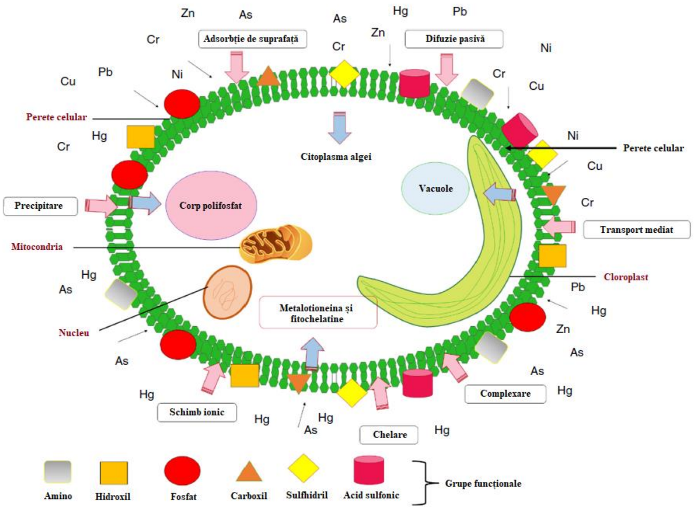

Fig. 5.41. Mechanism of heavy metal uptake in microalgae [316].

In recent years, particular attention has been paid to the application of biotechnology for the removal of heavy metal ions from wastewater. Biosorption is an alternative process using certain natural materials of biological origin, including fungi, bacteria, algae, yeasts, etc. Biosorbents have metal ion-trapping properties and can be used even when metal ion concentrations in wastewater are very low.

Enteromorpha is a green alga belonging to the class *Chlorophyceae* and order Ulvales [328]. The genus *Enteromorpha comprises* different species of green algae, namely E. *prolifera*, E. *linza*, E. *intestinalis*, E. *compressa* and E. *flexuosa*. E. *prolifera* has been shown to be the dominant species in the Chinese Yellow Sea [329]. It has been used as a functional food and traditional medicine [330 - 334].

5.2.2. Experimental part

Experimental research using *Sargassum fusiforme* and *Enteromorpha prolifera* algae is based on the use of a treatment system (Fig. 5.43.) consisting of the following components: (1) container, (2) waste water, (3) algae, (4) stand, (5) mechanical agitator.

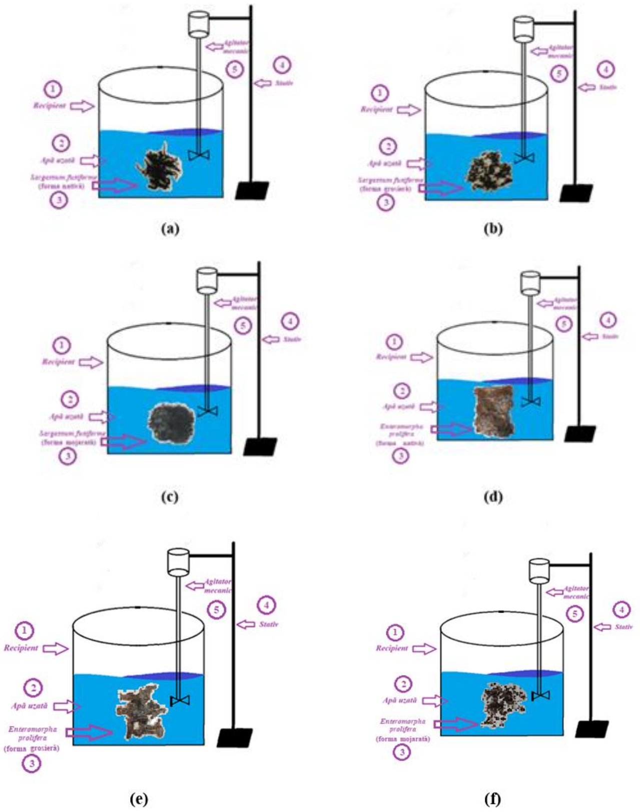

Fig. 5.42. Treatment system consisting of: (1) container, (2) waste water, (3) algae ((a) *Sargasssum fusiforme* - native form, (b) *Sargasssum fusiforme* - coarse form, (c) *Sargasssum fusiforme* - ground form, (d) *Enteromorpha prolifera* - native form, (e) *Enteromorpha prolifera* - coarse form, (f) *Enteromorpha prolifera* - ground form), (4) stand, (5) mechanical agitator.

During the experiments, the concentration of heavy metal ions in the wastewater was analysed for a clearly defined period of time (0-10 hours) and the standard treatment value was calculated for each analysis. The influence of operational parameters on the efficiency of the treatment process was studied.

Fig. 5.43. *Sargassum fusiforme (native form*).

Fig. 5.44. *Sargassum fusiforme (coarse form*).

Fig. 5.45. *Sargassum fusiforme* (ground form).

Fig. 5.46. *Enteromorpha (Ulva) prolifera (native form).*

Fig. 5.47. *Enteromorpha (Ulva) prolifera (coarse form).*

Fig. 5.48. *Enteromorpha (Ulva) prolifera (ground form).*

To remove copper, lead, manganese and nickel ions from the wastewater, 1 g of algae was used for each experiment. The initial concentration of copper ions studied was 2.05 mg/L, that of manganese ions was 1.64 mg/L, that of lead ions was 1.86 mg/L, for nickel ions two concentrations were studied, namely 1.30 and 2.74 mg/L. The phytoremediation process was carried out under continuous stirring at room temperature. Samples were taken every 2 hours and prepared for concentration determination using the PhotoLab S12 photometer.

Taking into account the determined concentrations, treatment efficiencies were calculated using the formula:

$$\eta = \frac{C_i - C_f}{C_i} * 100 \qquad (15)$$

unde: η - represents the treatment yield, %;

Ci- represents the initial concentration of copper, manganese, lead or nickel ions, mg/L;

Cf - represents the final concentration of copper, manganese, lead or nickel ions, mg/L.

5.2.2.1. Use of algae to remove copper ions from wastewater

The concentration of copper ions studied in these experiments was 2.05 mg/L and we used 1 g of *Sargassum fusiforme* algae in the water treatment process.

Figure 5.49. plots the variation of wastewater copper ion concentrations as a function of treatment time, starting from an initial copper ion concentration of 2.05 mg/L. Figure 5.50. also plots the treatment efficiencies recorded during the experiments using *Sargassum fusiforme* algae.

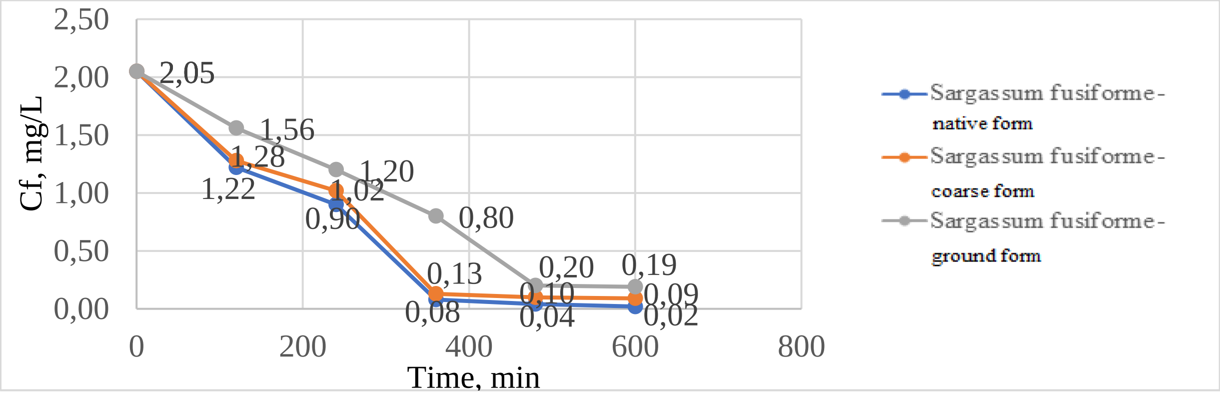

Fig. 5.49. Concentrations of copper ions in wastewater during experiments starting from the initial concentration of 2.05 mg/L using *Sargassum fusiforme* algae for treatment.

It can be seen in Figure 5.49. that *Sargassum fusiforme* algae were able to largely remove copper ion concentrations from wastewater. The final concentrations reached values of 0.02, 0.09 and 0.19 mg/L using the native, coarse and ground forms of *Sargassum fusiforme* algae, respectively.

