Fe plaque assisted aquatic U rhizofiltration by \textit{Phragmites australis} Trin ex Steud. – Performance and influence factors in natural-like wetland and mesocosm environments

Dissertation zur Erlangung des akademischen Grades
Doctor rerum naturalium (Dr. rer. nat.)

vorgelegt von
\textbf{M.S. Weiqing Wang}

geboren am 02.12.1985 in Huaian, China

Gutachter:
Prof. Dr. E. Gert Dudel, Technische Universität Dresden
Prof. Dr. Jan Vymazal, Czech University of Life Sciences Prague
Prof. Dr. Goddert von Oheimb, Technische Universität Dresden

Tag der Verteidigung: 27.01.2017
Erklärung des Promovenden

Die Übereinstimmung dieses Exemplars mit dem Original der Dissertation zum Thema:

„Fe plaque assisted aquatic U rhizofiltration by *Phragmites australis* Trin ex Steud. – Performance and influence factors in natural-like wetland and mesocosm environments“

wird hiermit bestätigt.

Dresden, 02.12.2017

……………………………………….….
Ort, Datum

Weiqing Wang

……………………………………….….
Unterschrift (Vorname Name)
Acknowledgement

The author wish to give address the particular mentions to the kind persons here, for their valuable support and advice to the research proceeding and Ph.D. dissertation modification.

The author firstly thanks to his doctoral supervisor, Prof. Dr. habil. E. Gert Dudel. His guidance accompanied the author all the time writing this dissertation. It will be impossible to successfully conduct the research and complete this dissertation, without the great support from his unsurpassed advices, enthusiasm and patience.

The author want to give thanks to Dipl.-Chem. Gisela Ciesielski, Dr. Arndt Weiske and Dr. Carsten Brackhage for their advice and support for the laboratorial analysis, data processing and dissertation writing. Their extraordinary work are indispensable for dissertation writing.

The author also thanks to the following persons and organizations for some special analysis and data support: Dr. Ernst Bäuer for root tissue mapping analysis; Dr. Stefan Ritzel and Dr. Andrea Sperrhacke for tailing water quality long term monitoring data supporting; Analysis and Test Center of Nanjing Normal University for FTIR analysis.

The author also thanks to the great supports from Dipl.-UWT Yan Lu, Mr. Lothar Keydel and Mr. Matthias Webering for the sample treatment and data recording in field investigation and mesocosm experiment.

The author gives the special thanks to the China Scholarship Council for the financial support in Germany.

Finally, the author want to thanks to his family for their support and accompany when writing this dissertation.
Contents

1. Introduction and main results ................................................................................................................. 1
   1.1 U contamination in tailing sites ................................................................. 1
       1.1.1 Natural U distribution in earth ............................................................. 1
       1.1.2 The U mining industry development .................................................. 1
       1.1.3 Formation of the U tailing sites .......................................................... 1
       1.1.4 U contamination in soil, aquatic sediment and water pathway of tailing sites .......... 3
   1.2 U contamination control technologies in tailing sites ................................................................. 4
       1.2.1 Physical methods .................................................................................. 4
       1.2.2 Chemical methods ............................................................................... 5
       1.2.3 Biological methods .............................................................................. 5
   1.3 Aquatic U removal via phytoremediation ...................................................................................... 6
       1.3.1 Definition of phytoremediation ............................................................ 6
       1.3.2 Sub-technologies of phytoremediation ............................................... 6
   1.4 Aquatic U retention via rhizofiltration ......................................................................................... 7
       1.4.1 Uptake of U by underground tissues of plant ......................................... 7
       1.4.2 Root retained U distribution ................................................................. 8
   1.5 Role of iron plaque (IP) on aquatic U rhizofiltration ................................................................. 8
       1.5.1 IP formation on root surface ................................................................. 8
       1.5.2 Metals/metalloids removal via IP assisted rhizofiltration ....................... 9
   1.6 Factors influencing IP assisted aquatic U rhizofiltration ......................................................... 10
       1.6.1 Fe and U availability in rhizosphere ..................................................... 10
       1.6.2 Biogeochemical conditions in rhizosphere ........................................ 10
       1.6.3 Plant transpiration ............................................................................... 14
   1.7 U retention by P. australis .............................................................................. 15
       1.7.1 Application of P. australis on metals/metalloids retention in wetlands ... 15
       1.7.2 U retention via phytoremediation (especially rhizofiltration) of P. australis ......... 16
   1.8 Main hypotheses ............................................................................................... 16
   1.9 References ....................................................................................................... 17

2. Rhizofiltration of uranium by plant root surface in a tailing wetland .................................................... 27

3. Fe plaque related aquatic uranium retention via rhizofiltration along a redox state gradient in a natural
   Phragmites australis Trin ex Steud. wetland ......................................................................................... 28

4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites
   australis Trin ex Steud. dominated mine tailing wetland ................................................................. 29
   4.1 Introduction ................................................................................................................. 30
   4.2 Material and methods ............................................................................................... 33
       4.2.1 Study site and sampling .......................................................................... 33
Figure legends

Fig 1.1 layout of a valley type tailing site in Neuensalz-Zobes, Western Ore Mountains, Germany.........................2
Fig 1.2 Deposition and migration of U in tailing site and its contiguous zone........................................................3
Fig 1.3 EDAX picture of the Fe plaque on root surface.............................................................................................9

Fig 4.1 Localization of sampling sites in a U tailing basin wetland, Zobes-Mechelgrün near Neuensalz, Western Ore Mountains Saxony, Germany...................................................................................................................34
Fig 4.2 Mean total concentrations of C and N in rhizosphere subhydric soil............................................................39
Fig 4.3 Proportions of U and Fe in different sequential extraction fractions in the rhizosphere subhydric soil........41
Fig 4.4 FT-IR spectra of organic functional groups formed in rhizosphere humic acids..........................................43
Fig 4.5 Mean concentrations of Fe and Fed retained in rhizosphere subhydric soil and root of P. australis.........45
Fig 4.6 Particle size related U and Fe distribution in the rhizosphere subhydric soil.............................................48

Fig 5.1 Description of sampling sites in the reed bad wetland in Neuensalz-Zobes, Western Ore Mountains .......60
Fig 5.2 Mean concentrations of aquatic U, Mg, Ca and As in water pathway of two sites from May to August .....64
Fig 5.3 Daily transpiration of reed with and without the shield of canopy (PS: completely open area with strong sunlight; PL: shaded area with low sunlight)........................................................................................................65
Fig 5.4 Mean concentrations of U retained in leaves and stems harvested in two developmental stages of P. australis ..................................................................................................................................................66
Fig 5.5 Mean concentrations of U in roots harvested in two developmental stages of P. australis...............67
Table legends

Table 4.1 Soil sequential extraction procedure................................................................. 35
Table 4.2 Mean concentrations of aquatic metals/metalloids in rhizosphere ......................... 37
Table 4.3a Water chemistry - geochemical conditions of leachate inflow and outflow (tailing pond water). ......... 37
Table 4.3b Water chemistry of rhizosphere - mean content of nitrogen and carbon........................ 38
Table 4.4 Band assignments of FT-IR spectra................................................................. 43
Table 4.5 Mean concentrations of elements within Fed..................................................... 46

Table 5.1 Mean values of biogeochemical conditions in water pathway............................... 63
1. Introduction and main results

1.1 U contamination in tailing sites

1.1.1 Natural U distribution in earth

As the heaviest element in natural environment, the average U abundance in earth crust is around 2.5 mg kg\(^{-1}\). The natural U is a mixture of three isotopes (U\(^{234}\), U\(^{235}\) and U\(^{238}\)) with the stable proportions, which are respectively 0.01%, 0.72% and 99.27%. All the isotopes can continuously contribute the radiation dosage to surroundings in the range of billion years.

