DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
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Biosorption of uranium in contaminated waters: the potential of four plant species for remediation of streams affected by acid mine drainages

Patrícia Joana Rodrigues de Oliveira Antunes

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Manuel Augusto Simões Graça (Universidade de Coimbra) e do Professor Doutor João António Mendes Serra Pratas (Universidade de Coimbra)

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Abstract

Several studies have demonstrated that plant biomass is able to remove heavy metals and radionuclides from aqueous media through a passive and metabolism-independent process, known as biosorption. The biosorption process consists on merely chemical and physical reactions that take place between the contaminants and certain chemical groups in structural compounds of the biomass, which may lead to significant contaminant retention in this material. The extent of the biosorption process is dependent on the biomass chemical composition and structure, thus it can vary greatly among different types of material.

This investigation analyses the capacity of passive uranium removal from waters by dead plant biomass derived from four plant species which are native and widely distributed in Portugal: *Callitriche stagnalis* Scop. (pond water-starwort), *Cytisus scoparius* (L.) Link, (common broom), *Erica arborea* L. (tree heath) and *Quercus robur* L (pedunculate oak). The study was conducted in a laboratorial microcosms system in which the plant material was exposed to approximately constant uranium concentrations, corresponding to the mean uranium concentration found in a local polluted stream (Ribeira da Pantanha, Nelas, Viseu).

Plants retained uranium in the order *C. stagnalis > Q. robur > C. scoparius > E. arborea*. The mean maximum uranium concentrations observed in the plant material were 632.06 ± 128.54 mg.kg^{-1} (dry weight) for *C. stagnalis*, 182.04 ± 27.37 mg.kg^{-1} (dry weight) for *Q. robur,* 127.26 ± 27.41 mg.kg^{-1} (dry weight) for *C. scoparius* e 97.01 ± 27.11 mg.kg^{-1} (dry weight) for *E. arborea*. These results indicate that the studied material acts as a uranium sink and therefore has the potential to effectively remove uranium from contaminated waters. However, the applicability of this material in

contaminated water treatment requires further investigation both in laboratorial and *in situ* conditions.

Key words: biosorption; uranium; mine drainages; remediation

Resumo

Vários estudos têm demonstrado que a biomassa vegetal possui a capacidade de remover metais pesados e radionuclídeos de meios aquosos através de um processo passivo e independente do metabolismo, conhecido como biosorção. O processo de biosorção compreende reacções meramente físicas e químicas que ocorrem entre os contaminantes e determinados grupos químicos em compostos estruturais da biomassa, e que podem conduzir à sua retenção no material em quantidades significativas. A magnitude deste processo é dependente da composição química e da estrutura da biomassa, pelo que pode variar grandemente entre materiais diferentes.

Esta investigação analisa a capacidade de remoção passiva de urânio de águas contaminadas por biomassa vegetal morta proveniente de quatro espécies vegetais nativas e de ampla distribuição em Portugal: *Callitriche stagnalis* Scop., (lentilhas-da-água) *Cytisus scoparius* (L.) Link (giesta), *Erica arborea* L. (urze-branca) e *Quercus robur* L. (carvalho-alvarinho). O estudo foi efectuado utilizando um sistema laboratorial de microcosmos, nos quais o material vegetal foi exposto a valores de concentração de urânio aproximadamente constantes, semelhantes aos encontrados, em média, numa ribeira local poluída (Ribeira da Pantanha, Nelas, Viseu).

As espécies estudadas retiveram urânio na ordem *C. stagnalis* > *Q. robur* > *C. scoparius* > *E. arborea.* Os valores máximos médios de concentração de urânio no material vegetal foram de 632.06 ± 128.58 mg.kg^{-1} (peso seco) para a espécie *C. stagnalis*, 182.04 ± 27.37 mg.kg^{-1} (peso seco) para a espécie *Q. robur*, 127.26 ± 27.41 mg.kg^{-1} (peso seco) para a espécie *C. scoparius* e 97.01 ± 27.11 mg.kg^{-1} (peso seco) para a espécie *E. arborea.* Estes resultados permitem concluir que o material vegetal estudado actua como um substrato para a retenção de urânio, podendo remover

efectivamente urânio de águas contaminadas. Contudo, a aplicabilidade deste tipo de material no tratamento de águas poluídas requer estudos mais aprofundados em condições laboratoriais e *in situ*.

Palavras-chave: biosorção; urânio; remediação; escorrências ácidas

Chapter 1

Introduction

1.1 Abandoned mines: a troubling legacy

As a result of a historical intense mining activity all over the world, several nations are today left with a legacy of numerous abandoned mines, many of them representing significant hazards both to the environment and to the welfare of the populations. It has been estimated that there are presently over 500.000 abandoned mines in the USA, 10.000 in Canada and 45.500 in Japan (Cidu, 2011). In Europe, the extent of the impact of abandoned mines remains undocumented and the magnitude of their impacts is still not well known, but countries with a long mining history include Czech Republic, Sweden and Germany (Cidu, 2011).

Abandoned mines are nowadays objects of concern mainly because appropriate measures were not taken during their active period. The adverse impacts of the mining activity on the environment arise with the first mining operations. These negative impacts last even after the cessation of the operations, especially when appropriate regulation is not considered and adequate rehabilitation measures are not undertaken. In fact, frequently during the exploration period, the concessions' management was defined mostly regarding economic criteria (Oliveira et al., 2002). Such practices, along with an uncontrolled abandonment of unproductive mines, have led to several environmental risk situations that further deteriorate with time (Oliveira et al., 2002).

Abandoned mines represent both environmental and public health hazards due to the release of toxic chemical compounds to soils, waters and the atmosphere (Salomons, 1995; Schöner, Sauter & Büchel, 2006; Jennings, Neuman & Blicker, 2008; Wu et al., 2010), and also result in physically disturbed landscapes, raising important aesthetic concerns (Mkandawire & Dudel, 2009).The excavations and consequent exposure of the minerals to external agents, as well as the accumulation of waste material in waste

dumps, results in the abnormal accumulation of chemical elements in the surrounding areas (Oliveira et al., 2002). These alterations invariably lead to the deterioration in the quality of soils, sediments and waters. The environmental impacts are worsened if mineral sulphides are present, as these are very unstable under the Earth's surface normal conditions and usually undergo a series of reactions that result in the formation of low pH waters. Acid waters, in turn, promote the solubilisation of the metals present in the ores and their consequent dispersion (Oliveira et al., 2002).

1.2. Acid mine drainages (AMD)

1.2.1. AMD generation

Tailing dumps seepage waters that originate from the infiltration of rainwater into the rejected material that results from the ore extraction and processing, as well as mine waters, which usually are in prolonged contact with mineralized veins, generally exhibit high concentrations of dissolved metals. This dissolution of the metals in mineralogical phases is often associated with high contents of sulphides (Oliveira et al., 2002). These compounds promptly react with water and oxygen, generating a low pH aqueous medium that promotes the solubilisation of chemical compounds in the ores and waste materials (Salomons, 1995; Oliveira et al., 2002; Johnson & Hallberg, 2005; Jennings, Neuman & Blicker, 2008). Many metals occur as sulphide ores, and these are generally associated with pyrite (FeS_2), which is the most abundant sulphide mineral on the planet (Johnson & Hallberg, 2005). Oxidation of pyrite with subsequent formation of acid waters can occur naturally, at a very low rate over a geological period (Jennings, Neuman & Blicker, 2008). However, in mines, due to the high concentration of exposed

sulphide minerals in the dumps material, this reaction occurs at a higher rate than that of the neutralisation reaction provided by alkaline compounds in the medium (Salomons, 1995; Jennings, Neuman & Blicker, 2008). The pyrite oxidation reaction, as described below, starts with the generation of an aqueous solution of ferrous iron and sulphuric acid;

$$2FeS_{2\,(s)} + 7O_2 + 2H_2O \longrightarrow 2Fe^{+2} + 4SO_4^{-2} + 4H^+ \qquad \text{(I)}$$
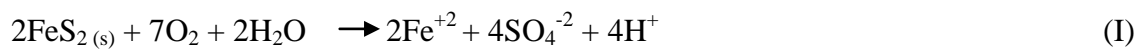
Ferrous iron can be further oxidised to ferric ion if oxygen is widely available;

$$2Fe^{+2} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{+3} + H_2O \qquad \text{(II)}$$

Ferric ion, in turn, can either react with water to form ferric hydroxide,

$$2Fe^{+3} + 6H_2O \longleftrightarrow 2Fe(OH)_{3\,(s)} + 6H^+ \qquad \text{(III)}$$

an orange-coloured precipitate frequently seen in waters contaminated by acid mine drainages, commonly known as "yellow-boy" or "ochre" (Kelly, 1988; Salomons, 1995; Johnson & Hallberg, 2005; Jennings, Neuman & Blicker 2008), or react with pyrite producing ferrous iron and acidity.

$$14Fe^{+3} + FeS_{2\,(s)} + 8H_2O \longrightarrow 2SO_4^{-2} + 15Fe^{+2} + 16H^+ \tag{IV}$$
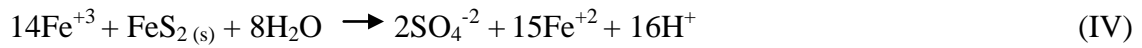
The rate of most of these reactions can be significantly augmented by lithotrophic iron- and sulphur-oxidising bacteria (Salomons, 1995; Johnson & Hallberg, 2005; Jennings, Neuman & Blicker, 2008), such as *Thiobacillus ferrooxidans* (Jennings, Neuman & Blicker, 2008) and *T. thiooxidans*, which are frequently found in abandoned mines.

Metal-laden acid waters then drain and may contaminate underground and surface waters. The impacts of this contamination on streams are complex due to the multi-factor nature of its effects (Gray, 1988), but they generally devastate aquatic systems in receiving waters, and this contamination can last for centuries (Pandey, Shama, Roy & Pandey, 2007) or millennia (Wu et al., 2010).

1.2.2. AMD impacts on aquatic systems

The pollution associated with AMD is characterized by acidic metalliferous conditions in waters (Jennings, Neuman & Blicker, 2008). Acidity can affect aquatic systems to a great extent (Kelly, 1988). Direct effects of acidity on the aquatic organisms include the impairment of cell transmenbranar ionic balance and hydrolysis or denaturation of cellular compounds (acidaemia) as well as dissolution of the shells of molluscs and some crustaceans (Kelly, 1988). Among the indirect effects of acid waters on aquatic organisms, it should be noted the impairment of the carbonate buffer system, a mechanism that prevents pronounced shifts in the water's pH, due to the enhanced loss of water carbonate to the atmosphere as carbon dioxide (Kelly, 1988). Low pH

values also enhance flocculation of suspended clay and silt particles (Kelly, 1988). Enhanced sedimentation has been associated to the inhibition of spawining and development of fish eggs and larvae, and also has the effect of smothering the benthic fauna (U.S. Environmental Protection Agency [USEPA], 2000; Jennings, Neuman & Blicker, 2008).