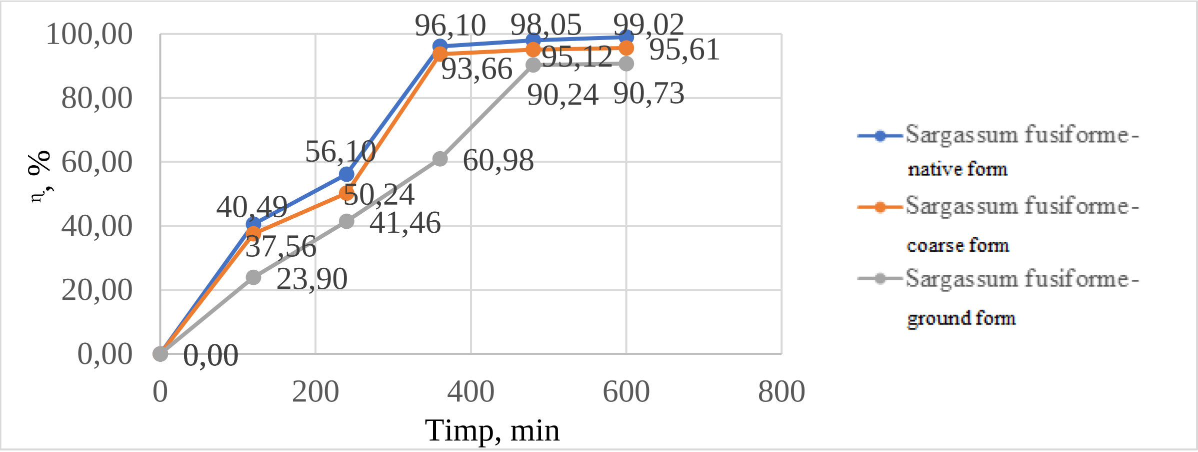

Fig. 5.50. Treatment yields obtained over a 10-hour contact time period for the removal of copper ions from wastewater with an initial concentration of 2.05 mg/L using *Sargassum fusiforme* algae.

According to the calculations made taking into account the values of copper ion concentrations in water during the treatment process using Sargassum fusiform algae, treatment efficiencies of 99.02, 95.61 and 90.73 % were obtained using the native, coarse and ground form of Sargassum fusiform algae respectively (Figure 5.51.).

5.2.2.2. Use of algae to remove manganese ions from wastewater

In Figures 5.51. and 5.52. show graphically the results obtained from the experimental investigation on the removal of manganese ions from wastewater using *Sargassum fusiforme* algae in three forms, namely the native form, the coarse form and the ground form. Figure 5.51. shows graphically the values of manganese ion concentrations determined from samples taken every 2 hours and Figure 5.52. shows graphically the treatment yields calculated using the yield formula according to equation (15).

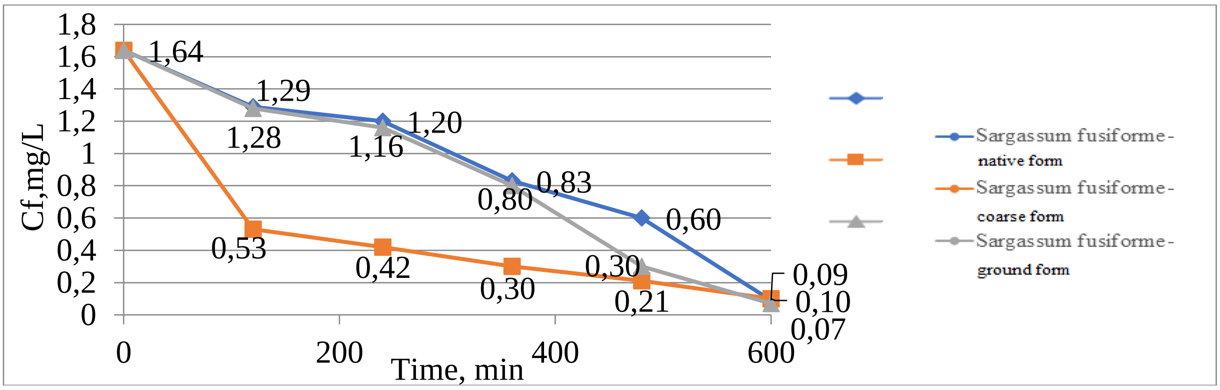

Fig. 5.51. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Sargassum fusiforme* for Ci = 1.64 mg/L.

Figure 5.52. shows the decrease in the concentration of manganese ions in the wastewater from the initial concentration of 1.64 mg/L to the final concentrations of 0.09; 0.10 and 0.07 respectively when *Sargassum fusiforme* algae was used in native, coarse and

ground form respectively. Concentrations gradually decreased over a 600 min contact time period. A more rapid decrease in manganese ion concentration is observed during the first 120 minutes of contact time when using the coarse form of the algae studied..

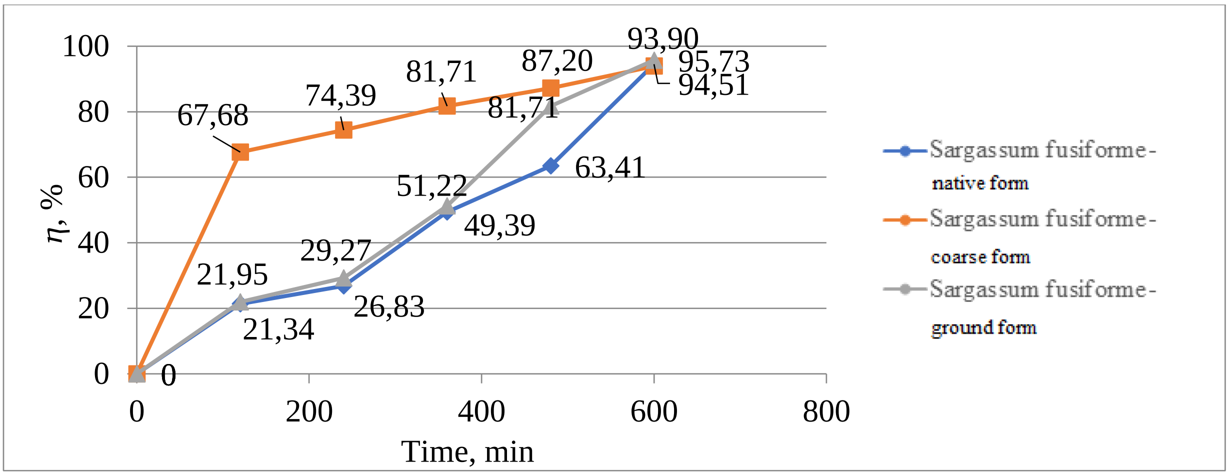

Fig. 5.52. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci = 1.64 mg/L.

The increase in treatment efficiency through the phytoremediation process is shown in Figure 5.53. At the end of the phytoremediation process, the highest treatment efficiency was obtained using *Sargassum fusiforme* algae in ground form, i.e. 95.73%. Also, at the end of the process, in all three cases, the yields were similar, i.e. 93.90; 94.51 and 95.73 %, using *Sargassum fusiforme* algae in coarse, native and ground form, respectively.

Figure 5.54. plotted the manganese ion concentrations determined throughout the phytoremediation process, and Figure 5.55. showed the treatment efficiencies using *Enteromorpha prolifera* algae in three forms, namely native form, coarse form and ground form.

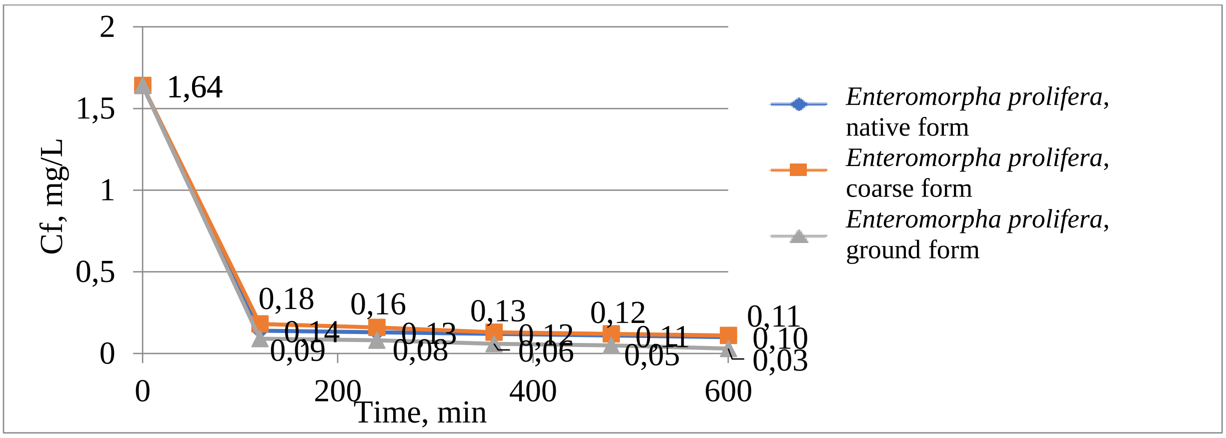

Fig. 5.53. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Enteromorpha prolifera* for Ci = 1,64 mg/L

The use of the alga *Enteromorpha prolifera*, in all forms presented above, showed rapid removal of manganese ions from wastewater within the first 120 minutes of contact time, reaching manganese ion concentrations of 0.14; 0.18 and 0.09 mg/L from an initial concentration of 1.64 mg/L Mn^{2+}. By the end of the phytoremediation process, manganese ion concentrations in the water gradually decreased to concentrations of 0.10; 0.11 and 0.03

mg/L, respectively, using the alga *Enteromorpha prolifera* in three forms, namely the native form, the coarse form and the ground form (Fig. 5.53.).