1.1.2 The U mining industry development

The purified U isotopes are widely used in power generation, medical service, manufacturing industry and nuclear weapons. Among these applications, the nuclear electric power industry is the main consumer of this element. Due to the increasingly shortage of petroleum supply, the nuclear energy has been considered as the reliable and relatively clean alternate resource for power demand. For this reason, different types of nuclear power plants are developed and established worldwide in recent decades. By the end of July in 2012, total 429 nuclear reactors are running in 31 countries, which have generated 2518 TWh of electricity in 2011 (Schneider and Froggatt 2012). The application of nuclear plant is also accompanied with some potential risks, including the environmental pollution for nuclear fuel generation, nuclear power plant construction and safe decommissioning. The danger of meltdown (e.g. GAU in Fukushima, Japan) make some countries such as German parliament confirmed the decommissioning of all operating nuclear power plants in future. However, the demand of nuclear energy is still increasing in other countries. The nuclear power capacity in China has increased by 3 times from 2013 to 2014 and will eventually reach to 88 GWe by the end of 2020 (Sun et al., 2014). To maintain the growth of the nuclear power plants, the demand for U as fuel is also increased. As a result, the scale of U mining industry is significantly developed facing this requirement.

1.1.3 Formation of the U tailing sites

The U mining industry are accompanied with the products of waste ores, contaminated water and dust. The waste ores are usually stored centralized in the idle area of mining site, including the utilized area in open pit and the abandoned mine shaft in the underground mine. Till the decommissioning of U mine, tailing dumps with considerable waste ores, contaminated sediments and water above and below ground are usually formed. According to various topographic features, those tailing sites are differentiated to several geographical types (valley type, flat type and mountain type etc.). The tailing site is usually periodically
renovated to make sure the safety of its structure and limit the adverse influence to the surroundings. The common measures include the site scale adjustment, soil refill, hydrological control, dam establishment and stabilization. The tailing site showed in Fig. 1.1 is a typical valley type tailing site treated with the mentioned measurements.

The area was exploited for U mining from 1956 to 1960 without any care for environment, till it was fully decommissioned up to 1977. The mine shaft nearby east upstream was flooded and the fine material was deposited in tailing pond. Moreover, the southeastern part of tailing site was later filled up with agricultural waste (compost). A natural wetland vegetation has been developed since then, which only sporadically disturbed by the rainstorm derived flooding. The underground leachate water from the eastern flooded mine is collected by the tailing pond in the western region before the dam. The bypass channel system surrounding the tailing has been completed after the flooding event in 2001. Since then the former *Typha angustifolia* habitat in south east has been replaced by *Phragmites australis* Trin ex Steud. as the “mono-species” community. However, in northwest part near the bank is also covered by *Salix* species (Fig 1.1). The tailing dam and bypass channel system keep the tailing a relatively separated hydrological position and stabilized the water level. This U tailing site is an example of a natural wetland vegetation development (succession) to a mono-species *P. australis* stand in relevant parts of contaminated tailing basin and pond over decades. It provides a world-wide generalizable working example for the rehabilitation/remediation of
1.1 U contamination in tailing sites

contaminated mining sites and passive water treatment, by applying the eco-technologies (e.g. phytoremediation) without artificial supply of energy and chemicals.

1.1.4 U contamination in soil, aquatic sediment and water pathway of tailing sites

However, the relatively isolated tailing sites still cause the serious contamination in situ and further to the surroundings. The waste ores in tailing site contain multiple metals/metalloids (Fe, Cd and As etc.), and radionuclides (U, Ra and Th etc.). The radionuclides has the unique radioactive hazard potential, whether they are retained inside the tailing site or output downstream to surroundings (Fig 1.2; Nassour et al., 2015).

Study on a U tailing site in Portugal has suggested that the radioactivity level of the tailing site is up to 200 times higher than the unaffected soil (Carvalho et al., 2007). The residual radiation in the tailing site also causes the direct threat to the surroundings. The investigation on a U deposit in Australia has showed that the residual U contributed the additional radiation dosage to surroundings around 0.67 mSv·h⁻¹ (Lottermoser and Ashley 2006). On the other hand, the chemical toxicity is a relative serious hazard the residual U (U²³⁵ and U²³⁸) may cause. The physicochemical processes (weathering, denudation and infiltration etc.) break down the waste ores to small particles containing U. It further results in the migration of U in forms of soluble complexes and suspended particles among the different soil or aquatic sediment layers. Studies have suggested that U can migrate to a considerable depth in soil up to 1 m (Maity et al., 2013). The investigation on the tailing site in Neuensalz-Zobes has
showed that the specific U concentration in aquatic soil and sediment layer of rhizosphere is around 35 mg·kg$^{-1}$, which is in the range of high grade ores (Chapter 3).

The U accumulation in top soil/aquatic sediment layer is influenced by different physical, chemical and biological factors. The surface water pathway and subsurface infiltration assist the U migration from top soil layer to water and further to surroundings (Chapter 2; Chapter 3). In some case, the U concentration in water pathway is higher than the recommended level even by hundredfold (Chapter 3; Kurttio et al., 2002). The U minerals dissolution is conducted by multiple solid and aquatic mediators. They result in the complicated occurrence of U incorporating with both inorganic compounds (Ca$^{2+}$, CO$_3^{2-}$ and HCO$_3^-$ etc.) and organic matter (OM). They may have different physical, chemical and biological properties and toxic potentials (Bernhard et al., 2001; Luo and Gu 2011). The high aquatic U concentration cause serious damage to human health, especially when it is introduced to the drinking water supply (Bayliss et al., 2012).

1.2 U contamination control technologies in tailing sites

1.2.1 Physical methods

Series of engineering methods are applied to control the U contamination in tailing site. The major methods include the soil capping or containment, soil washing and soil replacement (Lottermoser and Ashley 2006; Silver and Andersen 1981). The soil capping is a common method applied in tailing sites, by adding dense material layers (usually the original soil from mine process) on top of tailing site. It prevents the waste ores from the erosion effect of surface water (Olatuyi and Leskiw 2015). The soil washing is usually conducted by the hydraulic scouring to detach U from tailing site via introduced fluid (e.g. salted surface water and heat flow) (Kantar and Honeyman 2006). The soil replacement technology can permanently remove the U contaminated debris from the tailing site and refill the clean soil or other substances. These engineering methods can stably and effectively restrict the U contamination, but require the considerable costs of investment and work amount. For the sites covered by stand water, the additional dry-out is necessary to process before these physical methods are conducted. It will take long period and cannot be effect in short time. On the other hand, the simple physical treatment cannot change the chemical properties of U, which can still cause the risk to the environment in long term period.

1.2.2 Chemical methods

The chemical methods are developed to improve the disadvantages of the physical engineering measures. The aquatic U retention can be enhanced when certain chemical agents are introduced in the system. The phosphates (e.g. Ca$_5$(PO$_4$)$_3$(OH) and NaH$_2$PO$_4$·2H$_2$O) are
the effective flocculating agent with the strong complexation effect to aquatic U(VI). The aquatic U can be firmly precipitated in situ as sparingly soluble U-P minerals (Li et al., 2004; Mkandawire et al., 2007). By this means, the aquatic U contamination is thusly controlled. On the other hand, the U minerals can be dissolved out from soil/aquatic sediment particles by specific chemical agents. The bicarbonate or sodium carbonate result in the rapidly formation of high soluble U complexes and increase the U solubility in these forms (Zhou and Gu 2005). Despite of the inorganic mediators, certain organic substances are also proved effective to affect the U retention in soil/aquatic sediment. The OM can cause the strong U precipitation in form of organic aggregates (Crancon and van der Lee 2003). But some OMIs (e.g. citric acid) can also oppositely assist the U minerals dissolution to aquatic compounds (Choy et al., 2006; Schmidt 2003). The U contamination in soil/aquatic sediment and water pathway is supposed to be controlled via chemical solidification or elution by these inorganic or organic agents. However, the chemical treatments also has the adverse effect to the environment. The U within inorganic and organic agents can be dissociated and causes secondary pollution (Uyusur et al., 2015; Zhou and Gu 2005). From the view of the economy and environmental protection, the application of chemical agents for U decontamination is also expensive, especially in the large scale dimension of catchments and landscapes. For relatively lower concentration (below some hundred µg·l⁻¹) of aquatic U removal, the chemical technologies are also inefficient because exponentially increased cost/energy demand. Hence the passive/biological treatment technologies are indicated, especially in the low level U contaminated environment.