Acid-base reactions occurring, for instance, when contaminated acid waters join unpolluted waters, may bring the pH to values at which the iron released during the sulphide oxidation process is no longer soluble (Kelly, 1988; Oliveira et al., 2002). Consequently, precipitation of part of the iron in the form of ferric hydroxides and oxihydroxides ("yellow-boy"; Fig.1) occurs (Kelly, 1988; Oliveira et al., 2002; Smucker & Vis, 2011). Deposition of these precipitates in the substrate eventually leads to smothering of benthic organisms (Kelly, 1998).

Figure 1 – Ferric hydroxide precipitates in waters affected by AMD (Tutu, McCarthy & Cukrowska, 2008).

Naturally occurring substances at the mine areas are the major source of the pollutants transported by acid drainages. The mined ore, as well as the waste rock and tailings, contains varying concentration of several compounds, depending on the local geology (USEPA, 2000). Contaminants coming from a mine site are mainly metals (such as lead, copper, silver, manganese, cadmium, iron, zinc, and others), but also

radionuclides and other compounds (USEPA, 2000). Unlike organic compounds, metals cannot be removed from the environment through chemical or biological transformation (Prasad & Freitas, 2003). The impact of metals on aquatic systems is largely determined by metal speciation, i.e. the balance between the aqueous phase (free ions or complexes), the solid phase (colloids and particles) and the biological phase (adsorbed metals on biological surfaces, or incorporated in cells) (Kelly, 1988; Gerhardt, 1993). The free ionic form is generally the most toxic form (Kelly, 1988, Gerhardt, 1993; Salomons, 1995) and it is frequently taken up directly from the water by organisms (Gerhardt, 1993). Metals accumulated in the sediments have the potential of being re-dissolved into the water column, thus providing an additional long-term source of pollution that may lead to a chronic contamination of the streams (USEPA, 2000). Metal speciation depends on several factors such as water temperature, pH, stream flow, and the presence of complexing agents, as well uptake and adsorption mechanisms (Kelly, 1988; Gerhardt, 1993; Salomons, 1995).

Metals can have a variety of deleterious effects on organisms as they often interfere with several metabolic pathways (Kelly, 1988; Sutcliffe & Hildrew, 1989; Burkart, 1991). Metal ions can also affect gene expression and have mutagenic activity (Burkart, 1991). Synergistic effects between different metals in solution can also occur (Kelly, 1988; Gerhard, 1993; Pandey, Shama, Roy & Pandey 2007). Indirect effects of metals on organisms may include decrease of dissolved oxygen concentration due to metal oxidation and rise in osmotic pressure.

Streams affected by acid mine drainages usually have impoverished biota and altered community structure when compared to unpolluted streams (Kelly, 1988; Sutcliffe & Hildrew, 1989). A general reduction in the number of species and individuals has been reported (Kelly, 1998; Sutcliffe & Hildrew, 1989; Gerhardt, 1993; Pandey, Shama, Roy

& Pandey, 2007), with fish and some taxa of aquatic invertebrates (Ephemeroptera, Plecoptera and Trichoptera) showing pronounced sensitivity to acidity and high metals concentrations (Kelly, 1988; Lefcort, Vancura & Lider 2010) . Hence, contaminated streams communities may be reduced to a few tolerant species (Sutcliffe & Hildrew, 1989).

In addition to the direct effects of the water chemical and physical parameters on the organisms, other hypotheses have been suggested to explain altered community structure (Sutcliffe & Hildrew, 1989). For instance, the depletion of the flora in contaminated areas, along with impoverished microflora in acid waters, may have a negative impact on aquatic herbivorous invertebrates due to the lack of suitable food (Sutcliffe & Hildrew, 1989).

1.3. Abandoned mines and uranium environmental contamination

Uranium, a non-essential element for living organisms, is highly toxic for humans and animals (Malczewska-Toth, Myers, Shuey & Lewis, 2003). In most abandoned uranium mines, pollution discharges to the environment contain radionuclides and other contaminants such as cadmium, lead and arsenic. In addition to the environmental and public health hazards posed by abandoned mines, uranium mines represent an increased risk due to the radioactivity associated with the wastes and tailings (Dinis & Fiúza, 2003; Mkandawire & Dudel, 2009).

1.3.1 Uranium properties and geochemistry

Uranium is a metallic chemical element which is ubiquitous in nature. It is the heaviest element occurring naturally in weighable amounts (Kalin, Wheeler & Meinrath, 2005). Uranium concentration on the Earth's crust is estimated in 3 mg.kg^{-1}; it forms 160 mineral species and accounts for 5% of the total known minerals (Kalin, Wheeler & Meinrath, 2005).

Uranium occurs naturally as three isotopes with atomic masses of 234, 235 and 238, with relative abundances of 0.005%, 0.72% and 99.27%, respectively (Gravilescu, Pavel & Cretescu, 2009; Kalin, Wheeler & Meinrath, 2005). All the isotopes undergo radioactive decay through a complex isotope chain that ultimately results in stable lead (Malczewska-Toth, Myers, Shuey & Lewis, 2003). Uranium most common isotopes, ^{235}U and ^{238}U, have radioactive half-lives of 704 x 10^6 and 446 x 10^7 years, respectively. Natural uranium is considered a weakly radioactive element (Bleise. Danesi & Bukart., 2003).

Uranium occurs in several oxidation states, but in nature it exists mainly as U^{4+} and U^{6+} (Arnold et al., 2011). The U(IV) mineral phases, which prevail in reducing conditions, are almost insoluble, but the oxidised hexavalent uranium species are highly soluble and reactive. Because U(VI) species have an extreme affinity to oxygen (Kalin, Wheeler & Meinrath, 2005), uranyl ions UO$_2$$^{2+}$ formation occurs if oxygen is available (Kalin, Wheeler & Meinrath, 2005; Mkandawire & Dudel, 2009). Uranyl is the most bioavailable species of uranium (Srivastava, Bhainsa & D'Souza, 2010) due to its high solubility it is also highly mobile in the environment (Winde, Wade & van der Walt, 2004).

In waters, the mobility of dissolved uranium depends on the oxidation state, pH and Eh conditions, and types of available chelating agents such as carbonates, phosphates, vanadates, fluorides, sulphates and silicates (Kalin, Wheeler & Meinrath, 2005; Mkandawire & Dudel, 2009). Under oxidising conditions, hexavalent uranium in the form of uranyl ions form carbonate species as a function of pH and CO_2 pressure (Mkandawire & Dudel, 2009). Uranium carbonate compounds found in carbonate-containing waters are mainly $UO_2(CO_3)^{4-}$ and $UO_2(CO_3)_2^{2-}$ (Mkandawire & Dudel, 2009). If carbonates are not present at significant concentrations, mainly U(VI) oxides are formed. The carbonate species predominate under neutral and alkaline conditions, while hydrolysing in very acidic conditions (Mkandawire & Dudel, 2009). Under reducing conditions, the tetravalent species prevail, which tend to precipitate (Mkandawire & Dudel, 2009). In the presence of chelating agents such as phosphates and silicates, highly insoluble phosphate and silicate uranium compounds are formed (Kalin, Wheeler & Meinrath, 2005).

In natural waters, the mechanisms that participate in uranium immobilization in natural aqueous environments include the precipitation of less soluble uranium compounds, precipitation of salt crusts due to evaporation, co-precipitation along with iron and manganese oxyhydroxides or with calcium carbonate, and adsorption onto negatively-charged surfaces such as organic matter or clay minerals (Winde, Wade & van der Walt, 2004). In fact, the high affinity of uranium for organic compounds is well documented (Kabata-Pendias & Mukherjee, 2007; Schöner, Sauter & Büchel, 2006; Trenfield et al., 2011; Winde, Wade & van der Walt, 2004).

As a chemotoxic heavy metal, uranium poses a serious threat even at low concentrations (Das, 2012). Uranium, as all actinides, is easily bioaccumulated in aquatic organisms (Kabata-Pendias & Mukherjee, 2007). The uranyl ion is capable of

forming highly stable complexes with oxygen binding centres in proteins (Srivastava, Bhainsa & D'Souza, 2010). It also has a strong affinity for phosphate and sugar alcohol groups in nucleotides and polynucleotides, thus it can cause DNA damage (Srivastava, Bhainsa & D'Souza, 2010).

1.3.2. Impacts of the uranium mining industry

The mining industry is considered the major source of uranium environmental contamination (Mkandawire and Dudel, 2009). Because the uranium content in ores is typically between 0.1 and 0.7%., large amounts of rocks are excavated when extracting uranium in open-pit or underground mines, generating massive piles of waste rock and tailings (Mkandawire & Dudel, 2009). The wastes and tailings are associated with radioactive risk because about 85% of the uranium decay progenies remain in these materials (Mkandawire & Dudel, 2009). Although the radioactivity in most uranium tailings is considered to be relatively low, some radiological hazard will last practically forever due to the long half-lives of the radionuclides involved (Dinis and Fiúza, 2006). Wastes and tailings also release radon (^{222}Rn), a noble gas derived from radium (^{226}Ra), which is a natural product of uranium decay (Mkandawire & Dudel, 2009). Radon is radioactive and toxic if inhaled (Dinis and Fiúza, 2006).

Since uranium is easily washed and transported by waters, the water pathway is the major route for uranium environmental contamination. The solubilisation of uranium in wastes and tailings dump at abandoned mines is currently the contamination source of major concern (Mkandawire & Dudel, 2009).

1.3.3. Uranium mining in Portugal

The Portuguese mining industry had a marked development in the early 20th century, driven by external investments. During World War II, intense mining was performed in order to obtain tungsten, which was widely used to produce armament (Nunes, 2002), making the country the main producer of this metal in Europe. Nevertheless, the period of most intense mining activity in Portugal was the post-war period, between 1950 and 1980, when radioactive ores were highly sought (Cerveira, 1951). Also during this period, the Iberian Pyrite Belt, an important deposit of polymetallic sulfides of great extent in the south of the Peninsula, was highly explored. After 1980, new dynamics on the metal market led to the gradual cessation of the mining activity and consequent abandonment of the majority of the existent mines, leaving behind a high number of degraded structures. Nowadays, there are 175 abandoned mines in Portugal (Empresa de Desenvolvimento Mineiro [EDM], 2011; Fig.2). Many of these mines have been sources significant of environmental pollution.