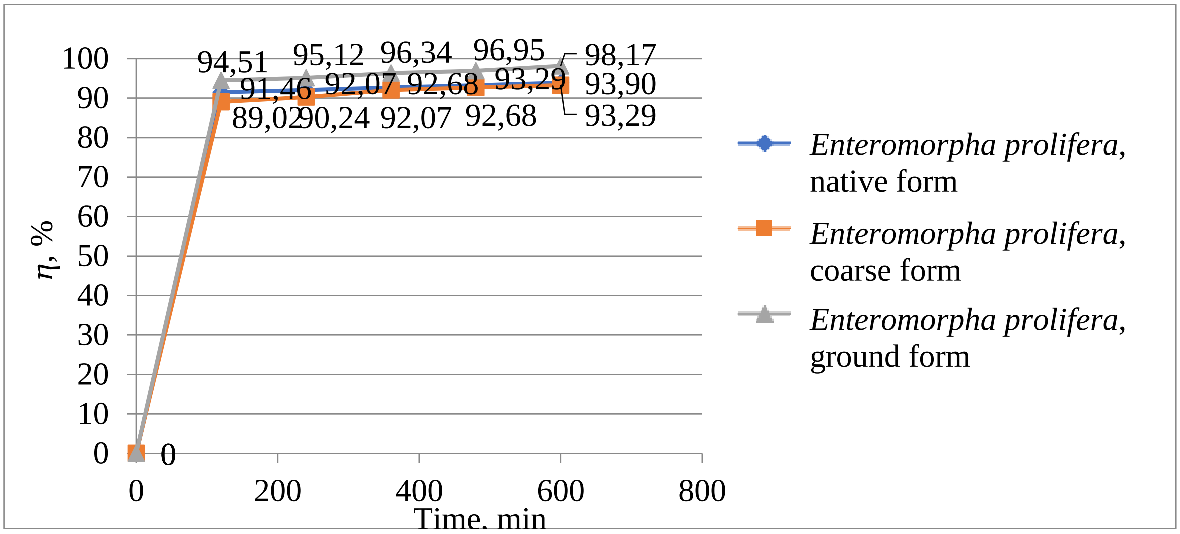

Fig. 5.54. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci = 1.64 mg/L.

It can be seen from Figure 5.54. that the treatment yields quickly reached the values of 91.46; 89.02 and 94.51% in the first 120 minutes of contact time using Enteromorpha prolifera algae in three forms, namely native form, coarse form and ground form. Treatment yields gradually reached 93.90; 93.29 and 98.17 % in 600 minutes of contact time, respectively.

The comparison of the percentage of treatment achieved using the two algae, in the three forms studied, for the removal of manganese ions from wastewater was shown in Figure 5.55.

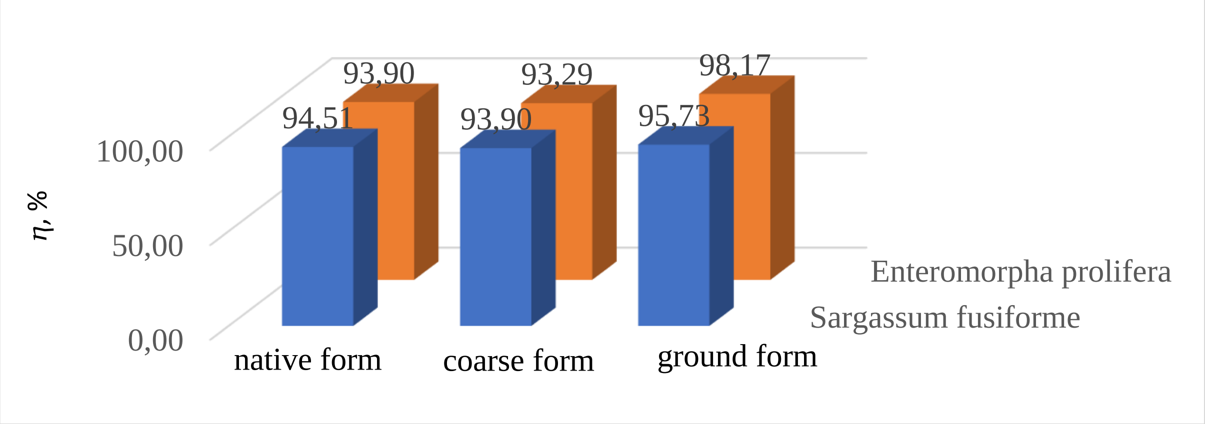

Fig. 5.55. Comparison of manganese ion water treatment efficiencies using the algae *Sargassum fusiforme* and *Enteromorpha prolifera.*

Comparing treatment yields for the removal of manganese ions from wastewater using *Sargassum fusiforme* and *Enteromorpha prolifera* algae, the results are quite similar, with yields ranging from 93.90% using *Enteromorpha prolifera* in coarse form to 98.17% using *Enteromorpha prolifera* in ground form. The highest treatment yield obtained using *Enteromorpha prolifera* algae was 98.17%.

5.2.2.3. Use of algae to remove lead ions from wastewater

The removal of lead ions from wastewater was studied using the same algae presented above, namely *Sargassum fusiforme* and *Enteromorpha prolifera*. The results obtained were presented graphically in Figures 5.56. and 5.58. representing the variation of lead ion concentrations over time and in Figures 5.57. and 5.59. showing the variation of treatment yields over time.

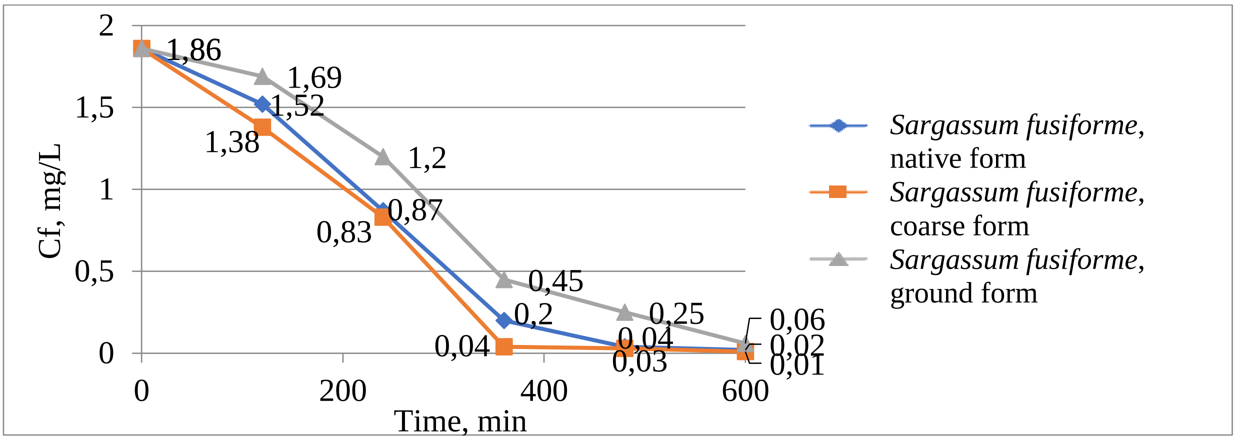

Fig. 5.56. Variation of Pb(II) ions in wastewater over time in a treatment system containing *Sargassum fusiforme* for Ci =1.86 mg/L.

Figure 5.56. shows the gradual decrease of lead ion concentrations in wastewater from the initial concentration of 1.84 mg/L to the final concentrations of 0.02; 0.01 and 0.06 over 600 minutes of contact time when using *Sargassum fusiforme* algae in native, coarse and ground form, respectively.