1.2.3 Biological methods

The biological technologies have been developed recently as an alternative option for U contamination control. The microbial communities in U contaminated soil and sediment layer have showed the high efficiency for aquatic U uptake and retention. The aquatic U(VI) can firmly precipitated on the membrane surface of some bacteria species (Strandberg et al., 1981). The absorbed U(VI) compounds in certain bacteria species (e.g. Pseudomonas spp.) can even form the intracellular precipitates (Kazy et al., 2009). Some sulfate reducing bacteria species can utilize organic carbon respectively hydrogen as the electron donor and assisted the U(VI) bioreduction (Khan et al., 2013). The U(VI) bioreduction can also be processed by some heterotrophic bacteria species and precipitated as insoluble U(IV) compounds (Lovley et al., 1993). Although the microbial communities have showed the capacity on aquatic U retention, there are still some disadvantages in this environmental biotechnology. The small size and disperse distribution of bacteria species inhibit the effective recovery of aquatic U precipitated with them. On the other hand, their rapid metabolism and decay rate cause the strong desorption of precipitated U back to the environment in short time.
1. Introduction and main results

(Gadd 2009). In this case, a long term and stable aquatic U removal cannot be ensured. In comparison, the phytoremediation via macrophytes is a feasible environmental technology for the long term and effective biological U contamination control, especially in the aquatic environments (e.g. natural/constructed wetlands).

1.3 Aquatic U removal via phytoremediation

1.3.1 Definition of phytoremediation

The phytoremediation is an environmental biotechnology to uptake, stabilize and deactivate the contaminants via plant tissues and the related attachments (microbial community, litter and secretions etc.) (Salt et al., 1995). The phytoremediation is also a passive technology that remove the aquatic contaminants without significantly interfering the physical structure and chemical balance of environment. The phytoremediation is also an cost-effective technology, especially in the large scale land decontamination (Mench et al., 2009). The phytoremediation is feasible for aquatic radionuclides (e.g. U) removal (Li et al., 2011). Research on the Armoracia rusticana has also showed that up to 86-98% of aquatic U was diminished through the phytoremediation using this species (Soudek et al., 2011). Favas et al. (2014) have indicated that sorts of submerged, free-floating and rooted emergent plants are capable to remove high amounts of aquatic U from environment. The field investigation in a U contaminated site in Portugal has suggested that some native aquatic plants (Callitriche stagnalis, Lemna minor, and Fontinalis antipyretica) were capable to accumulate considerable amount of aquatic U.

1.3.2 Sub-technologies of phytoremediation

The phytoremediation is usually contributed by different sub-technologies, including phytoextraction, phytovolatilization, phytostabilisation, phytodegradation and rhizofiltration (Dushenkov 2003; Newman and Reynolds 2004; Robinson et al., 2006). They are performed by plant species as different strategies in changed environmental conditions (e.g. chemical structures and aggregation states of contaminants). The phytoextraction is a technology utilizing the above ground biomass of plant to extract and retain contaminants inside the related tissues (e.g. stem and leaves.). Some plant species, so-called hyperaccumulators, are capable to retain high concentrations of certain contaminants in their above ground biomass (McGrath and Zhao 2003; Sun et al., 2007). The cultivation of Brassica juncea in U contaminated soil results in the accumulation of U in its leaves up to 2000 mg·kg$^{-1}$ (Chang et al., 2005). The phytoextraction provides a feasible way to permanently remove part of residual U from tailing site via harvest of plant. It is a major advantage of this technology. However, there are still some disadvantages for its practical application. The available plant
species with strong phytoextraction to multiple contaminants are usually limited. In most of cases, the certain plant species can only highly effective to specific contaminants species. It restricts the phytoextraction efficiency for the purpose of composite contamination control. Furthermore, introducing the certain hyperaccumulators from their origin area to a new ecosystem may restrict their efficiency and even damage the local ecosystem.

The phytostabilisation is not supposed to completely detach the contaminants and remove them with the harvestable biomass. Instead, it retains the contaminants inside the soil/aquatic sediment layer (e.g. rhizosphere) (Vangronsveld et al., 1995). The phytostabilisation can be conducted by different mediators from plant, including plant litter, microbial communities in rhizosphere and root tissue with the inorganic/organic compounds on its surface (Dousset et al., 2001; Marmiroli et al., 2005). The root is a major mediator contributing the phytostabilisation in form of rhizofiltration. The root and its subsidiary surface objects (precipitates, organic secretions and microorganism etc.) provide the capacity to uptake and retain the metals/metalloids from rhizosphere. The rhizofiltration is a common technology widely observed in many terrestrial and aquatic plant species for metals/metalloids removal, especially in the aquatic environment (Dushenkov et al., 1995). The rhizofiltration is also proved a feasible and effective way for aquatic U retention by some plant species. The hydroponic culture of sunflower and bean has showed that over 50% of natural U was accumulated by root of sunflower and 60-80% by root of bean (Lee and Yang 2010; Tome et al., 2008). Studies on some aquatic plant species (Callitriche stagnalis, Potamogeton natans and Potamogeton pectinatus) have also revealed the highly efficient U retention capacities of their roots, by removing 56-85% of aquatic U from the environment (Pratas et al., 2014). As a common macrophyte distributed worldwide, the P. australis was also effective to retain the multiple metals/metalloids via rhizofiltration (Chapter 2; Chapter 3; Vymazal et al., 2007).

1.4 Aquatic U retention via rhizofiltration

1.4.1 Uptake of U by underground tissues of plant

The underground biomass of plant is usually composed by the main root (or the rhizome for some aquatic macrophytes) and fine root attached on it. Studies have revealed that the rhizofiltration can be contributed by both these underground tissues (Liu et al., 2014). The fine root was main tissue for the water and nutrients uptake for some macrophytes, because its cell walls are sealed only slightly. It usually has higher capacity for aquatic metals/metalloids uptake than rhizome, stem and leaves (Bonanno and Lo Giudice 2010; Klink et al., 2009; Vymazal et al., 2007). The fine root is also possibly the main mediator supporting the U retention via rhizofiltration in both terrestrial and aquatic environments. The hydroponics on the Brassica juncea has showed that its fine root can take up 20-23% of U from the solution.
(Eapen et al., 2003). The investigation on the *P. australis* has also revealed that the fine root had the highest U retention efficiency than other tissues of this plant species (Chapter 2; Chapter 3).

### 1.4.2 Root retained U distribution

Few studies have focused on the U partition between the surface and inner parts of root. Studies have suggested that the root surface is relatively crucial position than the inner root tissue contributing the rhizofiltration. The 82.5% of U retained by root of hydroponically cultured *Landoltia punctata* was on its surface (Nie et al., 2015). The solution culture of bean suggested that the aquatic U rhizofiltration is mainly based on the U precipitation on root surface (Yang et al., 2015). The rhizofiltration via root of *P. australis* also showed the dominant U retention efficiency on root surface than the inner tissue (Chapter 2; Chapter 3). Although the former studies consider the root surface as the major position conducting the rhizofiltration of aquatic U, the specific mechanisms are still not clear yet. The inorganic and organic mediators in the rhizosphere are potentially involved and contributed in this process. Some reports have showed that the aquatic U retained on the root surface is presented as insoluble complexes with low mobility (Yang et al., 2015). For some macrophytes, the root derived aquatic U retention is significantly correlated with the aquatic Fe uptake from rhizosphere (Jha et al., 2016). It was also observed that the aquatic U precipitation on root surface of *Sparganium americanum* was enhanced in a Fe(II) rich solution (Chang et al., 2014). These studies have suggested a potential correlation between the aquatic U and Fe retention via rhizofiltration.