More than one third of the total number of abandoned mines corresponds to former radioactive ore mines (Fig. 3). These mines are located in the central region of the country, more precisely in the Uraniferous Region of Beiras, which encompasses the districts of Viseu and Guarda. The exploitation of radionuclides in Portugal began with the discovery of the uranium and radium deposit of Urgeiriça, (Viseu), in 1907. The production of uranium (U_3O_8) at the ore treatment facilities of the Urgeiriça mine reached 200 tons per year (EDM, 2010). The mine became inactive in 1991 (EDM, 2010). The Urgeiriça mine and other uranium mines, such as Cunha Baixa, Quinta do Bispo or Vale de Abutriga mines, have been considered as rehabilitation priorities by

the authorities and have been included in major rehabilitation projects (EDM, 2008a, 2008b, 2010).
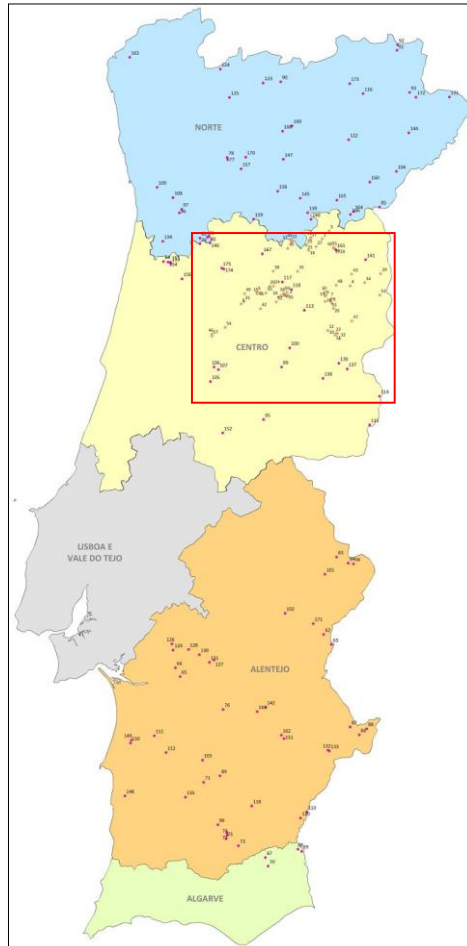
Figure 2 – Abandoned mines in Portugal. Orange points represent radioactive ore mines, whereas purple points represent other types of ore mines. The highlighted area is shown in Fig. 3. (adapted from EDM - http://www.edm.pt/html/ambito.htm).
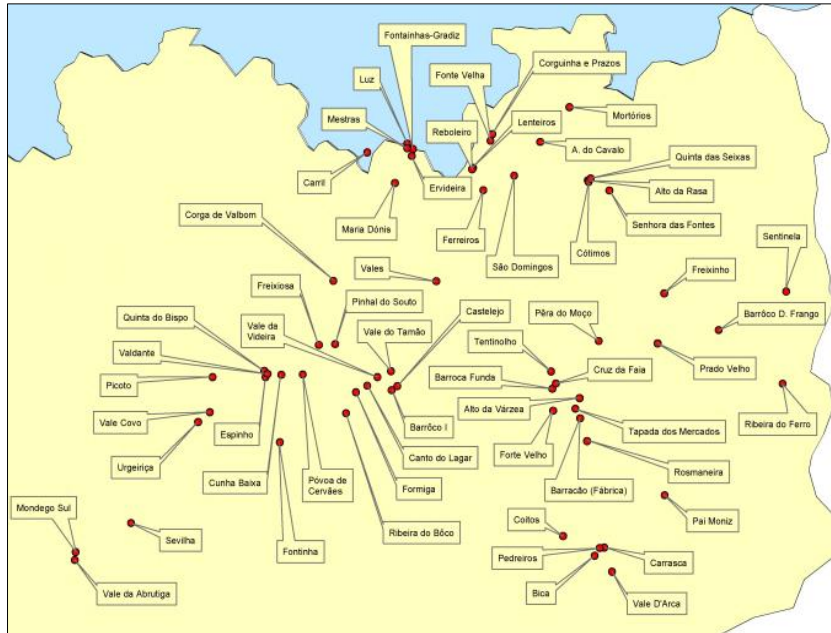
Figure 3 – The 61 abandoned radioactive ore mines in Central Portugal
(adapted from EDM - http://www.edm.pt/html/enquadramento.htm).

1.4. Rehabilitation of polluted streams

Conventional mitigation measures to reduce the impacts of AMD in streams include active and passive treatment systems involving the use of acid-neutralising reagents and metal removal by ion exchange resins, chemical precipitation, sedimentation, microfiltration, reverse osmosis and adorption onto activated carbon (Al-Masri, Amin, Al-Akel & Al-Naama, 2010; Johnson & Hallberg, 2005; Rai, 2009). Although they effectively reduce acidity and metal contents in waters, these methods generally generate large volumes of toxic by-products such as waste effluents and sludge (Rai, 2009), thus having a negative impact on the environment. In addition, conventional methods are extremely costly processes (Rai, 2009). These implications of conventional methods have led to an increase demand for innovative technologies.

Alternative methods to reduce metal concentrations in contaminated waters include the use of microorganisms (bioremediation) or living plants (e.g. rhizofiltration) to actively remove the metals from the aqueous environment. Nevertheless, the observation that the biomass is able to remove metals from waters through a passive and metabolism-independent process, known as biosorption, has propelled several studies regarding the use of this feature as an alternative technology for water remediation (Gadd, 2009).

Biosorption comprises a number of chemical and physical reactions that take place between the metals and active chemical groups mainly in the surface of the biomass, and which result in metal accumulation in the biological material. Such reactions include ion exchange, chelation, complexation, physical adsorption and microprecipitation, as well as ion diffusion an entrapment in inner spaces of the biomass (Park, Yun & Park, 2010; Volesky & Holan, 1995). It is known that ion exchange involving weakly acidic and basic groups present in the biomass is one of the main processes of heavy metal removal from waters (Kratochvil & Volesky, 1998). The active groups involved in metal binding and removal from solution include the amine, carboxyl, hydroxyl, imidazole, phenolic, phosphonate, phosphodiester, sulfonate and sulfhydril groups (Table I; Park, Yun & Park, 2010).

Table I – Some of the main active groups involved in biosorption (adapted from Volesky, 2007)

Binding group	Structural formula
Hydroxyl	- OH
Carboxyl	- C = O | OH

Table I (continued)

Binding group	Structural formula
Amine	$- NH_2$
Sulfhydryl	$- SH$
Sulfonate	$\begin{array}{c} O \\ \parallel \\ S = O \\ \parallel \\ O \end{array}$
Phosphonate	$\begin{array}{c} OH \\ \mid \\ P = O \\ \mid \\ OH \end{array}$
Phosphodiester	$\begin{array}{c} >P = O \\ \mid \\ OH \end{array}$

Since the active groups involved in metal sequestering are ubiquitous in the biomass, virtually all types of biomass are able to remove metals from aqueous environments to a certain degree (Gadd, 2009). Some types of biomass are broad range, accumulating the majority of metals, whereas others may be exhibit some specificity for certain types of metals (Volesky & Holan, 1995). It should be noted that the presence of some active groups in the biomass does not assure an effective metal sequestration since steric, conformational or other types of barriers may be present (Park, Yun & Park). Therefore, the extend of the biosorption process by a particular type of biomass depends not only on its chemical composition, which determines the type and number of active metal-sequestering groups, as well as the affinity of these groups for the pollutants (i.e.

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binding strength), but also on its structure, which influences the accessibility of the sites (Park, Yun & Park). Differences in the chemical composition and structure can greatly influence the biosorption performance of different types of biomass (Volesky & Holan, 1995).

Although the biosorption capacity is a property of both living and dead biomass (Volesky, 2001), the use of non-living materials is advantageous with respect to living biomass because they are cheaper, require less care and maintenance, are not affected by toxic ions and can be easily regenerated and reused (Das, 2012), with possibility of recovering the sequestered metal (Park, Yun & Park, 2010; Volesky, 2007). Various types of naturally abundant and inexpensive dead biomass have been studied regarding the possibility of use of such materials water remediation, including fungal (Sag, 2011), bacterial (Vijayaraghavan & Yun, 2008) and algal (Gupta, Shrivastava & Jain, 2007) biomass; plant-derived biomass such as litter (Dundar, Nuhoglu & Nuhoglu, 2011), dried branches and roots (Prasad & Freitas, 2003), or peat (Lourie & Gjengedal, 2011); and agro-based and industrial wastes such as tea waste products (Malkoc & Nuhoglu, 2005), crab shells (Vijayaraghavan, Palanivelu & Velan, 2006) fruit peels (Schiewer & Patil, 2008), or waste beer-yeast by-products (Soares & Soares, 2012).

Biosorption has been reported to be the most appropriate technology for radionuclides removal from waters (Das, 2012). Early studies on biosorption suggested that uranium is particularly susceptible to biosorption uptake, which is related to its large atomic weight and ionic radius (Volesky & Holan, 1995). In recent years, several types of biomass have been demonstrated to bind and remove uranium in solution, such as algae (Khani, Keshtkar, Ghannadi & Pahlavanzadeh, 2008; Yang & Volesky, 1999), fruit peels (Li et al., 2012; Zou, Zhao and Zhu, 2012) and dead plant material (Al-Masri, Amin, Al-Akel & Al-Naama, 2010; Shawky, Geleel & Aly, 2005).

1.5. Objectives

The main goal of this work was to evaluate the uranium biosorption applicability of selected plant species, in order to evaluate their potential to be used in alternative techniques regarding streams affected by AMD. The selection of the plant species was made having in view an eventual practical application of plant wastes produced during forests cleaning and management; therefore we selected four abundant and native plant species in Portugal, which are also commonly found in other regions of the world.

The specific objectives of this work were:

    i.   To evaluate uranium biosorption performances by plant material from each selected plant species in laboratory conditions of approximately constant uranium concentrations and pH;

   ii.   To compare uranium biosorption capacities among plant biomass from different species;

  iii.   To discuss future perspectives on the use of the studied type of material in phytoremediation strategies involving the mitigation of AMD effects.

Chapter 2   Materials and Methods

2.1 Reference study site: Ribeira da Pantanha

In the Urgeiriça mine, large amounts of wastes and tailings were accumulated in waste piles and in two tailing dams, named Barragem Nova and Barragem Velha. The total amount of solid wastes that has been disposed in these dumps and dams is calculated in three millions of tones (Carvalho, Oliveira & Lopes, 2006a). From the ore extraction and processing operations resulted acid waters that were initially discharged into a nearby small stream, Ribeira da Pantanha (Fig. 4; Fig. 5), without any previous treatment (Carvalho, Oliveira & Lopes, 2006a). In the following years, the discharges were made after acid neutralisation and precipitate decantation in a water treatment plant at Barragem Nova (Carvalho, Oliveira & Lopes, 2006a; EDM, 2010). In addition to the discharges, the stream eventually received contaminated waters resulting from rainwater leaching of the exposed wastes (Carvalho, Oliveira & Lopes, 2006b). Although the Urgeiriça mine area has been received extensive rehabilitation works since 2005, Ribeira da Pantanha still receives water drainages from a remaining discharge point (EDM, 2010; Rodrigues, Pratas, Tavares & Branches, 2010). Carvalho, Oliveira and Lopes (2006a) reported that its waters contained high levels of uranium and products of its decay, which could amount to 1000 times their natural concentrations. The average uranium concentration in Ribeira da Pantanha has been indicated as 200 ppb (200 µg/l) (Paulo, 2006), which is near 10 times the maximum uranium concentration recommended by USEPA for drinking water (0.3 µg.l^{-1}) (USEPA, 2012).
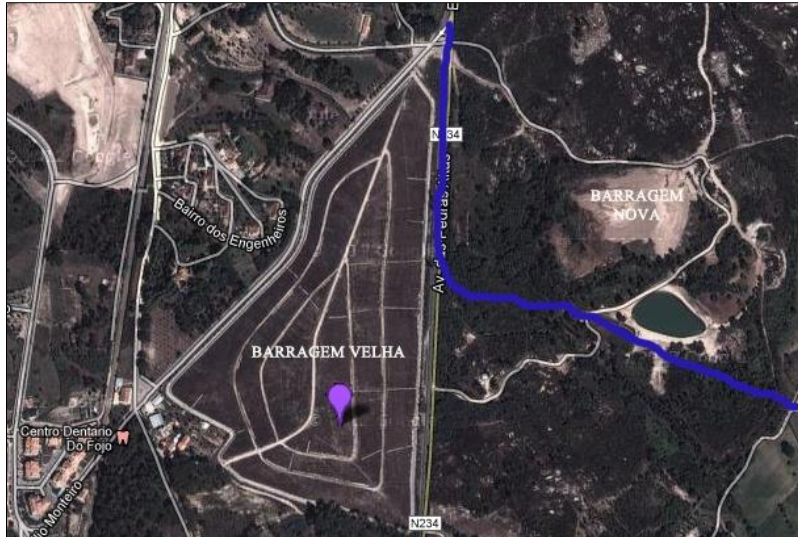
Figure 4 - Approximate location of Ribeira da Pantanha in the mine area (in blue). Ribeira da Pantanha is an effluent of the Mondego River (adapted from Google Maps, 2012).