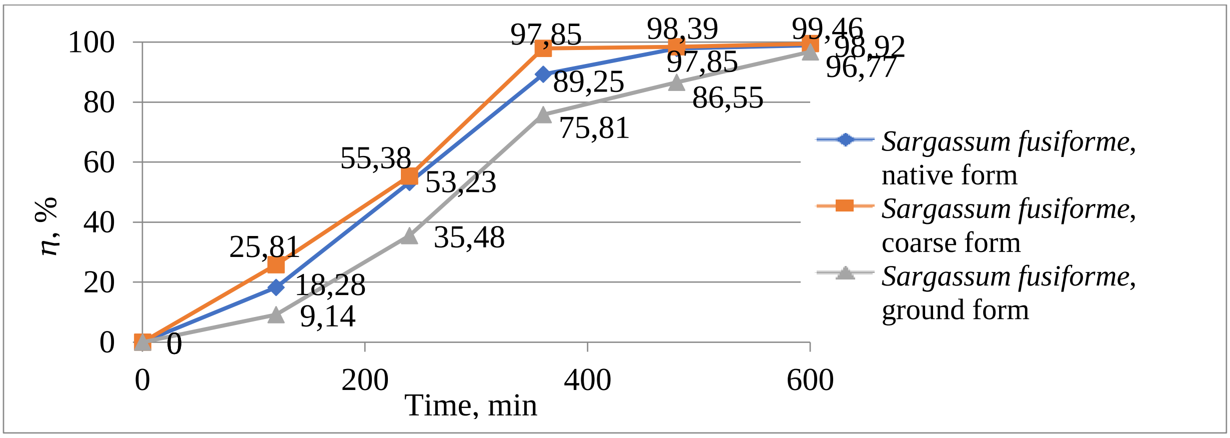

Fig. 5.57. Variation of treatment yield over time for wastewater containing Pb(II) ions, Ci = 1.86 mg/L.

The increase in treatment efficiency by the phytoremediation process for the removal of lead ions from wastewater is shown in Figure 5.57. At the end of the process, the treatment efficiency closest to the maximum efficiency (i.e. 99.46%) was achieved using coarse form of Sargassum fusiform algae. However, in all three cases, the treatment efficiencies were similar, i.e. 99.46, 98.92 and 96.77 % using *Sargassum fusiforme* algae in coarse, native and ground form, respectively.

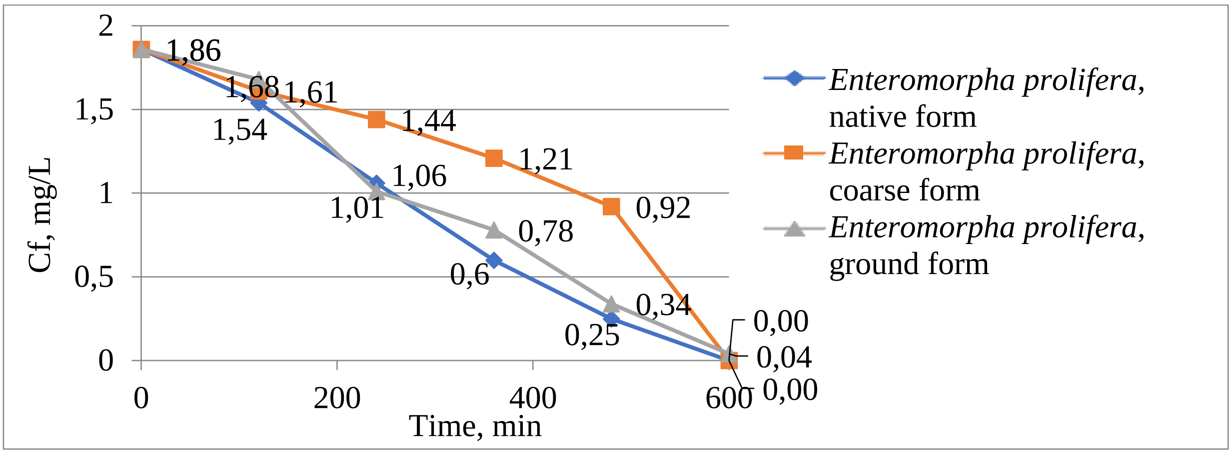

Fig. 5.58. Variation of Pb(II) ions in wastewater over time in a treatment system containing *Enteromorpha prolifera*, for Ci =1.86 mg/L.

Lead ion concentrations gradually decreased until lead ions were completely removed from wastewater when *Enteromorpha proliferfera* was used in its native, coarse form. The final concentration of lead ions in wastewater when using *Enteromorpha prolifera* algae in its coarse form was 0.04 mg/L (Fig. 5.58).

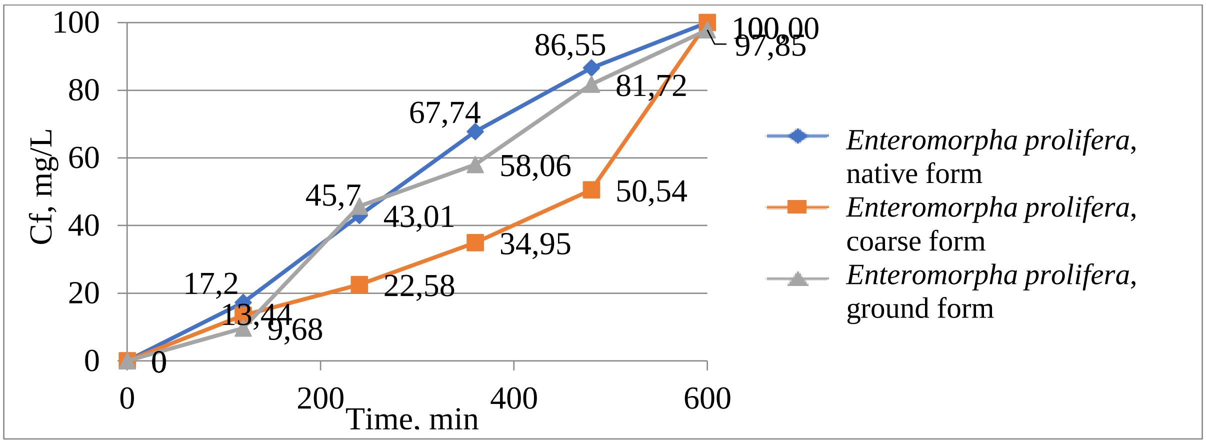

Fig. 5.59. Variation of treatment yield over time for wastewater containing Pb(II) ions, Ci = 1.86 mg/L.

It can be seen from Figure 5.59. that the maximum treatment yields were obtained using *Enteromorpha prolifera* algae in native and coarse form in a period of 600 minutes contact time.

The comparison of the percentage of treatment obtained using the two types of algae presented above, in the three forms studied, for the removal of lead ions from wastewater has been represented in Figure 5.60.

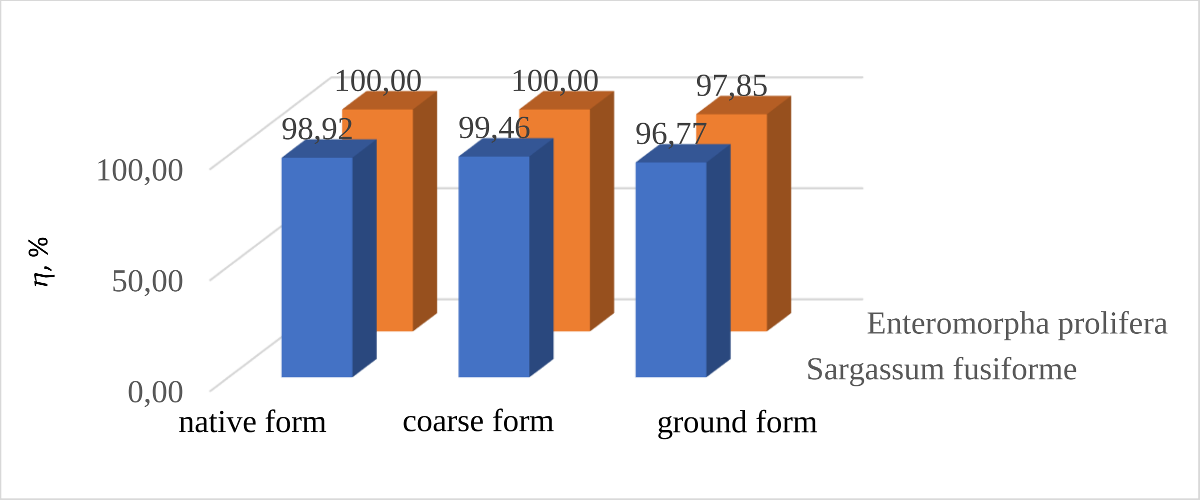

Fig. 5.60. Comparison of lead ion water treatment yields using the algae *Sargassum fusiforme* and *Enteromorpha prolifera*.

In the case of removal of lead ions from wastewater, comparing the results, the treatment efficiency was in all three cases higher when using *Enteromorpha prolifera* algae than when using *Sargassum fusiforme* algae.

5.2.2.4. Use of algae to remove nickel ions from wastewater

The removal of nickel ions from wastewater has been studied using the same algae presented above, namely *Sargassum fusiforme* and *Enteromorpha prolifera*. The results obtained were presented graphically in Figures 5.61. and 5.63. representing the variation of lead ion concentrations over time and in Figures 5.62. and 5.64. showing the variation of the treatment yields over time.