### 1.5 Role of iron plaque (IP) on aquatic U rhizofiltration

#### 1.5.1 IP formation on root surface

The Fe uptake and precipitation on root surface is mostly presented in form of IP. It is a complex composed by different Fe (hydr)oxides (Liu et al., 2006). Furthermore, this complex is also involved some bacteria species (also their detritus) and performed like the plane biofilm (Chang et al., 2014). The recent studies have identified the different Fe (hydr)oxides (ferric hydroxides, goethite, siderite and lepidocrocite *etc.* ) within IP (Chen et al., 1980; Hansel et al., 2001). The ferric hydroxides are the major Fe source for IP formation. Hansel et al. (2001) has observed that the ferrhydrite took the 63% of total Fe content within IP on root surface of *Phalaris arundinacea*. Over 81% of IP on the root surface of *Oryza Sativa* was also observed formed by ferrihydrate (Liu et al., 2006). These results support the opinion that the IP formation is mainly based on the microbial oxidation of root uptake Fe(II), when root aeration is available (Chang et al., 2014; Wu et al., 2012). It is usually presented as a dense
layer covering the root surface (Fig 1.3).

![Fig 1.3 EDAX picture of the Fe plaque on root surface](image)

### 1.5.2 Metals/metalloids removal via IP assisted rhizofiltration

The IP is proved an effective mediator assisting the uptake and retention of metals/metalloids by root. Investigation on different plant species has indicated that the increased content of IP improves the Cu and Zn retention via rhizofiltration (Jiang et al., 2009). The hydroponics on the *Oryza sativa* has also revealed a positive correlation between the concentrations of Fe, Al and Cd within IP (Chen et al., 2006; Liu et al., 2007). Some reports have also focused on the possible interaction between IP and aquatic U removal. Study on *Sparganium americanum* has suggested the positive effect of IP on aquatic U retention by stimulating the microbial bioreduction of aquatic U(VI) and its retention within IP (Chang et al., 2014). It is also possible for *P. australis* to remove the aquatic U via IP assisted rhizofiltration (Chapter 2; Chapter 3; Chapter 6).

The co-existing elements (*e.g.* P and As) also possibly influence the aquatic U retention within IP. The phosphates within IP potentially associate the co-precipitating of U (Chang et al., 2014). The As in rhizosphere has a high affinity to hydrated Fe(III) oxides and can be easily absorbed by IP on root surface (Fritzsche et al., 2006). It may cause the potential negative influence on the real efficiency of aquatic U retention within IP, when they stay in same environment (Chapter 2; Chapter 3). These studies have suggested a complicated process of IP (including the related microbes) assisted aquatic U rhizofiltration. It is possibly affected by their oxidation states and other mediators in rhizosphere, which is up to date largely unknown.
1.6 Factors influencing IP assisted aquatic U rhizofiltration

1.6.1 Fe and U availability in rhizosphere

The coupled Fe and target metals/metalloids contents in rhizosphere significantly influence the IP formation and related metals/metalloids absorption capacity. Liu et al. (2007) have suggested that the increase aquatic Fe availability enhanced IP formation and further encouraged the Cd co-precipitation on root surface. The similar effect was also observed in the Fe rich environment with the other metals/metalloids (Zn, Cu and As etc.) (Lee et al., 2013; Zhang et al., 1998). In Fe-U co-existing rhizosphere, the IP formation coupled with aquatic U uptake by root is possibly benefited from the increased Fe availability in rhizosphere (Chapter 3; Chapter 6). The increased aquatic U availability in rhizosphere has the uncertain influence on Fe and U co-precipitation on root, especially in the natural environment. The significantly different contents of U retained by root and also within IP could be observed based on the similar aquatic U levels (Chapter 4). In some cases, the U retention within IP can even be enhanced in rhizosphere with the relatively lower aquatic U availability (Chapter 3; Chapter 6). It suggested that the interaction between the IP and U is not only controlled by their contents in the rhizosphere soil but influenced by environment factors.

1.6.2 Biogeochemical conditions in rhizosphere

Studies have revealed that the IP formation was affected by certain biogeochemical conditions in rhizosphere. The redox state (represented by Eh value) is a comprehensive biogeochemical indicator describing the possibility of different biotic and abiotic redox processes in natural or controlled ecosystems. The redox state is also correlated with the aquatic Fe availability in rhizosphere (Chapter 3; Chapter 6). However, it is not directly affected by the redox gradient, but the redox processes derived oxidation state change of Fe (Grenthe et al., 1992). The changed Fe availability under the redox state gradient further influences the related IP formation on root surface (Chapter 6).

The redox state is also crucial to estimate the oxidation state of U and related interaction with its redox partners. Two major oxidation states of U are observed in natural environment, which are respectively U(IV) and U(VI). The U(VI) is mainly stable as the highly soluble and bioavailable uranyl cations in rhizosphere with strong oxidation ability. On the contrary, it can be chemically and biologically reduced to the sparingly soluble U(IV) minerals (e.g. UO$_2$) in reductive environment (Bailey and Ragnarsdottir 1994; Duff et al., 1997). The oxidation state change of U is usually coupled with the organic/inorganic mediators and related redox processes. The microbial respiration under different redox state conditions decompose the
OMs and released CO$_2$ as the metabolic product. The OMs retained U is also released in this process. The aquatic CO$_2$ promotes the formation of soluble uranyl carbonate compounds (Elless and Lee 1998). The aquatic U availability in rhizosphere is then possibly benefited from the OMs decomposition. However, it is still uncertain due to disparate microbial respiration mechanisms under the redox state gradient. Some anaerobic bacteria communities utilize the aquatic U(VI) as the electron acceptor for OMs oxidation and resulted in the precipitation of U(IV) (Khan et al., 2013). In this case, the redox state depended chemical state change of U possibly cause the different U occurrence in the soil/aquatic sediment layer. It may influence the certain bioavailability of U in rhizosphere for effective root uptake and also retention within IP (Chapter 4). The redox state gradient also potentially affect the interaction between Fe and U in rhizosphere. Stewart et al. (2009) have found that the aquatic U(VI) can be absorbed and reduced to U(IV) within the soluble Fe(II) oxides in the initial reduction cycle and maintained stable as U(VI)-ferrihydrite precipitate in the following oxidation cycle. The similar process may also be the possible cause for U retention within IP under the fluctuant redox state, since the ferrihydrite is the major compound within IP. However, the real performance of IP assisted aquatic U rhizofiltration under different redox state conditions is still not clear in recent studies.

The U occurrence in soil/aquatic sediment is presented in different forms, including exchangeable compounds, carbonates, Fe/Mn oxides, OMs and sparingly soluble minerals (Radenkovic et al., 2008; Schoener et al., 2009). The exchangeable ions and carbonates bound U have been considered the highly labile forms with strong mobility and bioavailability (Arey et al., 1999; Bednar et al., 2007; Elless and Lee 1998). On the contrary, the Fe/Mn oxides and other sparingly soluble minerals, even some OMs (e.g. plant litter), assist the firm U retention in soil/aquatic sediment (Spirakis 1996; Stewart et al., 2009). Comparing with the minerals and Fe/Mn oxides, the bioavailability of organic bound U can be relatively improved via microbial OMs decomposition. However, the composition of OMs in soil/aquatic sediment is highly complicated and varied under changeable biogeochemical conditions (Nguyen 2000). The humus in soil is effective to retain both aquatic U and Fe and form the stable organic mineral aggregates (Thiry et al., 2005). The aquatic U and Fe availability is then diminished when it is stabilized in organic sediment particles (Chen et al., 2014; Kleber et al., 2007). It is beneficial for the aquatic U and Fe removal, but possibly adverse for the root uptake of these elements (Chapter 4). Some OMs (e.g. citric acid from root exudates) are effective to dissolve the U minerals to aquatic forms for root uptake (Shahandeh and Hossner 2002). The different occurrence of OMs cause the uncertain influence on the chemical properties of organic U (Landais 1996). It possibly changes the availability of U within these OMs for IP assisted rhizofiltration, but has not been confirmed yet. The Fe(III) minerals in rhizosphere firmly retain the aquatic U in the soil/aquatic
sediment (Duff et al., 2002). However, the U retention with Fe(III) minerals is also not always a permanent process. Under the suitable biogeochemical conditions, the desorption of U from Fe(III) minerals is also feasible (Du et al., 2016). The high content of Fe(III) minerals is also crucial to support the Fe rich environment. The sufficient Fe supply in rhizosphere is further benefited the Fe retention by root and related formation of IP.