Figure 5 – Ribeira da Pantanha; ferric hydroxide precipitates are visible. (photos taken in May 2012).

2.2. Selected plant species

In order to study the applicability in uranium water removal of plant material derived from Portuguese forests, four plant species were selected: pond water-starwort

(*Callitriche stagnalis* Scop.), Scotch broom (*Cytisus scoparius* (L.) Link), tree heath (*Erica arborea* L.) and pedunculate oak (*Quercus robur* L.). These species are autochthonous and common in Portugal.

Pond water-starwort, *Callitriche stagnalis* Scop.: *C. stagnalis* (Fig. 6) is an amphibious macrophyte native to Europe and widespread in this continent. It has been introduced in North America where in some cases it is considered an invasive species. These annual plants are found in clear and shallow non-moving to slow-moving waters in streams, lakes and wetlands, and also in very moist soils along streams and lakes margins (Murillo, 1990). The aquatic forms are small submerged-root caulescent plants with very slender stems growing 10 to 30 cm and forming mats of floating leaves at the water surface (Murillo, 1990).
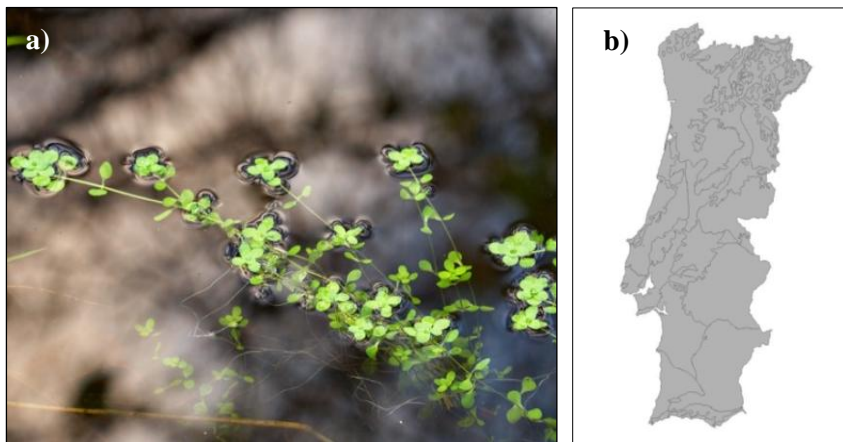
Figure 6 – *Callitriche stagnalis* and its distribution in Portugal (in gray)

(adapted from: a)

http://www.botanicalgarden.ubc.ca/potd/2006/11/callitriche_stagnalis.php;

b) Flora Digital de Portugal -

http://aguiar.hvr.utad.pt/pt/herbario/cons_reg_todos1.asp?ID=884).

Scotch broom, *Cytisus scoparius* (L.) Link: *C. scoparius* (Fig. 7) is a perennial leguminous shrub species native to Europe and Macaronesia, where it is widespread (Lozano, 2012; Flora Digital de Portugal, 2007a). It is commonly found throughout the Iberian Peninsula region, except for the East region, where it is less abundant (Lozano, 1990; Flora Digital de Portugal, 2007a). It was introduced in North America, Australia, South Africa, and India, and it is classified as an invasive species in these countries (Bossard & Rejmanek, 1992; Williams, 1981). *C. scoparius* plants are found in habitats ranging from mesic to seasonally dry, colonising disturbed sites such as roadsides and forests clearcuts, but also undisturbed sites such as grasslands, shrublands, river banks and open canopy forests (Lozano, 1990; Bossard & Rjmanek, 1992). Plants are 0.5 – 2 m tall, with long slender branches that form dense stands. The stem is five-angled (star-shaped in cross-section) (Lozano, 1990) and displays photosynthetic tissues (Bossard & Rejmanek, 1992). Leaves are lost during the summer months.
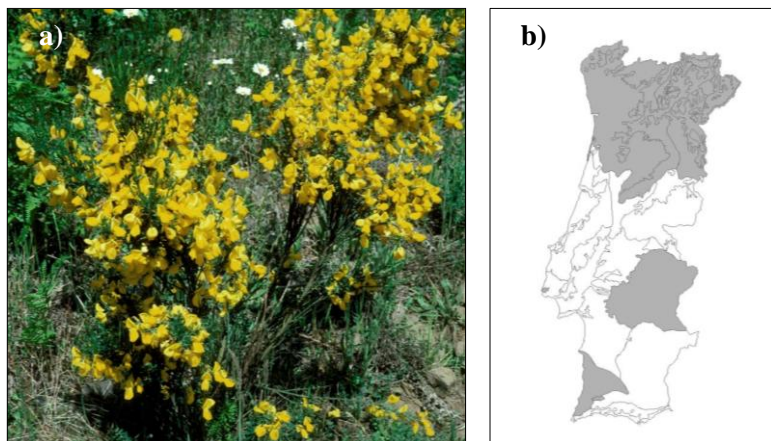
Figure 7 – *Cytisus scoparius* and its distribution in Portugal (in gray)

(adapted from: a) http://www.invasive.org/browse/subinfo.cfm?sub=4408,

b) Flora Digital de Portugal -

http://aguiar.hvr.utad.pt/pt/herbario/cons_reg_todos1.asp?ID=1393).

Tree heath, *Erica arborea* L.: *E. arborea* (Fig. 8) is a perennial shrub or small evergreen tree native to the Mediterranean region, Macaronesia, and North and East Africa. It is widespread in the Iberian Peninsula region (Bayer, 1990; Flora Digital de Portugal, 2007b). Plants are commonly found in dark and cool thickets and open woodlands, especially in acidic and siliceous soils (Bayer, 1990). Plants are 1 to 4 m tall, with some specimens reaching 7 m. Branches are slender, covered with hairs and very hard and heat-resistant. Leaves are small and narrow, 0.5 – 0.7 mm long (Bayer, 1990).
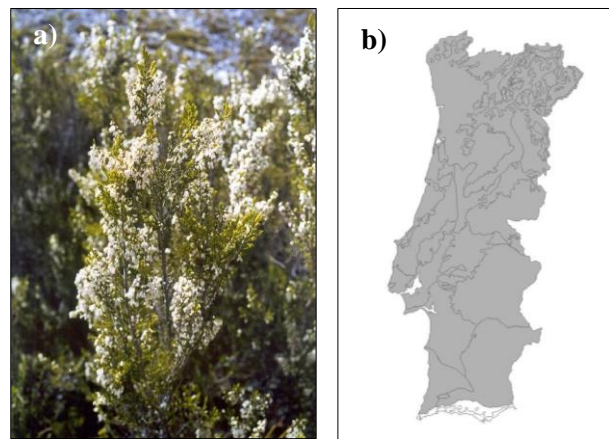
Figure 8 – *Erica arborea* and its distribution in Portugal (in gray) (adapted from Flora Digital de Portugal -

http://aguiar.hvr.utad.pt/pt/herbario/cons_reg_esp2.asp?ID=632).

Pedunculate oak, *Quercus robur* L.: *Q. robur* (Fig. 9) is a deciduous tree native to most Europe, the Balkans and the Ural Mountains region (Franco, 1990; Flora Digital de Portugal, 2007c). It is restricted to the North region of the Iberian Peninsula, and in Portugal it is found essentially in the North East region. *Q. robur* trees are found deep, cool and preferably siliceous soils, in temperate climates with short, if any, dry period (Franco, 1990). Trees are 30 to 40 m tall, with large wide spreading crowns.
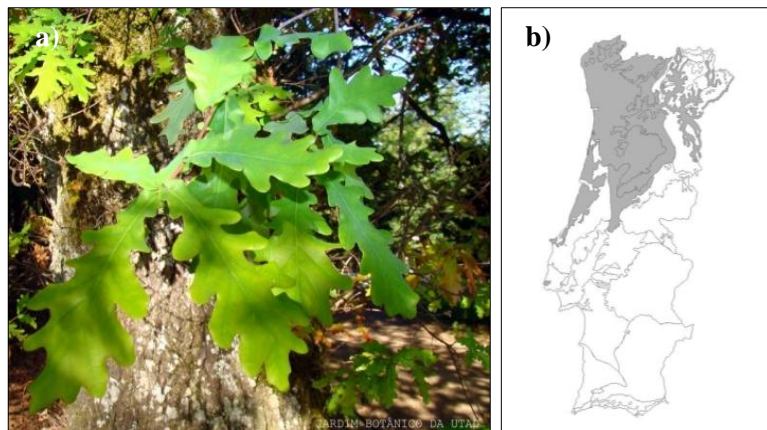
Figure 9 – *Quercus robur* and its distribution in Portugal (in gray) (adapted

from: a)

http://www.aphotoflora.com/af_quercus_robur_pedunculate_common_oak.h

tml; b) and c) Flora Digital de Portugal -

http://aguiar.hvr.utad.pt/pt/herbario/cons_reg_fam2.asp?familia=Fagaceae&I

D=847).

2.3. Evaluation of the plant material biosorption capacity

2.3.1. Plant material preparation

Senescent leaves of *Q. robur* were collected in Cioga do Campo (Coimbra) in 2008. Plant material from *C. scoparius* and *E. arborea* was collected in Vale de Canas (Coimbra), in September 2011. The aquatic plant *C. stagnalis* was collected from an unpolluted stream in Ançã (Ribeira de Ançã, Cantanhede, Coimbra), also in September 2011.

The plant material was processed at the Biogeochemistry Laboratory of the Department of Earth Sciences, University of Coimbra. Regarding the perennial species

C. scoparius and *E. arborea*, only the plant parts corresponding to the annual growth were used. In the case of *C. stagnalis*, whole plants were used.