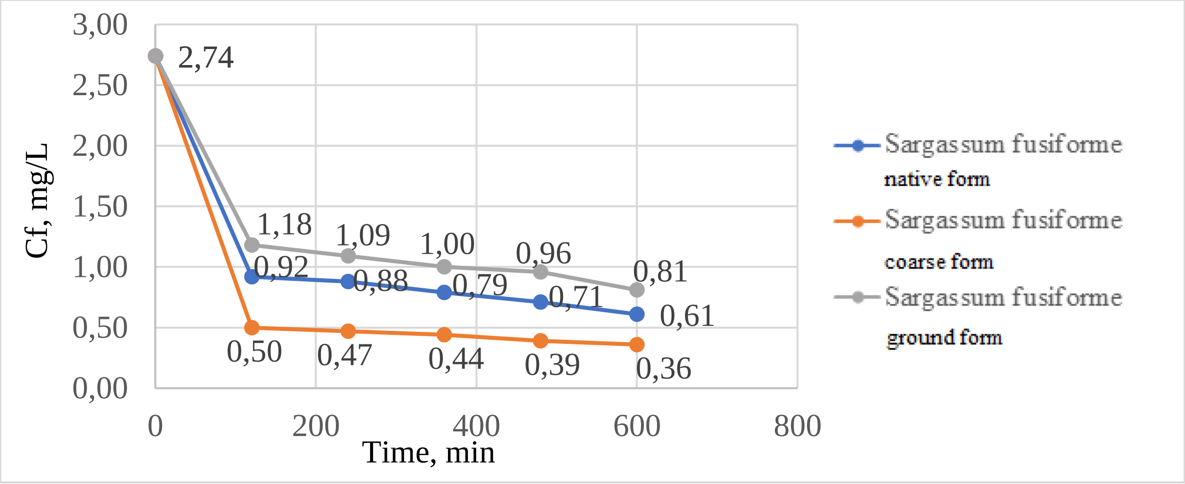

Fig. 5.61. Variation of Ni(II) ions in wastewater over time in a treatment system containing *Sargassum fusiforme* for Ci = 2.74mg/L.

Figure 5.61. shows the gradual decrease in wastewater nickel ion concentrations from the initial concentration of 2.74 mg/L to final concentrations of 0.61; 0.36 and 0.81 over 600 minutes of contact time when using *Sargassum fusiforme* algae in native, coarse and ground form, respectively.

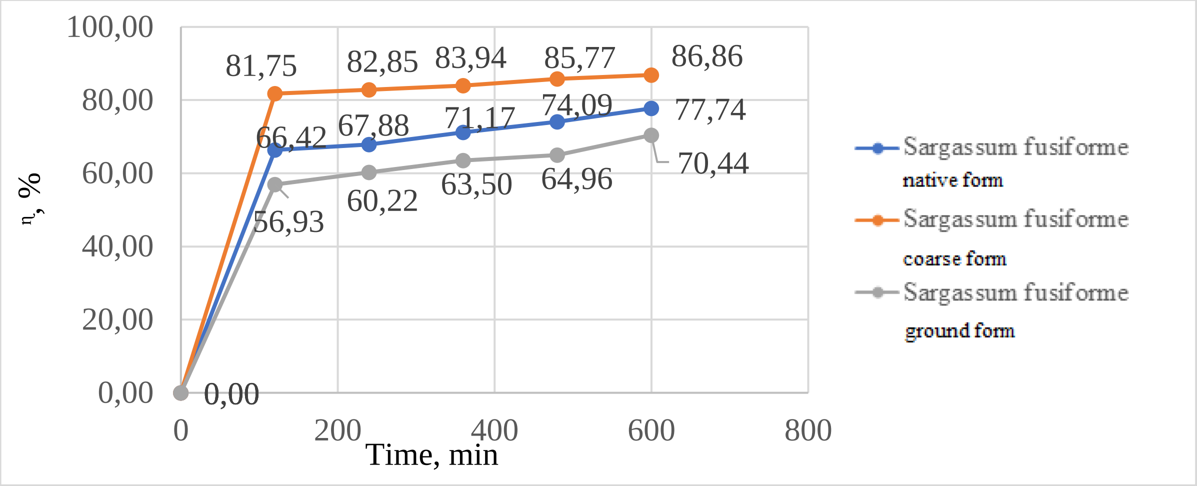

Fig. 5.62. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci =2.74mg/L.

The increase in treatment efficiency by the phytoremediation process for the removal of nickel ions from wastewater is shown in Figure 5.62. At the end of the process, the highest treatment efficiency obtained from these experiments (i.e. 86.86%) was obtained using *Sargassum fusiform* algae in coarse form. Using the native and ground forms of *Sargassum fusiform* algae, efficiencies of 77.74% and 70.44% were obtained.

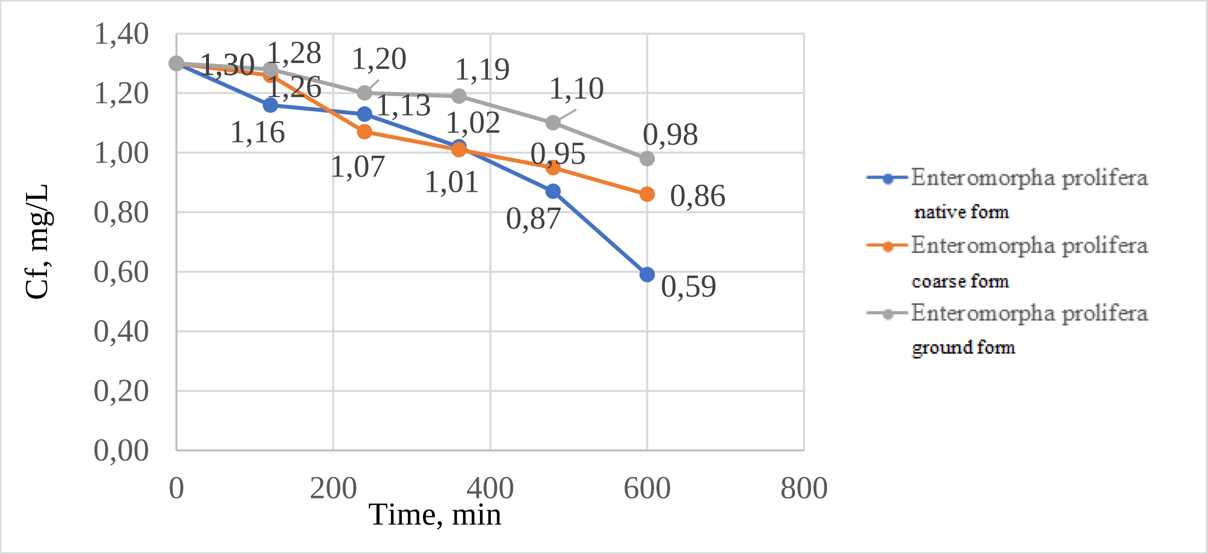

Fig. 5.63. Variation of Ni(II) ions in wastewater over time in a treatment system containing *Enteromorpha prolifera* for Ci =1.30 mg/L.

Figure 5.63. shows that using the three forms of *Enteromorpha prolifera* algae (native, coarse and ground) to remove nickel ions from wastewater, the final concentrations only reached 0.59; 0.86 and 0.98 mg/L, starting from a concentration of 1.30 mg/L.

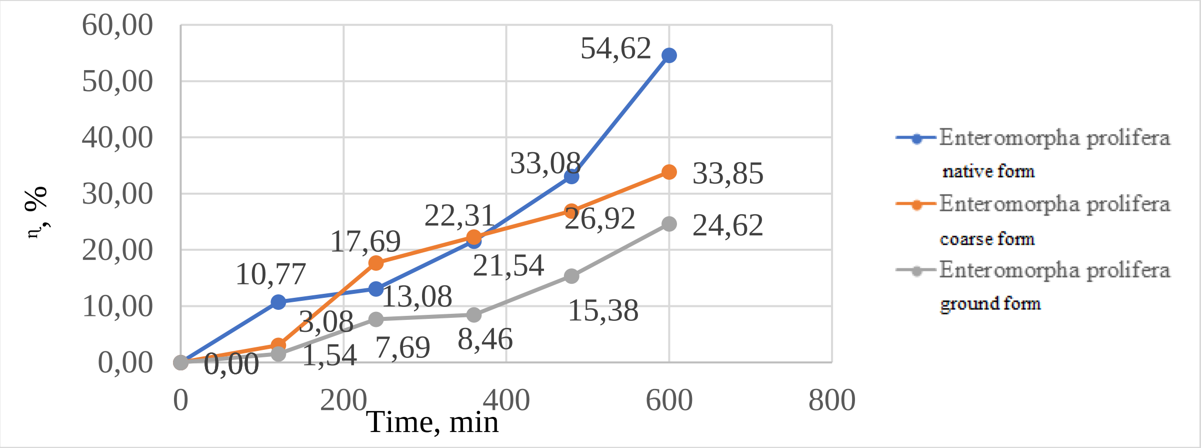

Fig. 5.64. Variation of treatment yield over time for wastewater containing Ni(II) ions, Ci =2.74mg/L.