The co-existing metals/metalloids and nutrients in rhizosphere also influence the oxidation state of aquatic U and its retention within IP. The direct contact with the solid MnO$_2$ can cause the rapid oxidization of UO$_2$ minerals and release the aquatic Mn(II) and U(VI) cations (Wang et al., 2013). Wang et al. (2014) has indicated that the high redox state promoted the oxidation of aquatic Mn(II) to Mn(III)/Mn(IV) oxides and rapidly recycled to Mn(II) by oxidizing the UO$_2$ minerals. It may also be conducted by some bacteria species through the bioreduction of Mn(III)/Mn(IV) oxides (Chinni et al., 2008). On the other hand, the Mn(II) is also an important mediator affecting the UO$_2$ dissolution in both aerobic and anoxic conditions. The Mn(II) in form of MnCO$_3$ can firmly precipitated on surface of UO$_2$ mineral and inhibits its dissolution by potential oxidizing agents. These interactions between U and Mn may cause the potential competitive effect to U retention with Fe oxides in rhizosphere. The Ca in rhizosphere is capable to restrict the U retention ability of hydrated Fe(III) oxides, especially when the U availability is low (Massey et al., 2014). The high Ca availability increase the solubility of U by forming the soluble Ca-uranyl-carbonate compounds (Zhou and Gu 2005). On the contrary, the P in rhizosphere assist the aquatic U retention by forming the sparingly soluble precipitates (H$_3$O$_2$PO$_4$·3H$_2$O, Ca(UO$_2$)$_2$(PO$_4$)$_2$ and Na(UO$_2$)$_2$(PO$_4$)$_2$ etc.) (Munasinghe et al., 2015). The P not only promote the aquatic U precipitation in soil/aquatic sediments, but also the U retention within the IP on root surface (Chang et al., 2014; Seder-Colomina et al., 2015). The high-grade U ores from both the organic rich sedimentary deposition and hydrothermal mineralization are associated with the abundant of As inside.

The As is then a common co-contaminant with high affinity to the hydrate Fe(III) oxides (Fritzche et al., 2006; Li et al., 2015). The aquatic As in rhizosphere can be adsorbed by ferrihydrite and form the stable Fe(III)-As(V) complexes (Essilfie-Dughan et al., 2013). The Al-Fe hydroxide nanoparticles also have the ability to incorporate with As (Kumar et al., 2016). The high As availability in U tailing site may cause the strong competitive adsorption effect to U retention within IP (Chapter 2; Chapter 3).

Some major nutrients (e.g. ammonium (NH$_4^+$) and nitrate (NO$_3^-$)) are also the common elements in U minging site (Finneran et al., 2002; Miao et al., 2013). These nitrogen species are the important mediators affecting the oxidation states of Fe and U in different ways. The high NH$_4^+$ availability is beneficial to maintain the lower oxidation ability in rhizosphere (Christensen et al., 2000). Some Fe(III) reducing bacteria can oxidize the NH$_4^+$ for Fe(III)
bioreduction to Fe(II) (Sawayama 2006). The aquatic U(VI) bioreduction is also possibly involved in the NH$_4^+$ depended microbial Fe(II) generation. It has been reported that the NH$_4^+$ oxidation by Acidimicrobiaceae bacteria was coupled with the simultaneous Fe(III) and U(VI) bioreduction (Gilson et al., 2015). The Fe(III) and U(VI) bioreduction in NH$_4^+$ rich environment may increase the aquatic Fe availability but oppositely diminish the aquatic U availability (Miao et al., 2013). For this reason, NH$_4^+$ is used in some U chemical ore extraction processes to recover the aquatic U(VI) in the form of U(IV) precipitates (Tomazic et al., 1969). The NO$_3^-$ is a effective oxidizer capable to enhance the oxidation ability and the related stability of Fe(III) minerals. The microbial NO$_3^-$ reduction also assist the aquatic Fe(II) oxidation to hydrated Fe(III) oxides by some nitrate depend Fe(II) oxidizing bacteria species (Miot et al., 2015). Some microbial NO$_3^-$ reduction processes (e.g. denitrification and dissimilatory reduction to NH$_4^+$) also provide the intermediates capable to dissolve the U(IV) minerals to aquatic U (VI) compounds (Senko et al., 2002). The microbial oxidation of U(IV) minerals is also coupled with Fe(III) (hydr)oxides by nitrate depend Fe(II) oxidizing bacteria species (Wu et al., 2010). The high NO$_3^-$ availability is beneficial for the improvement of aquatic U availability, but adverse for the aquatic Fe availability as well (Finneran et al., 2002). The U and Fe retention by root and the related co-precipitation within the IP on root surface is affected in different N species cultured rhizosphere (Chapter 6). However, it has not been fully revealed in the current studies. On the other hand, the root uptake and assimilation of different N species also influence the plant biomass accumulation and related partition between the below and above ground tissues (Li and Redmann 1992). The biomass accumulation of plant is crucial for the real capacity of phytoremediation (e.g. rhizofiltration) derived contaminants removal in a certain areal land (Vymazal and Brezinova 2015). In this case, the presence of different N species (e.g. NH$_4^+$ and NO$_3^-$) can not be ignored for the IP assisted aquatic U rhizofiltration (Chapter 6).

The OM accumulation in rhizosphere is also crucial for aquatic U removal via rhizofiltration and soil/aquatic sediment. The OM (e.g. plant litter) rich rhizosphere enhance the aquatic U retention in soil/aquatic sediment (Spirakis 1996). The litter from the fallen plant tissues is a major component in the aquatic environment with productive macrophytes. The litter surface supports the biofilm formed by microbial communities (Schaller 2013). The bacteria and fungi within the biofilm further promote the litter decomposition to particulate organic matter capable to retain the aquatic U (Schaller et al., 2008). The different environmental conditions and related microbial respiration mechanisms result in the various chemical components of OM degradation products (Chapter 4). They either retain the aquatic U in soil/aquatic sediment or assist the dissolution of U minerals. The litter retained U is possibly remobilized with the the decomposition products including the forms of dissolved organic carbon (DOC) and microbial exudates. It has been reported that the U concentration on litter surface...
1. Introduction and main results

decreased from 1427 mg·kg$^{-1}$ to 664 mg·kg$^{-1}$ when the litter was decomposed to DOC by *G. pulex* (Schaller *et al.*, 2008). As a result, the U occurrence in rhizosphere is possibly affected by the different quantity and quality of OMs (Chapter 4). It may further influence the soil to root translocation of U and related rhizofiltration efficiency.