The plant material was rinsed with tap water, cut and dried at 60 ºC for 30 hours. Samples of plant material were collected and analysed to determine initial uranium content, which was 0.991 ± 0.340 mg.kg^{-1} in *C. stagnalis*, 0.318 ± 0.129 mg.kg^{-1} in *C. scoparius*, 0.355 ± 0.165 mg.kg^{-1} in *E. arborea* and 0.282 ± 0.05 mg.kg^{-1} in *Q, robur*. Plant material of each species (4.0 g dry weight) was placed separately in mesh bags (PVC; approximately 13 x 15 cm, mesh diameter $\approx$ 2 mm) to be used in the laboratory assay.

2.3.2. Experimental design and procedures

The laboratory assay was entirely developed at the Biogeochemistry Laboratory of the Department of Earth Sciences, University of Coimbra. A total of 30 microcosms were constructed, which consisted of glass aquariums (17.5 x 20.3 x 30 cm) filled with 4.5 l of water and equipped with air pumps (0.5 l/min) to provide water oxygenation and homogenisation. Each microcosm contained plant material of the four selected species. The microcosms were kept at room temperature. The water evaporation rate in the microcosms was assumed to be constant and was determined as 0.027 l/day.

In order to attain an approximate initial concentration of 200 ppb (200 µg.l^{-1}), 1 ml of a 1000 ppm (1 g.l^{-1}) uranyl nitrate 6-hydrate ($N_2O_8U.6H_2O$) solution was added to the water in each microcosm. Measurements and readjustments of the uranium concentrations were made at approximately every two days during the experiment, in to approximate the system to a continuous flow system with continuous inputs of contamination. The water pH was measured using a pH meter and initially adjusted to

pH 5.5 in each microcosm by adding sulfuric acid (H_2SO_4) 1%. During the study period, the pH was measured and readjusted with the same regularity of uranium concentrations readjustments, by adding the necessary volumes of sulphuric acid 1% or potassium hydroxide (KOH) 1 M solutions.

Sampling was done at preset dates during the study period in order to assess uranium accumulation over time: 2 hours (I), 1 day (II), 2 days (III), 7 days (IV), 19 days (V) and 37 days (VI). For such purposes, the microcosms were arranged in six blocks of five units and at each sampling date one block was dismantled in order to collect the plant material, which was then prepared for analysis. To evaluate the distribution of uranium in the system, the contents of dissolved uranium and of uranium associated with suspended matter (mostly resulting from decomposition of the biomass) were also analysed. Samples of water were collected and filtrated using a filtering device composed of a 100 ml syringe and 0.45 µm glass microfiber filters (934-AH Whatman). The filtrated water was transferred to 50 ml bottles and acidified to pH 2 with nitric acid (HNO_3) 65%, in order to prevent uranium precipitation. Filtrated water samples and filters were then prepared for analysis.

2.3.3. Uranium determination methodologies

Uranium concentrations in waters, plant material and suspended matter were determined by standard fluorometric analysis in which the intensity of the delayed fluorescence of uranyl ions at $\lambda = 530$ nm is measured. This is a prompt analytical method with high sensitivity (2 $\mu g.l^{-1}$). The analyses were made using a Fluorat – 02 – 2M equipment (Lumex, Russia). Each analysis was preceded by equipment calibration

using uranium solutions of 2 ppb, 10 ppb, 100 ppb and 1000 ppb, prepared by diluting the initial 1000 ppm U solution in HNO_3 0.005%.

Uranium analyses in water: Solutions for measuring uranium concentrations in waters during the course of the experiment were prepared by adding 0.5 ml of microcosms water, 0.5 ml of polysilicate and 5 ml of HNO_3 0.005%. Solutions for analysis of the filtered water samples were prepared in the same way.

Uranium analyses in plant material: The plant material and the filters were prepared as described on the methodologies developed by Van Loon and Barefoot (1989). The process consisted of the following steps:

1) After removal from the microcosms, plant material was left to dry at room temperature or dried at 60 º C when necessary. Dry material was weighed in order to determine weight loss due to decomposition.

2) Dry plant material was ground using a crushing machine to produce homogenous samples. 1.0 g of the ground material was collected for further analysis.

3) Ground material was burned at 450 º C for 8 hours in order to eliminate organic contents.

4) Ashes were transferred to 100 ml Falcon tubes and 8 ml of nitric acid saturated with aluminium nitrate (prepared by dissolving 950 g of $Al(NO_3)_3.9H_2O$ in 600 ml of HNO_3 2.5 M) were added to each sample.

5) Samples were placed in a boiling bath and heated for one hour.

6) Tubes were cooled to the room temperature and 10 ml of ethyl acetate ($C_4H_8O_2$) were added to each tube.

7) Tubes were shaken for 2 minutes in a shaking machine.

8) Samples were let stand for minutes to allow phases separation and 5 ml aliquots of ethyl acetate were pipetted to porcelain crucibles.

9) Each crucible's content was ignited and burned until its complete consumption.

10) After ignition, 7 ml of HNO_3 0.005% were added to the crucibles in order to dissolve the residues on the crucibles' material.

11) The final solutions for uranium analysis were prepared by transferring 0.5 ml of these solutions to 25 ml plastic recipients and adding 0.5 ml polysilicate and 5 ml distilled water.

Since the fluorometer provided only uranium measurements less than 1000 ppb, samples with high content of uranium were diluted 10 times or 100 times in distilled water. The diluted solutions were used for analysis as described in 11).

Uranium analyses in suspended material: The analyses of the material collected through water filtration were performed resorting to an adapted methodology similar to that described by Van Loon and Barefoot (1989) for determination of uranium contents in plant samples. The main differences comprised the initial steps of the process:

1) Filters were burned at 450 º C for 8 hours.

2) Filters with the remaining material were transferred to 100 ml Falcon tubes and 8 ml of nitric acid-aluminium nitrate reagent were added to each tube.

3) Tubes were shaken for 2 minutes in a shaking machine to allow the complete reaction of the reagent with the particles on the filters.

4) Tubes were placed in a boiling bath and heated for one hour.

5) Samples were cooled to room temperature and 10 ml of ethyl acetate were added to each tube.

6) Tubes were shaken for 10 minutes in a shaking machine.

7) Filters were removed from the tubes.

The next steps taken in suspended material analyses were performed as described in steps 8) to 11) of the plant material analyses methodology.

Uranium contents determination: The fluorometric analysis is a direct method for determining uranium concentrations in water samples. For estimating the uranium total contents in the plant material the following formula was used

$$U\ content\ (\mu g/kg\ DW) = \frac{U \times 14}{m} \times d$$

in which U represents the uranium concentration value obtained by fluorometric analysis, m is the mass of the sample (g dry weight) and d represents the dilution of the sample solution when this was needed.

The same formula was used to estimate uranium contents in the suspended matter. However, in such cases the volume of water filtered in order to produce the samples was used instead of the samples mass.

2.4. Statistical analysis

Differences in uranium immobilisation abilities among plant species were tested using uranium concentrations in the plant material as well as total uranium amounts in the plant material over time. These data were log-transformed in order to meet the assumptions of normality and homogeneity of variances, which were verified using

Shapiro-Wilk test and Levene's and Hartley's test (Field, 2009). The assumptions of ANCOVA were also verified and this analysis was conducted in both set of data, using time as covariate. Pairwise comparisons were conducted afterwards using Sidak-corrected α values.

Differences in weight loss due to decomposition at 37 days were analysed using one-way ANOVA, followed by a Tukey test.

The significance level was held at 0.05 for all the statistical evaluations (except for the pairwise comparisons with Sidak correction). All the statistical analyses were performed using SPSS v.15.0 software.

Chapter 3   Results

3.1. Uranium removal from waters

Throughout the experiment, regular additions of variable amounts of uranium to the microcosms' water were performed, in order to maintain the water uranium concentration at values near 200 µg.l^{-1}. During 37 days, a total of 6.592 mg of uranium was added to the system (Fig. 10).
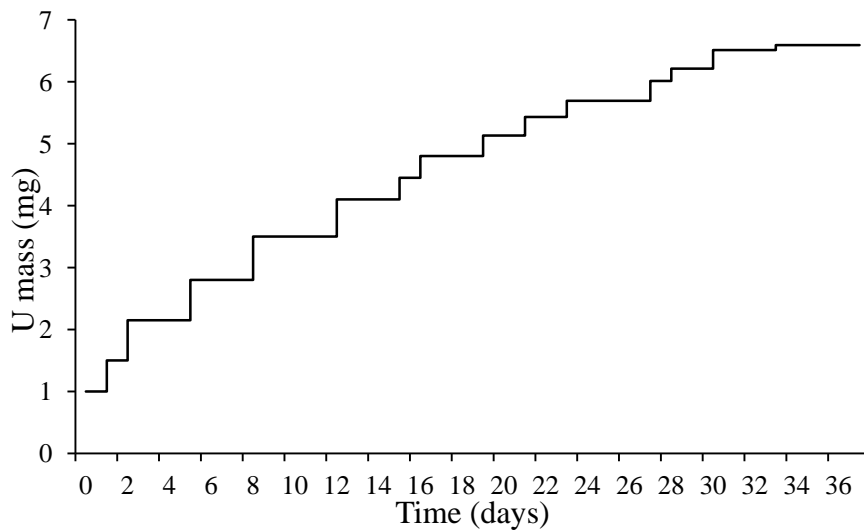
Figure 10 – Addition of uranium to the microcosms' water along the experiment.