The treatment yields obtained using the three forms of *Enteromorpha prolifera* algae (native, coarse and ground) to remove nickel ions from wastewater were 54.62; 33.85 and 24.62% in a time period of 600 minutes. Unlike *Sargassum fusiforme* algae, *Enteromorpha prolifera* algae were not as effective.

The comparison of the percentage of treatment obtained using the two types of algae presented above, in the three forms studied, for the removal of nickel ions from wastewater was represented in Figure 5.65.

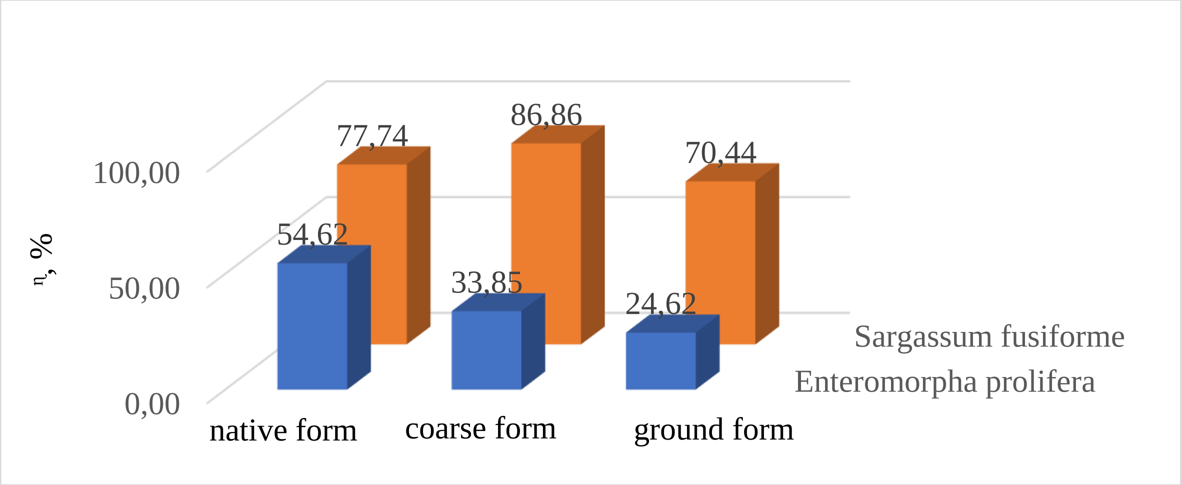

Fig. 5.65. Comparison of nickel ion water purification yields using the algae *Sargassum fusiforme* and *Enteromorpha prolifera.*

Comparing the results obtained for the removal of nickel ions from wastewater using Sargassum fusiforme and *Enteromorpha prolifera* algae shows that *Sargassum fusiforme* algae were much more efficient than *Enteromorpha prolifera* algae, with the highest removal efficiency of 86.86% when the coarse form of the algae was used.

5.2.2.5. Use of algae for simultaneous removal of copper and manganese ions from wastewater

In this experimental study, *Sargassum fusiforme* algae were studied in three different forms: native, coarse and ground to evaluate their efficiency in removing copper and manganese ions from wastewater. The use of these three forms was based on the understanding that the specific surface area of the algae varies according to their form. Consequently, a higher specific surface area tends to lead to higher treatment efficiency. The native shape of the *Sargassum fusiforme* algae used in the experiments reflects their natural state. To obtain the coarse form of *Sargassum fusiforme*, the algae were cut to a size of 2 cm, and to obtain the ground form, the native algae were ground using a grinder and a pistil. Standard copper and manganese ion solutions of 1000 mg/L were used to prepare the wastewater used for the experiments. The wastewater was prepared in the laboratory is composed of bidistilled water and copper and manganese ions. To ensure proper mixing, continuous stirring of the wastewater was maintained using a mechanical stirrer. The parameters of the phytoremediation process were: amount of algae 1 g, pH value of waste water 6, room temperature. The initial concentrations of copper and manganese ions were 1.10 and 1.05 mg/L respectively. These concentrations were prepared by dilutions using 1000 mg/L standard solutions. Figures 5.67. and 5.68. show graphically the concentrations of copper and manganese ions obtained from samples taken every 2 hours. Variations in metal ion concentrations were plotted for all three forms of Sargassum fusiforme algae used, i.e. native, coarse and ground.

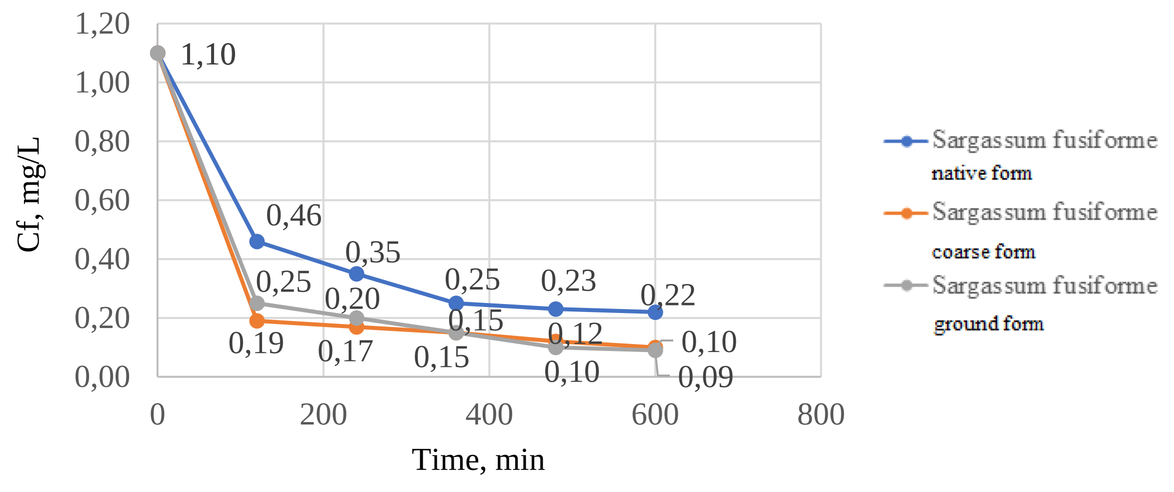

Fig. 5.66. Variation of Cu(II) ions in wastewater over time in a treatment system containing *Sargassum fusiforme* for Ci =1.10 mg/L.

Figure 5.66. illustrates the gradual decrease in copper ion concentration in the wastewater over a contact time of 600 minutes. Initially, the concentration was 1.10 mg/L, which subsequently decreased to final concentrations of 0.22 mg/L, 0.10 mg/L and 0.09 mg/L when *Sargassum fusiforme* was used in its native, coarse and ground form.

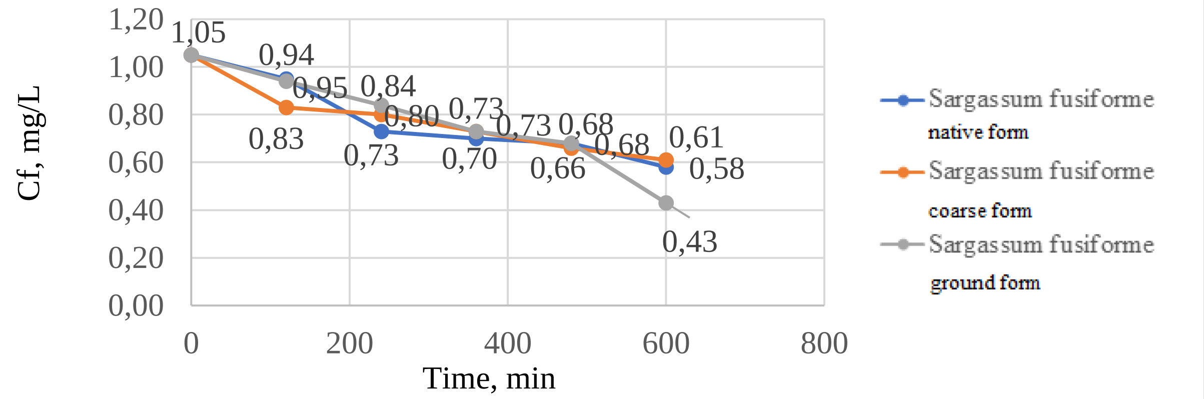

Fig. 5.67. Variation of Mn(II) ions in wastewater over time in a treatment system containing *Sargassum fusiforme* for Ci =1.05mg/L.