1.6.3 Plant transpiration

Studies have suggested that the plant transpiration is beneficial for the contaminants removal via rhizofiltration. The root uptake of Cd and Zn has been enhanced under strong transpiration effect of terrestrial species *Triticum aestivum* (Grifferty and Barrington 2000; Salah and Barrington 2006). The Cu retention by root of *Fagopyrum esculentum* was also increased under higher transpiration condition (Tani and Barrington 2005). The uptake and assimilation of soluble nutrients (*e.g.* NH$_4^+$ and NO$_3^-$) in plant are also promoted by the enhanced transpiration (Phlak 2003). The increased nutrients uptake benefit the plant biomass accumulation and possibly the contaminants (*e.g.* U) retention capacity (Chapter 6). Studies have suggested that the root Fe uptake by roots of some terrestrial plant species (*Vitis vinifera, Zea mays* and *Oryza sativa* etc.) was enhanced along with the increased transpiration rate (TR) (Boselli *et al.*, 1998, Sandor and Zoltanne 1981; Shrestha *et al.*, 2015). The oxygen transfer via root aerenchyma is also promoted by the enhanced transpiration coupled photosynthesis (Armstrong and Armstrong 2005). The sufficient root radial oxygen loss promote the chemical/microbial Fe(II) oxidation to hydrated Fe(III) oxides in root zone. In this case, the strong plant transpiration is possibly positive for IP formation (Chapter 6). However, the real effect of transpiration on IP formation is still largely unknown in current studies. Few results have showed the related aquatic U uptake and retention in plant, especially for aquatic plants, under different transpiration conditions. It has also been indicated that the concentration of U in leaves was positively correlated with the increased TR condition (Aranjuelo *et al.*, 2014). It has not concerned the U partition in underground biomass of plant with the TR variation. Moreover, it is the observation on the hydroponically cultured terrestrial plant species. The potential effect of transpiration on the phytoremediation of aquatic plant species, especially the emergent macrophytes, is still largely unknown. Furthermore, few studies have focused on the effect of plant transpiration on aquatic U rhizofiltration, especially when it is coupled with the IP formation. The IP assisted aquatic U rhizofiltration is possibly benefited from the strong transpiration effect (Chapter 6). The aquatic U retention via IP assisted rhizofiltration is also possibly affected by changed TR conditions, especially for the macrophyte species taking the rhizofiltration as the major aquatic U removal technology (*e.g.* *P. australis*) (Chapter 5 and Chapter 6).
1.7 U retention by \textit{P. australis}

1.7.1 Application of \textit{P. australis} on metals/metalloids retention in wetlands

The \textit{P. australis} is a macrophyte species mainly inhabiting as single dominant species in the shallow water environments (pond, beach and estuary etc.). It is composed by the leave blade (with sheath), stem, rhizome and root. The subspecies of \textit{P. australis} are distributed worldwide in most of continents (Koppitz 1999). The wide distribution and growth habit make the \textit{P. australis} an important species in most types of wetlands worldwide. Studies have revealed that the \textit{P. australis} can inhabit in both acidic and alkaline wetlands (Batty and Younger 2004; Garde et al., 2004). Although the \textit{P. australis} prefers the shallow water environment, it also showed the high tolerance to the arid environment (Gong et al., 2014). The wide distribution of \textit{P. australis} also diminish the potential ecological risk than introducing the exogenous plant species for phytoremediation purpose. Instead, the \textit{P. australis} can be utilized as local species providing the similar and effective service of contamination control. The \textit{P. australis} is capable to retain multiple metals/metalloids within both its below and above ground tissues. Studies have suggested the root of \textit{P. australis} is the most efficient tissue for different metals/metalloids retention than other tissues (Vymazal and Brezinova 2015). Investigation on the Ni, Cr, Cu, Fe and Pb bioaccumulation in \textit{P. australis} has also revealed the highest concentrations of these contaminants in root than other tissues (Keller et al., 1998, Peverly et al., 1995). Study on the constructed wetlands in Czech also showed an average root to leaf concentration ratio of multiple metals in \textit{P. australis} around 20 (Vymazal et al., 2007). Many studies have supported the opinion that the rhizofiltration is the major sub-technology for \textit{P. australis} derived aquatic metals/metalloids removal. However, it is not a certain conclusion valid in all environmental conditions. The various couples of certain metals/metalloids and biogeochemical conditions in applied environments potentially affect the partition of the contaminants in \textit{P. australis}. The concentration of Zn retained by \textit{P. australis} can be oppositely higher in leaves and stem (Larsen and Schierup 1981; Peverly et al., 1995). The bioaccumulation of metals/metalloids in \textit{P. australis} also cause the adverse stress to plant. The toxicity of retained Cu significantly restricted the biomass accumulation of \textit{P. australis} (Ali et al., 2002; Ye et al., 2003). A similar restriction to \textit{P. australis} development is also observed with the increased Zn and Pb contents in the environment. (Ye et al., 1997). The biomass accumulation of \textit{P. australis} is not only affected by the metals/metalloids, but also other factors (nutrients availability, seasonal change and transpiration etc.) (Li and Redmann 1992; Saltonstall and Stevenson 2007). The difference on the biomass accumulation then influence the real capacity of \textit{P. australis} for the metals/metalloids retention (Vymazal and Brezinova 2015).
1.7.2 U retention via phytoremediation (especially rhizofiltration) of *P. australis*

The current studies has paid less attention on the certain effect of *P. australis* derived phytoremediation and related rhizofiltration for aquatic U removal. The current knowledge in this field has suggested that the *P. australis* is capable to retain aquatic U within the different tissues (Cerne et al., 2011). However, the performance of respective sub-technologies (*e.g.* rhizofiltration and phytoextraction) for aquatic U retention is still largely unknown, especially in the natural aquatic environment. Some studies focus on the aquatic U retention within the above ground biomass of *P. australis* (Cerne et al., 2011). However, this plant species is not an effective hyperaccumulator for many metals/metalloids (Vymazal et al., 2007). The real efficiency of phytoextraction may be less in *P. australis* for U retention (Chapter 3; Chapter 5). The root of *P. australis* is supposed to be the crucial tissue for aquatic U uptake and retention (Chapter 2; Chapter 3; Chapter 5). Few studies have reported on this possible path. Furthermore, the certain performance of aquatic U rhizofiltration coupled with some other mediators or agents (*e.g.* IP) is still unknown, especially under fluctuating environmental conditions. The gap of knowledge in this field makes the *P. australis* a valuable species to estimate its specific role on aquatic U removal.

1.8 Main hypotheses

The current studies have revealed that the aquatic U contamination derived from its deposition site (*e.g.* U mine and/or ore processing tailing site) cause hazardous impact to surrounding ecosystem and population. It is necessary to develop economic and effective technologies to stabilize the aquatic U in situ. The phytoremediation, especially the sub-technology of rhizofiltration, is a feasible option for this purpose. The aquatic U retention via phytoremediation has not been widely considered in current applications and studies, especially for aquatic plant species. It leads to the incomplete understanding on aquatic U removal via different sub-technologies of phytoremediation (*e.g.* rhizofiltration and phytoextraction) coupled with possible mediators (*e.g.* IP). In order to fill this gap and bring the further knowledge to improve the efficiency of phytoremediation on aquatic U retention, field investigation and mesocosm experiments were conducted choosing the *P. australis* as major carrier for aquatic U uptake and retention. In this study, the following hypotheses were verified during the investigation:

a. The aquatic U was effectively retained via *P. australis* derived phytoremediation mainly contributed by rhizofiltration.

b. The IP formed on root surface is crucial assisting the aquatic U retention via rhizofiltration of *P. australis*. 

16
c. The redox state related U and Fe occurrence and co-existing inorganic/organic compounds in rhizosphere are crucial to the IP assisted aquatic U rhizofiltration.

d. The different inorganic N species (e.g. NH$_4^+$ and NO$_3^-$) respectively main nutrients are crucial mediators to regulate the efficiency of IP assisted rhizofiltration and area-related U retention capacity via rhizofiltration.

e. The transpiration of *P. australis* is an effective above ground factor for IP assisted aquatic U rhizofiltration. It not only affect the aquatic nutrients (e.g. N), U and Fe availability for root uptake and assimilation, but also the biomass production of *P. australis* and related U retention capacity.

1.9 References


1. Introduction and main results

Bonanno G, Lo Giudice R (2010): Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators. Ecological Indicators 10, 639-645.


1. Introduction and main results

conditions. Biodegradation 26, 475-482.


Jha VN, Tripathi RM, Sethy NK, Sahoo SK (2016): Uptake of uranium by aquatic plants growing in fresh water ecosystem around uranium mill tailings pond at Jaduguda, India. The Science of the total environment 539, 175-84.