Uranium removal from waters was noticeable throughout the experiment. Uranium concentration reduction was more pronounced during the first 48 hours of experiment (Fig. 11; Fig. 12). The observed decrease in uranium concentrations was gradually less marked over time.
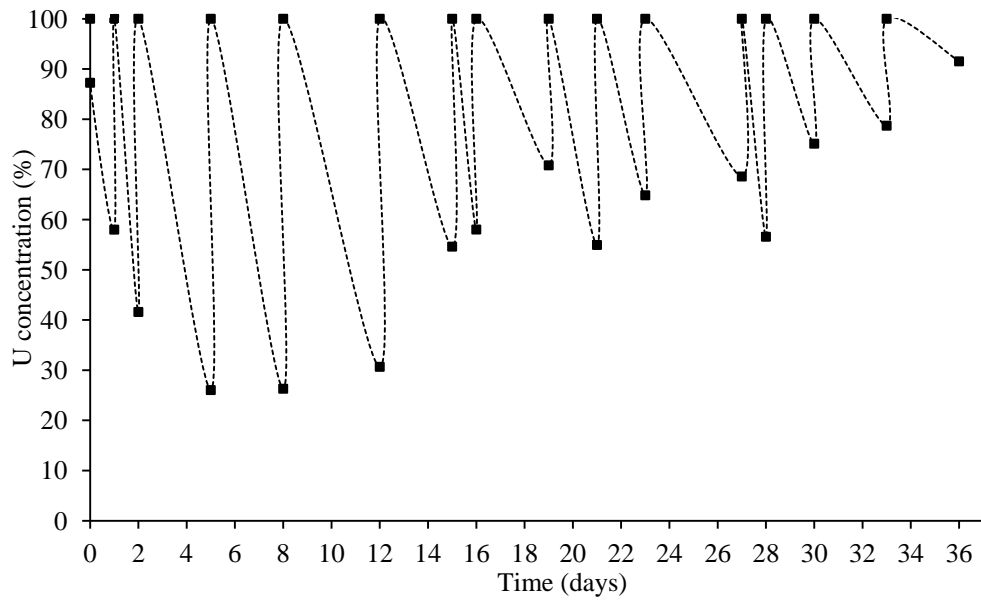
Figure 11 – Variation of the uranium concentrations in the water of the microcosms during the experiment, expressed as percentages of initial concentrations, considering regular uranium replenishments.
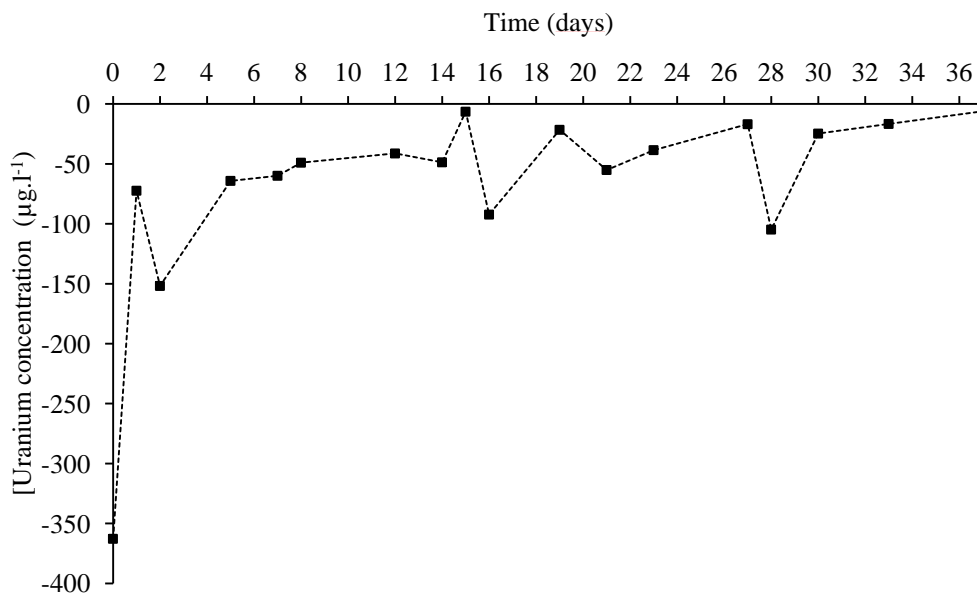
Figure 12 – Rate of uranium concentration reduction, calculated as the variation of uranium concentration as a function of time variation ((U concentration$_{t1}$- U concentration$_t$) / (t$_1$ – t)).

3.2. Accumulation of uranium in the plant biomass

Uranium was retained in the biomass of the four selected species (Table II; Fig. 13). The highest uranium concentrations were found in *Callitriche stagnalis* (632.06 ± 128.54 mg.kg^{-1}), followed by *Quercus robur* (182.04 ± 27.37 mg.kg^{-1}), *Cytisus scoparius* (127.26 ± 27.41 mg.kg^{-1}) and *Erica arborea* (97.01 ± 27.11 mg.kg^{-1}). Differences in uranium accumulation among species were significant (ANCOVA, $F_{(3, 113)} = 39.65$, $P < 0.001$). Pairwise comparisons revealed that uranium accumulation in *C. stagnalis* biomass was significantly higher than accumulation in the remaining plant species ($P < 0.001$), whereas no significant differences in uranium accumulation were observed between *Q. robur* and *C. scoparius* ($P = 0.237$) and between the later and *E. arborea* ($P = 0.545$).

Table II – Uranium concentrations in the biomass (mg.kg^{-1} dry weight) of the four plant species along 37 days (means ± standard deviations, $n = 5$ except for *Cytisus scoparius* at 19 and 37 days, when $n = 4$).

	Callitriche stagnalis	*Cytisus scoparius*	*Erica arborea*	*Quercus robur*
I: 2 hours	13.94 ± 10.40	2.82 ± 0.53	2.05 ± 0.67	7.27 ± 2.45
II: 1 day	31.73 ± 18.51	5.65 ± 0.78	5.51 ± 0.93	9.47 ± 0.93
III: 2 days	31.72 ± 15.42	10.79 ± 2.96	5.96 ± 2.35	9.65 ± 4.33
IV: 7 days	96.80 ± 21.23	26.03 ± 12.79	19.44 ± 11.80	27.32 ± 2.68
V: 19 days	336.64 ± 72.65	40.66 ± 9.933	55.98 ± 9.55	72.94 ± 7.34
VI: 37 days	632.06 ± 128.54	127.26 ± 27.41	97.01 ± 27.11	182.04 ± 27.37
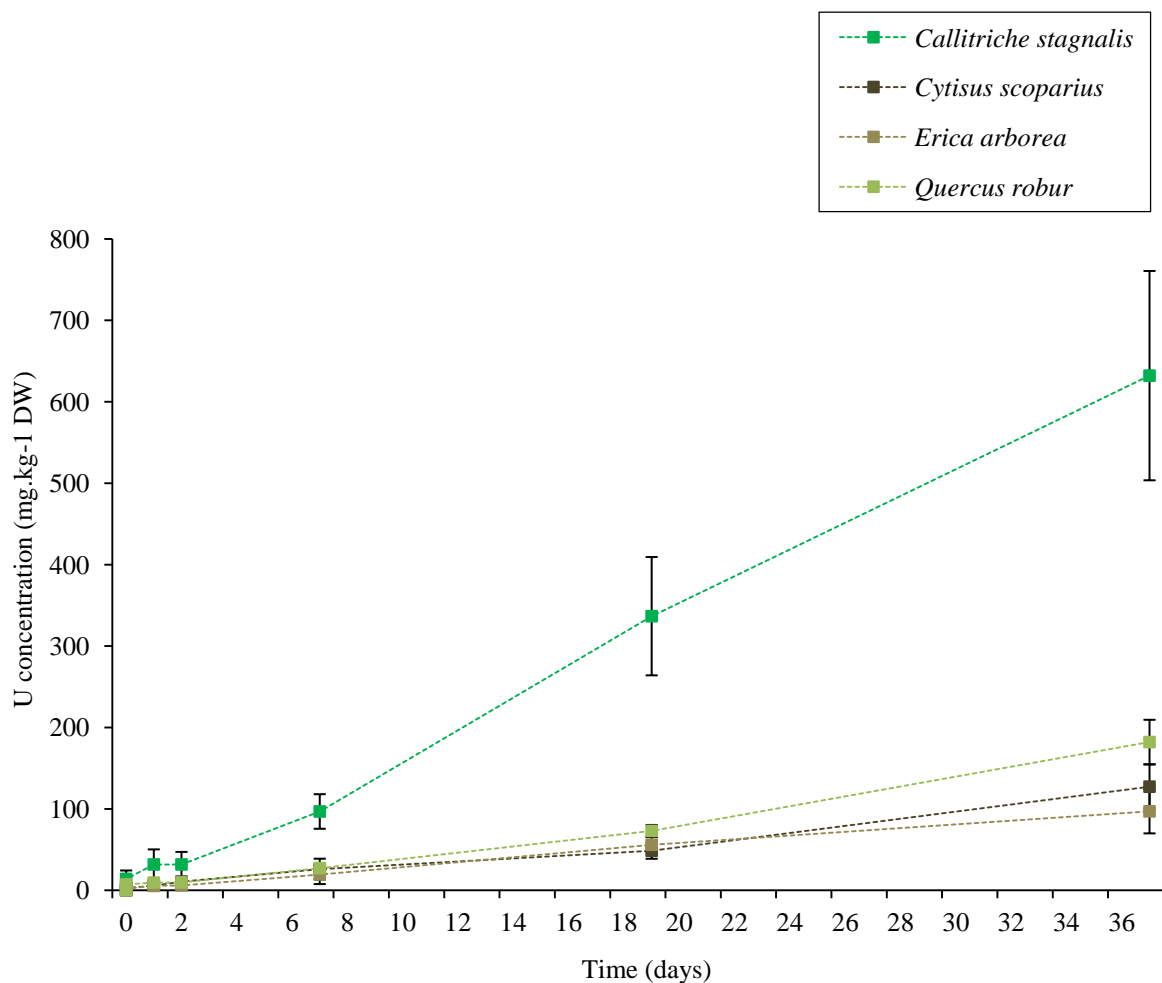
Figure 13 – Uranium accumulation in the biomass of the four studied plant species over time (means ± standard deviations, *n* = 5 except for *Cytisus scoparius* at 19 and 37 days, when *n* = 4).

Since plants lost weight during the experiment due to decomposition, increasing uranium concentrations do not prove that there was a continuous uranium accumulation. It is also necessary to evaluate the total uranium amounts in the plant material during the study period; table III shows the estimated overall uranium amounts in the biomass of the four studied plant species.

Table III – Overall amounts of uranium (mg) in the plant biomass at each sampling date and the proportion of increase in relation to the uranium amounts measured in the previous sampling date (means ± standard deviations, $n = 5$ except for *Cytisus scoparius* at 19 and 37 days, when $n = 4$.

	Callitriche stagnalis		*Cytisus scoparius*		*Erica arborea*		*Quercus robur*	
I: 2 hours	0.046 ± 0.035		0.010 ± 0.002		0.008 ± 0.003		0.032 ± 0.011	
II: 1 day	0.100 ± 0.056	2.18	0.020 ± 0.003	1.97	0.021 ± 0.004	2.67	0.041 ± 0.004	1.28
III: 2 days	0.102 ± 0.050	1.02	0.035 ± 0.035	1.74	0.022 ± 0.008	1.02	0.041 ± 0.019	1.01
IV: 7 days	0.285 ± 0.061	2.80	0.084 ± 0.037	2.42	0.069 ± 0.041	3.16	0.114 ± 0.010	2.76
V: 19 days	0.766 ± 0.165	2.69	0.142 ± 0.037	1.69	0.183 ± 0.027	2.65	0.279 ± 0.036	2.44
VI: 37 days	1.396 ± 0.276	1.82	0.355 ± 0.087	2.50	0.322 ± 0.090	1.76	0.688 ± 0.117	2.47

The overall amounts of uranium in the plant biomass increased during the study period, confirming that uranium accumulation was continuous over time. Differences in the overall uranium immobilisation were significant among species (ANCOVA, F

(3,113) = 33.58, $P < 0.001$). Pairwise comparisons using these data showed different results in relation to pairwise comparisons using uranium concentrations data. Total uranium accumulation in *C. stagnalis* biomass was significantly higher than accumulation in the biomass of *Q. robur*, *C. scoparius* and *E. arborea* ($P < 0.001$). However, total uranium accumulation in oak leaves was also significantly different from the remaining species ($P < 0.05$), whereas no significant differences were observed between *C. scoparius* and *E. arborea* ($P = 0.934$). Different results may be related to different decomposition rates of the biomass, which resulted in different weights among species over time. Since concentration data do not consider the effects of different weights, the analysis of metal concentrations alone may not provide accurate conclusions regarding metal accumulation.