Figure 5.67. shows the progressive reduction of manganese ion concentrations in wastewater over a period of 600 minutes. The initial manganese ion concentration was 1.05 mg/L, which gradually decreased to final concentrations of 0.61 mg/L, 0.58 mg/L and 0.43 mg/L when Sargassum fusiforme in its coarse, native and ground form was used.

Figures 5.68. and 5.69. show graphically the removal efficiencies of copper and manganese ions from wastewater using the three forms of *Sargassum fusiforme* algae tested during this research.

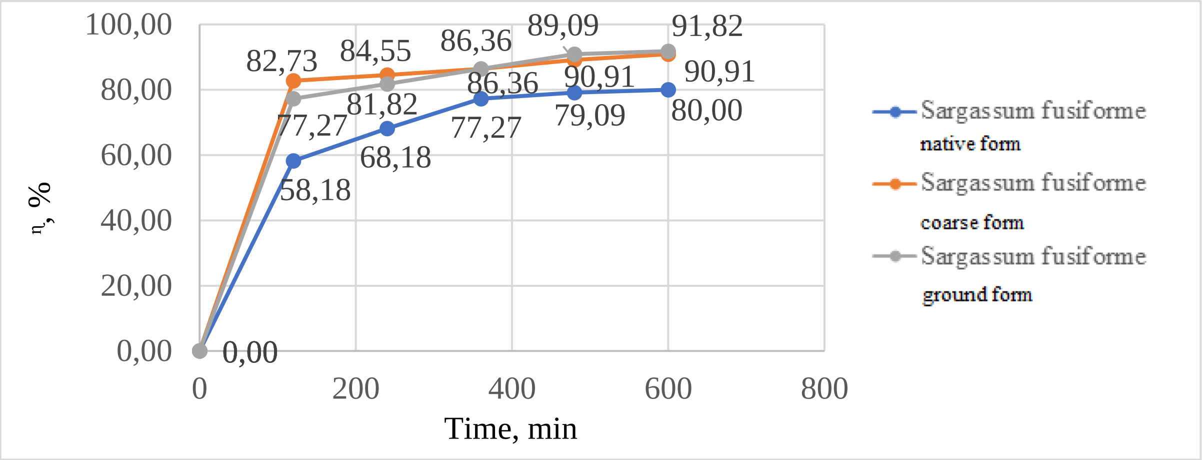

Fig. 5.68. Variation of treatment yield over time for wastewater containing Mn(II) ions, Ci =1.05mg/L.

Figure 5.68. illustrates the increase in treatment efficiencies achieved through the phytoremediation process. During the initial 120 minutes of contact time, *Sargassum fusiforme* in its coarse form showed remarkable efficiency in removing copper ions from wastewater, reaching 82.73%. In contrast, the other two forms of *Sargassum fusiforme* showed a lower efficiency of 77.27% and 58.18% during this period. However, at the end of the phytoremediation process, the highest treatment efficiency was observed when Sargassum fusiforme in the ground form was used, reaching a remarkable treatment efficiency of 91.82%.

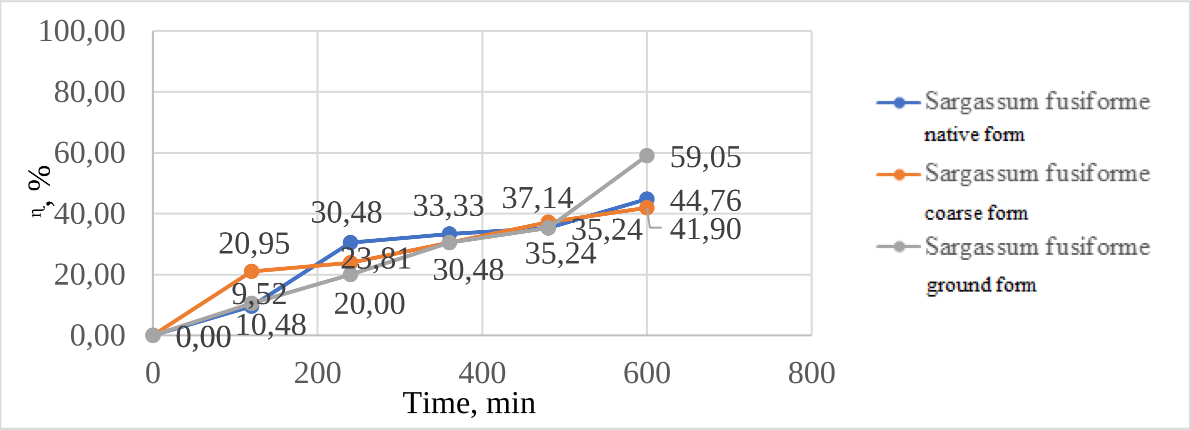

Fig. 5.69. Variation of treatment yield over time for wastewater containing Cu(II) ions, Ci =1.05mg/L.

The treatment efficiency for the removal of manganese ions from wastewater using Sargassum fusiforme algae in ground, native and coarse form did not increase as much as for copper ions, reaching values of 59.05, 44.76 and 41.90% during the 600-minute experiment.

Figure 5.70. illustrates a comparison of treatment efficiency for the removal of copper and manganese ions from wastewater using the three forms of *Sargassum fusiform* algae studied. It can be seen that the removal efficiencies of copper ions from wastewater (80.00; 90.91; 91.82%) were much higher than the removal efficiencies of manganese ions (44.76; 41.90; 59.05%).

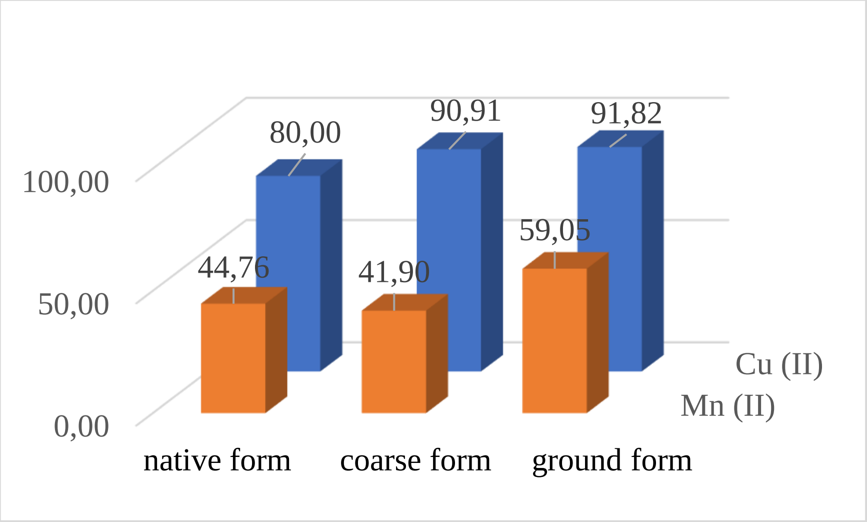

Fig. 5.70. Comparison of copper and manganese ion removal yields using native, coarse and wetted *Sargassum fusiforme.*

5.2.3. Conclusions

The use of plants, especially algae, to purify water containing heavy metal ions has proven effective. Phytoremediation, an increasingly used method, offers notable advantages, including cost-effectiveness and exceptional treatment efficiency.

The results achieved in removing metal ions from wastewater are closely linked to the metabolic bioaccumulation mechanism exhibited by algae. Living algal cells play a crucial role in controlling metal uptake.

The results showed a gradual decrease in metal concentration over the whole period. In the case of Ni(II) ions, *Sargassum fusiforme* can be successfully used in the water treatment process, with yields of 86.86% using the coarse form, 77.74% using the native form and 70.44% for the ground form over a period of 10 h.

Concentrations of Mn(II) and Pb(II) ions in wastewater decreased rapidly, so that the two types of algae *Sargassum filiforme* and *Enteromorpha (Ulva) prolifera* can be successfully used as heavy metal biosorbents, with yields of over 95.00% for the removal of manganese ions and 96, 00% for the removal of lead ions using *Sargassum fusiforme* algae in ground form and over 98.00% for the removal of manganese ions and 100.00% for the removal of lead ions from waste water using *Enteromorpha (Ulva) prolifera* in ground form.

It can be concluded that these algae, *Sargassum filiforme* and *Enteromorpha (Ulva) prolifera*, can be successfully used in the removal of heavy metals from industrial wastewater.

A possible explanation for the use of algae in decomposition may be the extracellular metabolic products that form complexes with biomass sorbing metals and for the development of suitable technologies that can be applied in water treatment.

CHAPTER 6. ORIGINAL CONTRIBUTIONS WITHIN THE DOCTORAL THESIS. FINAL CONCLUSIONS. PERSPECTIVES

6.1. Original contributions from the PhD thesis

The basis of my PhD thesis is the study of the literature, which provided me with all the necessary information for the application of phytoremediation for the treatment of water containing heavy metal ions.