Kumar PS, Quiroga Flores R, Sjostedt C, Onnby L (2016): Arsenic adsorption by iron-aluminium hydroxide coated onto macroporous supports: Insights from X-ray...
1.9 References


plants (Oryza sativa L.). Environmental Science & Technology 40, 5730-5736.


1. Introduction and main results


1. Introduction and main results


2. Rhizofiltration of uranium by plant root surface in a tailing wetland

Weiqing Wang, Carsten Brackhage, Ernst Bäuker, E. Gert Dudel

*Institute of Plant and Wood Chemistry, Dresden University of Technology, Plienner Strasse 19, D-01737 Tharandt, Germany*

Published in: Merkel, Broder J., Arab, Alireza (Ed) Uranium - Past and Future Challenges (2015), Springer, Switzerland, pp 497-508

Article link address: [https://doi.org/10.1007/978-3-319-11059-2_57](https://doi.org/10.1007/978-3-319-11059-2_57)
3. Fe plaque related aquatic uranium retention via rhizofiltration along a redox state gradient in a natural *Phragmites australis* Trin ex Steud. wetland

Weiqing Wang, E. Gert Dudel

*Institute of Plant and Wood Chemistry, Dresden University of Technology, Pienner Strasse 19, D-01737 Tharandt, Germany*


Article link address: [https://doi.org/10.1007/s11356-017-8889-5](https://doi.org/10.1007/s11356-017-8889-5)
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a *Phragmites australis* Trin ex Steud. dominated mine tailing wetland

Weiqing Wang, E. Gert Dudel

*Institute of Plant and Wood Chemistry, Dresden University of Technology, Pienner Strasse 19, D-01737 Tharandt, Germany*

Manuscript prepared for submission

**Abstract** Studies have revealed that the U retention in soil is a major path for aquatic U removal. However, the U occurrence in rhizosphere formed over decades in macrophyte habitat is largely unknown. Hence, the U retention in rhizosphere subhydric soil was quantified in a eutrophic *Phragmites australis* Trin ex Steud. dominated wetland exposed to a relative continuous aquatic U contamination over decade. Two sites with different redox state (54 to 302 mV) and related oxidizers (*e.g.* DO and nitrate) availability conditions were selected in oxidizing water pathway (PO) and more reductive tailing pond (PR). The soil retained U concentrations differed relatively low in two sites (51.6 mg·kg⁻¹ in PO vs. 48.5 mg·kg⁻¹ in PR). The organic matters (OMs) were highly competitive for U retention (proportion of organic bound U: 68.7% in PO vs. 21.3% in PR) in eutrophic soil with productive *P. australis*. It also favored the organic U remobilization into labile uranyl carbonates compounds (proportions of U in carbonate soil fraction: 16.4% in PO vs. 10.5% in PR), especially in relatively oxidizing rhizosphere. Furthermore, the absorption of U and other elements (*e.g.* Fe) within OMs also impaired the U retention capacity of related inorganic mediators. The share of organic bound U was diminished with the attenuated nutrients (TN concentrations: 8.3 g·kg⁻¹ in PS vs. 1.5 g·kg⁻¹ in PS) and related oxidation ability (*e.g.* from aquatic nitrate: 4.1 mg·l⁻¹ in PL vs. <0.01 mg·l⁻¹ in PS). It oppositely encouraged the U retention within inorganic mediators as sparingly soluble minerals. It was likely that the anaerobic OMs decomposition promoted free Fe oxides (Fed) formation (soil Fed/Fe concentration ratios: 0.17 in PO vs. 0.35 in PR). The Fed could effectively retain U and partially increased its mobility in form of suspended (exchangeable) soil particles (proportions of U in exchangeable fraction: 1.0% in PO vs. 8.1% in PR). The Fed precipitated on root of *P. australis* also mediated the aquatic U uptake by root. The Fed assisted U retention might be affected by other co-existing elements. The Ca and As caused the potential competitive effect to U retention within Fed, while the presence of P possibly assisted the interaction of U and Fed.

**Keywords** organic matter, oxidation state, nitrate, sequential chemical extraction, free Fe oxide, humic matter quality

**Acknowledgement** The authors would like to thank the support provided by Technical University of Dresden and China Scholarship Council for this study (Grant No. 2011671061).
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

The authors appreciate the assistance of the colleagues (particularly Dr. Arndt Weiske, Dipl.-Chem. Gisela Ciesielski and Yan Lu) in processes of sampling and analysis. The authors also particularly thanks Dr. Stefan Ritzel and Dr. Andrea Sperrhacke from Saxon State office for Environment and Agriculture (LfULG) Dept. Natural Radioactivity for the long-term water monitoring data provision and compilation and the FTIR analysis supported by the Analysis and Test Center of Nanjing Normal University.

4.1 Introduction

Considerable parts of residues remain on site as wastes in active and decommissioned uranium (U) mines and tailings (Nassour et al., 2015). The wasted U exposed or buried in these deposit sites causes the potential environmental risk to surroundings mainly due to its chemical toxicity (Bayliss et al., 2012; Lourenco et al., 2012). The local surface water pathway and underground leachate can export high content of U (soluble or particle bound) from the deposited site to open water and aquifers, even to the source of drinking water. In certain area, the specific concentration of aquatic U exceeded to the recommended level by hundredfold and causes severe health threat to surrounding population (Kurttio et al., 2002).

The chemical mine water treatment technologies have been applied to restrict the contamination of aquatic U by precipitating it from water, especially the water source in the highly acid water source. However, the efficiency of chemical U precipitation was restricted in alkaline water. Krestou et al. (2004) has observed that around 32% of U precipitated with hydroxyapatite (Ca$_5$(PO$_4$)$_3$(OH)) in acid and neutral solution was dissolved when the solution shifted to alkaline condition. The active remobilization of U precipitated with chemical agent results in the incomplete aquatic U removal and demands the especially high technical and economical efforts to control the aquatic U to required level in the alkaline water. Therefore, the alternative environmental friendly and economical biotechnologies based on the primary producers are proposed for aquatic U removal (Kalin et al., 2005). Among these environmental biotechnologies, the phytoremediation in natural and constructed wetlands have been applied successfully worldwide for the aquatic metals/metalloids removal (Vymazal et al., 2007). The phytoremediation is also proved feasible retaining the aquatic U by some plant species (Kropfelova et al., 2009; Pratas et al., 2012). However, the sustained operation of phytoremediation respectively sustainable function and permanent U retention is also controversial showed by other studies (Vandenhove 2013). The certain chemical properties and related bioavailability of U in soil and water pathway is possibly influence the performance of phytoremediation in different environmental conditions.

Two formal U oxidation states are found in the biogeosphere, which are respectively U(IV) and U(VI). The U(VI) occurs as the uranyl cation (UO$_2^{2+}$) in highly acidic environment and
the hydroxides and minerals associated with other elements (Si, Ca and P etc.) in relatively higher pH condition (Dreissig et al., 2011; Langmuir 1978; Ohnuki et al., 2004). U(IV) is mainly stable in the reductive environment as sparingly soluble UO₂ compounds formed through biotic and abiotic redox processes. The activity of dissimilatory metal reducing bacteria promotes the U(VI) bioreduction in anoxic environment (Liu et al., 2002). The microbial Fe(II) generation through Fe(III) reducing bacteria species in anoxic environment is also potentially accompanied with the U(VI) reduction to U(IV) compounds in both aqueous and solid phases (Fredrickson et al., 2000). The U(VI) bioreduction can also be induced by the sulfate reducing bacteria species utilizing U(VI) and Fe(III) together with the sulfate as the electron acceptors (Tebo and Obraztsova 1998). However, U was not only immobilized in the form of U(IV) compounds, the different U(VI) species can also be precipitated under certain environmental conditions. If anions (carbonates, sulfates and phosphates etc.) are available in slightly acidic to alkaline water, U(VI) can be precipitated and deposited as U(VI) minerals like autinite and natroautunite (Mehta et al., 2014). The formation of these minerals is even possible in oxidized environment. U(VI) can also retained by the mediators (e.g. hydrated Fe(III) oxides) under the cycled redox state changes (Stewart et al., 2009). Despite of the precipitation with anions in forms of inorganic minerals, the organic matters (OMs) (oxalates, citric acids and humic acids etc.) also have the ability retaining U in biogeo sphere (Lenhart et al., 2000; Mkandawire et al., 2006). The interaction between U and OMs can be assisted by the related bacterial communities. They utilize U as potential electron acceptors and OMs (e.g. humic substances) as electron shuttles for anaerobic respiration in subhydric organic soil (Finneran et al., 2002; Salome et al., 2013). For this reason, it is understandable that the increasing production and related respiratory decay of OMs has the potential to promote the U retention in soil as organouranium compounds (Bednar et al., 2007). In most of cases, the U occurrence in soil is interfered by the associated effects of OMs and inorganic mediators, especially in natural environment. The soluble Fe can be transformed to the labile precipitates, like the Fe plaque (IP) mainly composed by the hydrated Fe(III) oxides and associated bacteria species (Petrie et al., 2003). It is capable to retain the aquatic U within in rhizosphere and the decayed root litter enriched in ambient soil (Chang et al., 2014). The presence of Ca inhibits the U(VI) availability for microbial OMs respiration (Brooks et al., 2003). Stewart et al. (2007) have also suggested that the inhibition effect of Ca on U(VI) reduction is restricted when Ca is absorbed by goethite and hematite. They have further suggested that the Ca was capable to compete with the ferrihydrite on U incorporation.