Weight loss due to decomposition during 37 days was higher in *C. stagnalis* (44.5%) followed by *C. scoparius* (30.1%), *E. arborea* (17.0%) and *Q. robur* (14.1%) (Fig. 14). Differences in overall weight loss during the study period were significant among species (one-way ANOVA, $P < 0.001$), although no significant differences were found between oak leaves and heath (Tukey HSD test, $P = 0.657$).
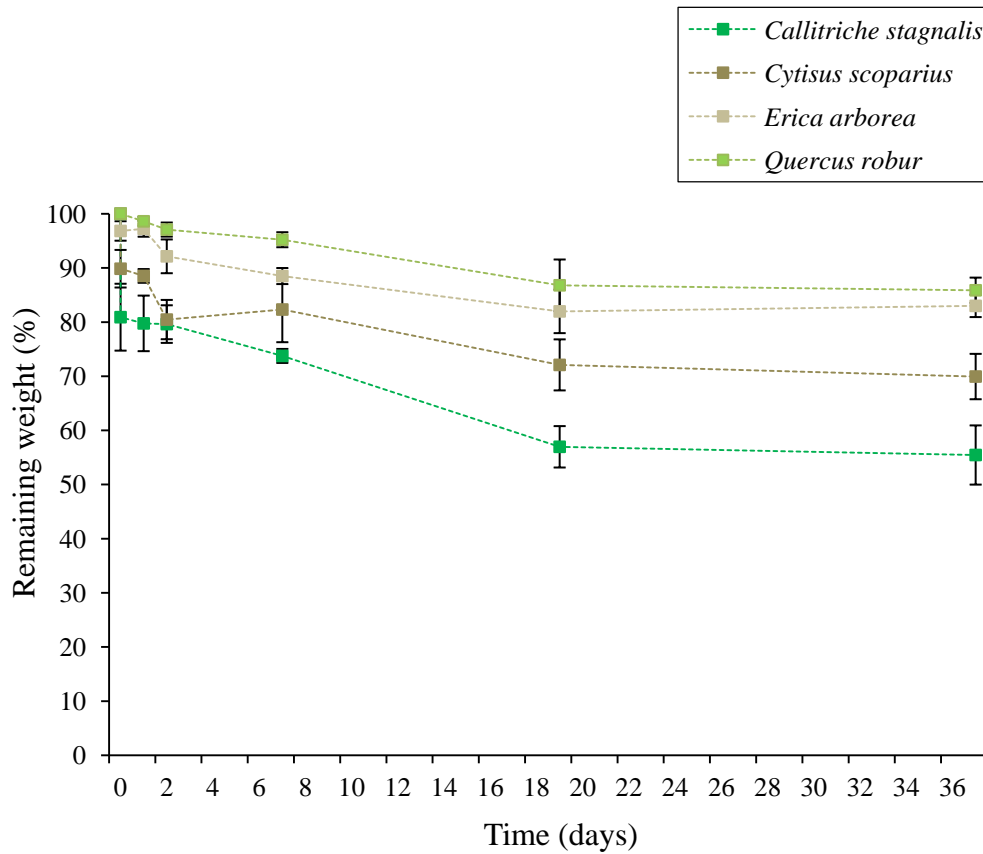
Figure 14 – Weight loss of the four studied species over 37 days (,
remaining weight, means ± standard deviations, $n = 5$).

3.3. Uranium in the water and associated with suspended matter

Dissolved uranium concentrations were lower than 200 µg.l^{-1} during the study period.
The analysed suspended matter was found to retain uranium in significant
concentrations, increasing in time to the maximum of 167.57 ± 11.67 µg.l^{-1} (Table IV;
Fig. 15).

Table IV – Uranium concentrations in water and in the suspended matter ($\mu g.l^{-1}$) during the experiment (means ± standard deviations, $3 \geq n \geq 5$).

	Water	Suspended matter
I: 2 hours	81.13 ± 9.08	75.32 ± 5.23
II: 1 day	83.83 ± 13.61	64.53 ± 11.98
III: 2 days	100.61 ± 1.62	46.46 ± 6.60
IV: 7 days	109.03 ± 32.25	82.70 ± 31.43
V: 19 days	153.75 ± 9.74	154.54 ± 31.22
VI: 37 days	120.30 ± 22.42	167.57 ± 11.67
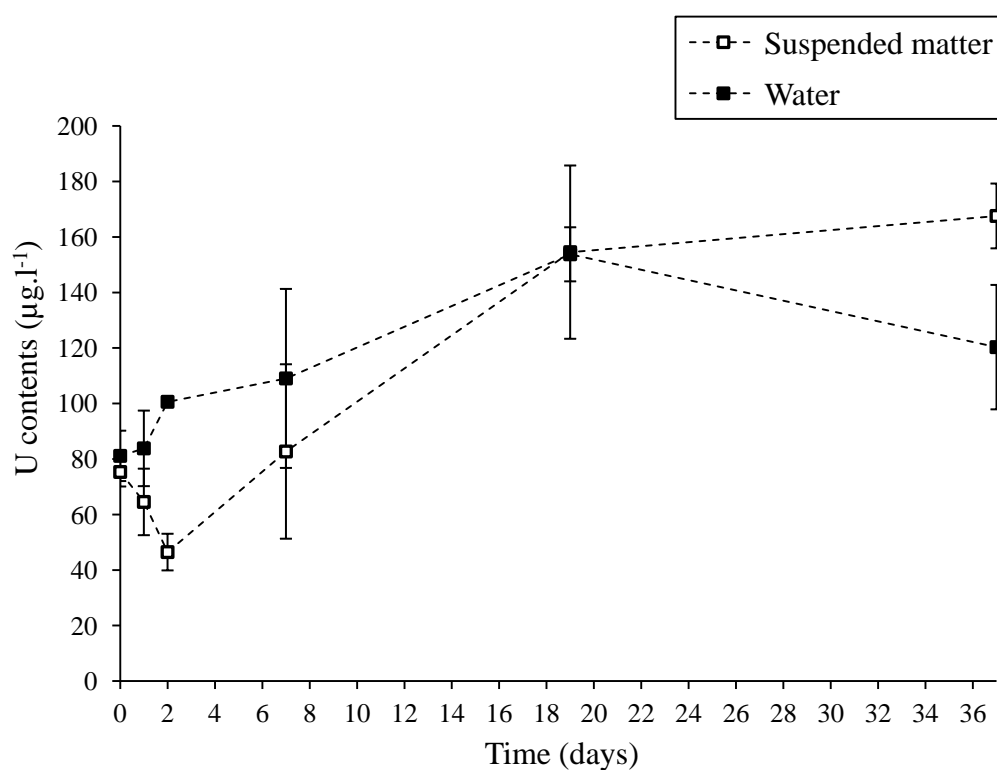
Figure 15 – Variation in concentrations of dissolved uranium and uranium associated with the suspended matter (means ± standard deviation, $3 \geq n \geq 5$).

3.4. Uranium distribution in the system

Overall, the proportion of the total mass of uranium in the system that was transferred to the plant material increased throughout the experiment, whereas the proportion of dissolved uranium and uranium associated with the suspended matter decreased (Fig. 16). At the end of the study period, about 42.7% of the total mass of uranium was in the plant biomass, whereas about 8.6% was associated with the suspended matter and 5.6% was in the water. The contribution of each plant species to the total uranium mass transferred to the plant biomass was similar along the experiment, with pond water-starwort retaining approximately 50% of the uranium in the plant material. A large fraction of the total uranium mass in the system was not accounted for in the performed analyses.
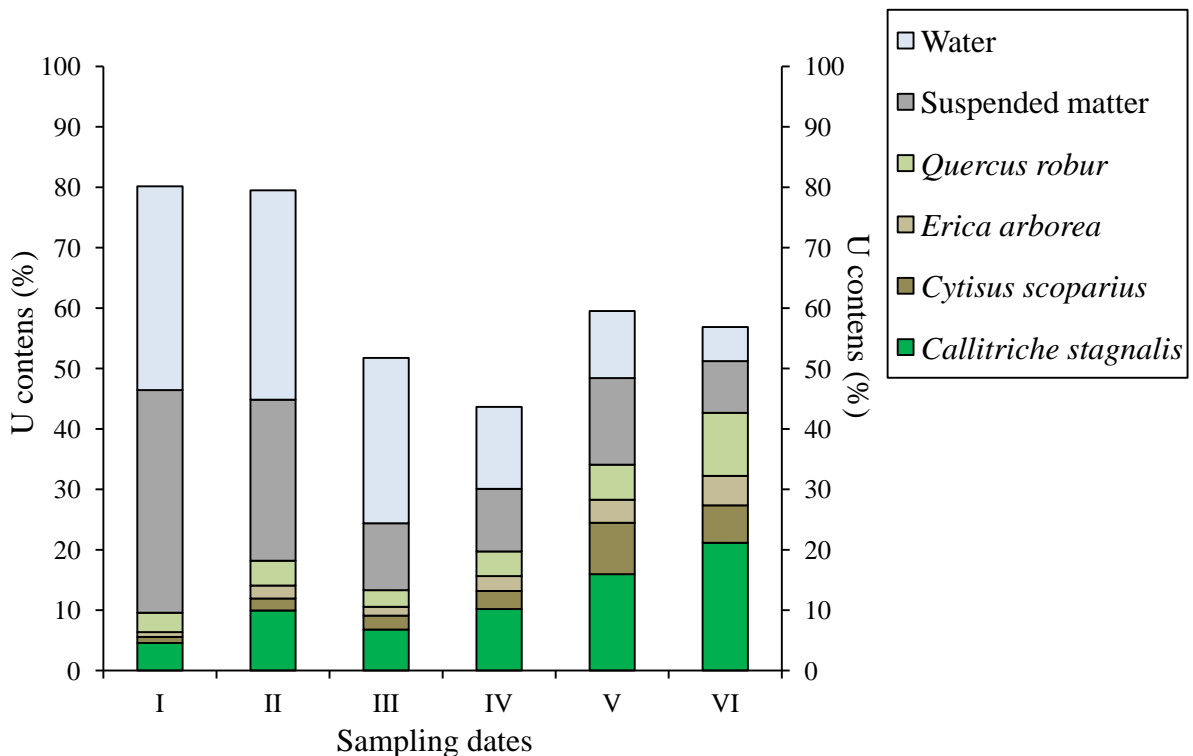
Figure 16 - Proportion of the total uranium mass (mg) transferred to each studied compartment during the experiment, expressed in percentages (means, $n = 5$).

Chapter 4   Discussion

4.1. Uranium removal from waters

It was observed that the plant material immobilized uranium; therefore the studied plant biomass has the potential to effectively remove uranium from contaminated waters.

The removal of uranium from was waters and its accumulation in the plant tissues was a biphasic process, with a rapid uranium removal phase during the first two hours of experiment being followed by a slower removal phase. Rapid removal rates are usually associated with the adsorption of the metal onto the external surface of the sorbent, whereas slower removal rates are usually related to the solute diffusion into the sorbent's micropores that follows saturation of the external biding sites (Gadd, 2009; Vadivelan & Kumar, 2005). Other factors contribute to slow biosorption rates such as eventual changes in the chemical composition of the medium throughout the experiment and nucleation - precipitation reactions (Gadd, 2009). After 37 days of exposure, the rates of uranium concentration decrease in the water were low, suggesting that the system was progressing towards equilibrium.