As conventional water treatment methods have various disadvantages such as high costs or the production of large quantities of sludge, it was necessary to implement unconventional water treatment methods. Thus, phytoremediation has been applied to remove metal ions from wastewater, as it is a cheap, environmentally friendly method with high treatment efficiencies.

- The Typha angustifolia plant was used for the adsorption of copper, manganese, nickel and lead ions from wastewater, investigating metal ion concentrations different from those found in the literature;
- At the same time, the algae *Sargassum fusiforme* and Enteromorpha prolifera were used for the absorption of copper, manganese, nickel and lead ions from wastewater, studying different concentrations of metal ions from those found in the literature;
- Treatment systems were developed for experimental research using the plant Typha angustifolia and the algae *Sargassum fusiforme* and *Enteromorpha prolifera.*

6.2. Final conclusions

Phytoremediation is an environmentally friendly and sustainable method of wastewater treatment. This method uses plants as a means of removing toxic and polluting substances from water, thereby improving water quality and reducing environmental impact.

Phytoremediation can contribute to the ecological restoration of ecosystems by removing pollutants and creating an environment conducive to the regeneration of local vegetation and fauna.

Overall, the use of plants for water treatment is a promising and effective method that can be successfully implemented to improve water quality and protect the environment.

Algae are living organisms that can absorb and metabolise a wide range of chemicals, such as nitrates, phosphates and heavy metals, thus reducing pollution levels in water. In addition, algae produce oxygen through photosynthesis, thus improving oxygen levels in the water and helping to eliminate unpleasant odours.

The use of algae for water treatment still needs further research to develop more efficient technologies and improve performance under different environmental conditions. The environmental impact and food safety aspects of using algae for water treatment also need to be considered.

Overall, the use of algae for water treatment represents an exciting and promising opportunity to develop innovative and sustainable water treatment technologies, thus contributing to environmental protection and water quality improvement.

Experimental research results presented in the PhD thesis demonstrated that plants and algae can be successfully used as bioabsorbents for the removal of metal ions from industrial wastewater. A possible explanation for the use of algae and plants may be the extracellular metabolic products that form complexes with the metals absorbing biomass and are effective for the development of suitable technologies that can be applied in water treatment.

In the case of the experiment on the removal of copper ions from wastewater, the initial concentrations of copper being 0.60; 1.10 and 2.0 mg/L, high treatment yields were obtained, namely: when using 5 Typha angustifolia plants, the treatment efficiency was

87.00%, considering the highest initial concentration studied, as opposed to treatment efficiencies of 75.00% when using 3 plants and 11.50% using a single Typha angustifolia plant, the treatment time required being 2400 minutes, for each concentration and for any number (1, 3 and 5 respectively) of Typha angustufolia plants used.

According to the calculations made in the experimental research, considering the initial concentration of 0.60 mg/L of copper ions in water during the treatment process using Typha angustifolia, treatment efficiencies of 93.33% were obtained using 1 plant and 100.00% using 3 and 5 Typha angustifolia plants.

At the same time, for the removal of nickel ions from wastewater the treatment efficiency of Typha angustifolia plants was 84.00% when using 5 plants and 71.09% and 76.95% when using 3 and 1 Typha angustifolia plant respectively, considering the highest initial nickel ion concentration used in the experiment.

The results obtained from experiments testing the efficiency of the Typha angustifolia plant for the removal of lead ions from wastewater at the initial concentration of 0.65 mg/L were 100% when using 5 and 3 Typha angustifolia plants respectively, and 98.00% when using one Typha angustifolia plant after the same contact time of 40 hours.

When testing the capacity of Typha angustyfolia plants for the removal of manganese ions from wastewater, when using 1 and 3 plants respectively, considering initial concentrations of 2.00 and 1.74 mg/L, the highest treatment efficiency was recorded when using 3 Typha angustifolia plants, with treatment yields of 77.50% and 93.68% respectively.

When 1, 3, 5 Typha angustifolia plants were applied in the experimental investigations as biomaterials for simultaneous removal of copper and nickel ions from wastewater, having initial concentrations of 1.14 and 0.74 mg/L, respectively, the results showed that copper ions were removed down to a concentration of 0, 20 mg/L when one plant was used, 0.19 mg/L when 3 plants were used and 0.07 mg/L when 5 plants were used, compared to nickel ions which were removed to concentrations of 0.37 mg/L when one plant was used, 0.36 mg/L when 3 plants were used and 0.26 mg/L when 5 plants were used. Thus, Typha angustifolia had a higher bioavailability for copper ions than nickel ions.

In conclusion, considering the initial concentrations of heavy metal ions applied in the experimental investigations where Typha angustifolia plant was used as biomaterial for the removal of heavy metal ions from wastewater, efficiencies of 100.00% of heavy metal removal from wastewater were obtained for Pb^{2+} ions when 3 and 5 plants were used at an initial concentration of 0.65 mg/L, as well as for Cu^{2+} ions at an initial concentration of 0.60 mg/L.

Following the analysis of the experimental research, making a hierarchy in terms of the order of removal of heavy metal ions applied in the experimental research, when the Typha angustifolia plant was used as biomaterial, taking into account the highest concentrations of heavy metal ions in wastewater, the removal efficiency of heavy metal ions is as follows: $Cu^{2+} > Ni^{2+} > Mn^{2+}$.

Also, applying in the experimental research the algae *Sargassum fusiforme* and Enteromorpha prolifera as biomaterials for the removal of Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} ions from wastewater, the highest treatment efficiency was obtained when using the algae Enteromorpha prolifera, native and coarse forms, it was 100.00% for the removal of lead ions from wastewater, and when using *Sargassum fusiforme* algae, coarse form, the *highest* treatment efficiency obtained was 99.46%. Sargassum fusiforme, native form, showed a removal efficiency of copper ions from wastewater of more than 99.00%.

Following the application of the algae *Sargassum fusiforme* and *Enteromorpha prolifera* as biomaterials for the removal of manganese ions from wastewater, high removal efficiencies of 98.17% were obtained using the ground form of the algae *Enteromorpha prolifera* and 95.73% using *Sargassum fusiforme*, also the ground form.

The value of the treatment efficiency for the removal of nickel ions from wastewater by applying in the phytoremediation process the alga *Sargassum fusiorme*, coarse form as biomaterial was 86.86% .

For the simultaneous removal of copper ions and manganese ions from wastewater using *Sargassum fusiforme* algae as biosorbent, the calculations showed treatment yields of 91.82% for copper ions and 59.05% for manganese ions.

The order of removal of heavy metal ions from wastewater using *Sargassum fusiforme* and *Enteromorfa prolifera* algae as biomaterials is as follows: Pb^{2+}> Cu^{2+} > Mn^{2+} > Ni^{2+}.

The originality of this study is represented by the application and use of the number of Typha angustifolia plants, the different quantity of *Sargassum fusiforme* and *Enteromorfa prolifera* algae, the contact times between the biomaterials and wastewater used in the experimental research, the concentrations of heavy metal ions present in the wastewater, different from those investigated in the literature.

6.3. Perspectives

In the future, the plant *Typha angustifolia* and the algae *Sargassum fusiforme, Enteromorpha prolifera* will be tested to remove other heavy metal ions that are found in industrial wastewater and are toxic if they reach the human body.

The parameters of the treatment process will be taken into account: the time needed for treatment and the correlation between the number of plants needed or the amount of algae to remove different concentrations of heavy metals in the wastewater, the temperature and the time of harvesting the plants.

Analysis of plant organs (root, stem, leaves, flowers, seeds) after the uptake of heavy metal ions from wastewater to observe the capacity of each organ to retain pollutants.

LIST OF PUBLICATIONS

1. Loredana DIACONU, Cristina Ileana BUTNARIU, Alina Gina CATRINA, Gigel PARASCHIV, Water depollution using *Typha angustifolia*, ANNALS of Faculty Engineering Hunedoara – International Journal of Engineering, Tome XVII [2019] | Fascicule 3 [August], 199-200.
2. Loredana Ioana DIACONU, Cristina Ileana COVALIU-MIERLA, Oana PAUNESCU, Leon Dumitru COVALIU, Horia IOVU, Gigel PARASCHIV, Phytoremediation of Wastewater Containing Lead and Manganese Ions Using Algae, Biology 2023, 12, 773, 1-15, **F.I. = 5.168**.
3. Diaconu L., Covaliu-Mierlă I.C, Coz A. F., Paraschiv G., MANGANESE IONS REMOVAL FROM WASTEWATER USING TYPHA ANGUSTIFOLIA, INTERNATIONAL SYMPOSIUM ISB-INMA TEH 2021, 946-949.
4. Loredana Ioana DIACONU, Cristina Ileana COVALIU-MIERLĂ, Usage of Typha angustifolia for simultaneous removal of copper and nickel ions from wastewater, U.P.B,Sci.Bull., **F.I. = 0.5.**

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