4.2. Uranium biosorption ability of the plant biomass

Uranium was immobilised in concentrations the plant biomass of the four studied species, attaining maximum mean values of 632.06 mg.kg^{-1}. Despite the diminished rates of uranium removal by the end of the study period, uranium accumulation in the biomass apparently did not stabilise, suggesting that the plant material was not completely saturated. Thus, it may be assumed that the plant material can immobilise higher concentrations of this radionuclide.

The maximum uranium concentrations attained in the dead biomass may be considered low when compared with results obtained in other biosorption studies (Table V). However, comparisons between results have to be drawn with caution since typical biosorption studies are usually conducted under very distinct conditions, commonly involving much higher contaminant concentrations, more controlled physicochemical conditions (e.g. limited influence of competing ions) and some degree of preparation of the biomass (e.g. crushing or grinding for augmented surface area). Furthermore, the results of such studies frequently correspond to calculated maximum biosorption capacities, obtained through specific mathematical sorption models (e.g. Langmuir isotherm).

Table V – Maximum adsorption capacities of some types of biomass (q_{max}, predicted by the Langmuir isotherm) and associated experimental conditions.

Biosorbent material	Maximum adsorption capacity Q_{max}	Experimental conditions	References
Water hyacinth roots	5.15×10^{-2} mg.g^{-1}	U range 20 – 100 mg.l^{-1}; pH 5	Shawky, Geleel & Aly, 2005
Poplar leaves	2.3 mg.g^{-1}	U range 1 -10 mg.l^{-1}; pH 4	Al-Masri, Amin, Al-Akel & Al-Naama, 2010
Grapefruit peel	140.79 mg.g^{-1}	U range 50 – 500 mg.l^{-1}; pH 5	Zou, Zhao & Zhu, 2012

Table V (continued)

Biosorbent material	Maximum adsorption capacity Q_{max}	Experimental conditions	References
Pummelo peel	724.6 mg.g^{-1}	U range 30 – 80 mg.l^{-1}; pH 5.5	Li et al., 2012
Cystoseria indica algae	256 mg.g^{-1}	U range 50 – 500 mg.l^{-1}; pH 4	Khani, Keshtkar, Ghannadi & Pahlavanzadeh, 2008

The studied plant species exhibited different behaviours concerning the amounts of immobilised uranium in the plant biomass. These differences may be related to physical differences in the surface of the plant material, including distinct porosities and surface areas, as well as to differences in the chemical composition of the biomass. These differences ultimately influence the type, number and accessibility of the metal-sequestering chemical groups, which determine the binding strength of the metal to the biomass and the amounts of adsorbed metal (Park, Yun & Park, 2010; Volesky & Holan, 1995).

The highest uranium concentrations were found in pond water-starwort tissues. This plant accumulated the highest overall amounts of uranium along the experiment even though it has also registered the highest mass loss due to decomposition. These results demonstrate that decaying tissues of *C.stagnalis* can act as a sink for uranium in contaminated waters. Pond water-starwort is known to accumulate uranium in its tissues in high concentrations. This ability was demonstrated by Pratas, Favas, Paulo,

Rodrigues and Prasad (2012), who have reported maximum uranium concentrations of 1948.41 mg.kg^{-1} in living plants growing in polluted streams (uranium concentration in waters of 1220.4 µg.l^{-1}). The extent of active and passive mechanisms involved in uranium immobilisation in living pond water-starwort plants is unknown. The capacity of this aquatic plant to retain uranium through passive processes may be related, at least in part, with structural and morphological characteristics which constitute adaptations to the aquatic environment, such as the presence of a thin cuticle and a high surface area (Bornette & Puijalon, 2009). In fact, several studies have reported significant metal passive accumulation by various macrophyte species (Rai, 2009); aquatic plants seem to have strong affinity for uranium (Kalin, Wheeler & Meinrath, 2005).

The plant biomass derived from the terrestrial species retained considerably lower amounts of uranium where compared to pond water-starwort tissues. Of these, the highest amounts of uranium were found in *Quercus robur* leaves. Metal biosorption by leaves has been studied by several authors. Shafique et al. (2012) have calculated maximum arsenic accumulation capacity of 3.27 mg.g^{-1} in pinus leaves (*Pinus roxburghii* Sarg.), whereas Prasad and Freitas (2000) have measured maximum concentrations of 0.62 mg.g^{-1} in *Quercus ilex* L. leaves. Rodrigues, Pratas, Tavares and Branches (2006) observed that leaves can act as an effective uranium immobilisation substrate in natural waters. The extent of uranium accumulation was species-dependent, and the highest mean uranium concentrations (159.5 mg.kg^{-1}) were found in litter composed mainly by *Salix babylonica* L. leaves.

The hypothesis of different plant biomass composition and structure as the causes for different uranium retention abilities may be confirmed resorting to a variety of analytical tools, which are summarised in Table VI. These techniques provide important information concerning the composition of the biosorbent and the active sites involved

in the biosorption process, as well as on the form of the bound contaminant (Park, Yun & Park, 2010). Therefore, such analyses are essential for a comprehensive characterization of the specific biosorption mechanisms associated with each type of material, providing a better understanding of its options of use in contaminated water treatment.

Table VI – Some analytical tools used in biosorption studies (adapted from Park, Yun & Park, 2010).

Analytical thechniques	Remarks
Scanning electron microscopy (SEM)	Visual confirmation of the biosorbent surface morphology
Transmission electron microscopy (TEM)	Visual confirmation of inner morphology of biomass
Potentiometric titration	Determine active sites of the biosorbent and their amounts
Fourier-transformed infrared spectroscopy (FT-IR)	Determine active sites of the biosorbent
Electron spin resonance spectroscopy (ESR)	Determine active sites of the biosorbent
Energy dispersive X-ray spectroscopy (EDS)	Element analysis and chemical characterization of the metal bound to the biosorbent
X-ray photoelectron spectroscopy (XPS)	Determine oxidation state of the bound metal and its ligand effects

4.3. Overall uranium distribution in the system

Uranium was transferred to the plant biomass throughout the experiment, whereas the analyses performed to the water and filtrated matter indicated a decrease in these compartments over time. Since the high affinity of uranium for organic compounds is well known, a fraction of the uranium in the system presumably became associated with the solubilised compounds resulting from the plant material leaching (e.g. sugars, amino acids), during the first instants of experiment. As the plant material was decomposing, the released organic particles would also adsorb uranium.

A large proportion of the total uranium mass in the system (ranging from approximately 20 to 40%) was not detected in the performed measurements. This was particularly noticeable considering the results obtained after two days of experiment. It is likely that an important fraction of the total uranium mass in the system was associated with organic matter resulting from the decomposition process that was entrapped in the mesh of the PVC bags, which was not analysed in this study. The amount of organic matter retained in the mesh bags was noticeably high by the end of the experiment. It is also likely that part of the uranium in the system has formed insoluble complexes throughout the experiment, and the resultant material may have been deposited onto the component materials of the microcosms. These deposits could also have prevented the detection of some uranium in the system. Additionally, in control parallel experiments conducted concurrently with the presented study, it was observed that a small fraction of uranium was adsorbed onto the surfaces of the system's components, such as the mesh bags and the microcosm's glass (data not showed).

4.4. Influence of the experimental conditions and methodologies on uranium biosorption

Several parameters that are known to directly or indirectly influence the biosorption process, such as the presence of competitor ions, ionic strength of the medium, and redox potential, were not considered in the present study. Information on these parameters would have been useful in order to better understand the physicochemical processes that took place in the system.

The presence of other cations in solution (e.g. Ca^{2+}, Mg^{2+}, Na^+) may have had a negative influence on the uranium biosorption process mainly due to the competition for negatively-charged groups in the biomass (Park, Yun & Park, 2010). Anions in solution (e.g. Cl^-, SO_4^{2-}, CO_3^{2-}) may also have affected the uranium biosorption due to the formation of uranium complexes. The effect of counterions in uranium biosorption may differ concerning different types of biomass (Diniz & Volesky, 2005). Complexing agents such as PO_4^{2-} promote the formation of uranyl phosphate insoluble complexes. Precipitation of these compounds may have decreased the concentration of uranium in the water to which the plant material was exposed.

Since the biosorption process is strongly pH-dependent, a more frequent accurate control of this parameter would have been adequate. Changes in the pH conditions through time may have had important impacts in the biosorption process, due to its influence in the uranium chemistry in solution, the activity of the active groups and the competition with coexisting ions (Park, Yun & Park, 2010).

In order to better evaluate the ability of the biomass to adsorb uranium, crushing or grinding the plant material to obtain augmented contact surfaces would have been adequate. In the present study, this simple method of sorbent preparation could have

reduced the differences in uranium accumulation abilities among species by reducing the effects of distinct sorbent size and surface area.

Other factors that may have influenced the results include the use of mixture of species in the microcosms and the biomass thermal drying. The effects of combining different plant materials in the studied system are unknown, but may have included small additive or antagonistic effects (Salim, Al-Subu & Dawod, 2008). On the other hand, drying the plant biomass at high temperatures may have had significant negative impacts, depending on the type of material. Some authors have reported that natural-dried leaves showed higher adsorption capacity than oven-dried leaves, and indicated that this was due to the destruction of some adsorption sites when exposed to high temperatures (Al-Masri, Amin, Al-Akel & Al-Naama, 2009; Salim, Al-Subu & Dawod, 2008).

4.5. Further work

The present study analysed uranium immobilisation in raw biomass of four common plant species when exposed to realistic physicochemical conditions and uranium concentrations, with satisfactory results. Further investigation is required in order to understand the options of use of the studied material in bioremediation.

Future work could be directed towards the evaluation of the use of the material in the raw form water treatment systems such as surface water flow wetlands, where the plant biomass would function as a substrate for uranium immobilisation. In these systems, the decaying plant material could serve as a uranium long-term sink as the more recalcitrant part of the biomass, along with the retained contaminant, are buried within the sediments.

A more complex option would include the use of the studied biomass to produce biosorbent materials. For such purposes, further investigation of the uranium biosorption capacity of pond water-starwort tissues would be appropriate, since this species accumulated showed high uranium retention ability in the present study. The evaluation of the potential of certain materials to be used as biosorbents in larger scales (i.e. biosorption reactors/columns) relies on specific protocols and mathematical models to evaluate the sorbent's affinity for the contaminant and sorption capacities (Volesky, 2007). Moreover, these studies analyse the kinetics of the biosorption process considering varying conditions of pH, contaminant concentration, biosorbent size and dosage, among others, thus allowing the identification of optimum conditions for maximum biosorption. Some possibilities of biomass modification in order to increase the biosorption ability of the material, as well as its resistance to degradation may also be investigated (Park, Yun & Park, 2010). Therefore, these experiments would provide essential information concerning the suitability of the studied material to produce enhanced biosorbent materials.

In conclusion, the present study delivers preliminary information concerning the use of the studied material in the raw form, and constitutes a screening evaluation for the identification of suitable material for biosorbent production purposes. It provides a contribution towards an alternative, cost-effective and ecologically sustainable technology for the treatment of metal-bearing effluents, and future work in this field must now follow.

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