On the other hand, the U retained within these mediators can also be desorbed when environmental conditions are changed. The CO₂ and related (bi)carbonate released via microbial respiration have been considered the major promoter for the dissolution of some sparingly soluble U(IV) minerals (e.g. U₃O₈) under both moderately oxidizing and reductive
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

conditions (Ulrich et al., 2008). Li et al. (2013) have studied the absorption and desorption of U(VI) in oxidized soil and suggested that the multiple agents (EDTA, citric acid and HNO₃), especially the HNO₃ respectively H⁺ and NO₃⁻, can extract U(VI) from solid compounds. However, the nitrate has not destabilized the reduced U(IV) directly but with the assistance of bacteria. The microbial respiration, mainly the denitrification can mediate both the enzymatic and abiotic U(IV) oxidation to dissolved chemical species (Wu et al., 2010). In some cases, the hydrated Fe(III) oxides are also involved in the denitrification through nitrate-dependent-Fe(II)-oxidizing bacteria and resulted in the absorption of U(VI) in Fe-bacteria complexes (Chang et al., 2014; Senko et al., 2005).

The above-described adsorption, reduction, precipitation and incorporation processes of U bound in minerals and OMs are usually examined individually in isolated and simplified subsystems. For the application of aquatic U removal from complex environment (e.g. wetland habitats), the high retention rate and durable fixation of U in the soils and sediments is preferred. In other words, it is necessary to determine the certain share of the durable fixed and temporarily bound U in soil that may be dissolved and recycled in plant/microbe-soil-water system.

A set of sequential extraction methods are widely applied to identify the occurrence of metals/metalloids in soil. The procedure allowed the estimation of the re-solution potential or the binding stability of immobilized pollutants. By sequentially applying the chemical extractants, the typical chemical fractions corresponded to a defined chemical speciation of the target element can be obtained. They may include the fractions like interstitial water, easily exchangeable ions, carbonates, organic associated complexes, oxides, insoluble residual with crystal lattice and some other primary and secondary minerals (Wang et al., 2002). The incomplete extraction and redistribution of target element are the common disadvantages of proposed sequential extraction methods in different extent. For this reason, the Tessier’s method is a well-accepted procedure with relatively reliable sequential extraction result for metal speciation (Tessier et al., 1979). The Schultz’s method has been recently improved based on the Tessier’s method and achieved better reproducibility than the latter for common metals/metalloids speciation (Schultz et al., 1998). However, the Schultz’s method has shown lower reliability than Tessier’s method regarding U speciation. The comparative extraction using both Tessier’s and Schultz’s methods showed that the latter had the significantly higher variation coefficients (up to 50%) than Tessier’s method (less than 10%) in most of U chemical fractions (Blanco et al., 2004). In this case, the Tessier’s method is still a feasible and reliable procedure for operational U speciation. By applying the Tessier’s method, it is open to U chemical speciation for following ways: i) estimate the share of U in highly labile and bioavailable soil fractions binding with exchangeable ions and carbonates; ii)
evaluate the level of organouranium, which is degradable and has the potential to remobilize in certain conditions; iii) estimate the share of U in Fe/Mn oxides (not loosely adsorbed), which is a relatively durable U fixation fraction and also potential source for the formation of IP (IP) in rhizosphere; iv) estimate the share of highly firm U in residual fraction with recalcitrant matter and host material.

So far the effect of redox state on the stability of U within general fractions in soil/sediment has not been fully revealed. Further understanding in this knowledge can help to enhance the function of wetland for U bio-stabilization. It was hypothesized that the redox state related distinct composition of soil/sediment and U oxidation state caused the difference on U speciation in soil/sediment. The certain U speciation further affected the U retention stability in soil/sediments and related soil-plant translocation potential. To verify these hypotheses, a field investigation was conducted in a nature-like Phragmites australis Trin ex Steud. dominated eutrophic wetland fed by mine and seepage water, which has: i) regulated and stabilized water level and chemical conditions (monitored since last decade); ii) minor variations of U speciation input from the underground (anaerobic) water; iii) moderate U contamination in near neutral carbonate rich hard water and continuous nitrate supply; iv) undisturbed soil genesis mainly driven by P. australis litter since decades. These environmental conditions made it a feasible site to estimate the U retention in rhizosphere soil under redox state gradient related oxygen and nitrate respectively nutrient (TN) levels.

4.2 Material and methods

4.2.1 Study site and sampling

Field investigation was conducted in natural P. australis dominated wetland. The wetland was formed since 1977 on a U tailing basin refilled with sedimented silty material. Leachate from the flooded mine shafts located above in the east constantly effused with the underground pressure flow. It flowed via a preferential surface water pathway and collected by still tailing pond in the southwest of wetland (Fig 4.1).
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

Since 2003, the water level of tailing pond was finally regulated. The aside tailing dam and bypass channels decoupled the tailing basin hydraulically from the surroundings. They provided the possibility for undisturbed *P. australis* and related surface organic subhydric soil layer development at least since 2003. The *Typha latifolia* in south-east was replaced in last decade by *P. australis* and *Salix* species due to natural succession. Two represent sites (PO and PR) were selected along the water pathway to exclude the irrelevant environmental interferences (*e.g.* weak solar radiation in the *Salix* shaded area) for the research question. The site PO was located in preferential surface water pathway about 50m downstream leachate pond (mine water inlet). The site PR was selected near the joint point between the water pathway and sedimentation area of tailing pond with deeper water stand approx. 0.5 m above oulet into the bypass channel. The sampling period was set in autumn when *P. australis* was fully developed (nutrient translocation and deposition in rhizomes). The root of *P. australis* was randomly collected from rhizosphere of both sites and packed wet under water to avoid air contact. The attached subhydric soil particles on root were slightly detached and stored in PE vessels. All vessels were filled with the rhizosphere water to provide an environment-similar storage condition. The pH and redox state conditions in rhizosphere water were determined by the pH/ORP meter (TM-39 meter, Fa. Sensortechnik, Germany). The DO concentration and conductivity of water were determined by the oximeter (LF95 bzw. Oxymeter, Fa. WTW, Germany). The rhizosphere water was collected in situ before the subhydric soil sampling with 0.45μm syringe filter (Roth GmbH, Germany) and sealed in PE vessels. All samples were transferred and stored cool (4-6°C) in laboratory. The water chemistry long term monitoring data (2006-2012, Table 3a,b) were collected and analyzed by the Saxon Operating Company for Environment and Agriculture (Betriebsgesellschaft für landwirtschaft und Umwelt Sachsen) according to DIN-ISO certified standard procedures for leaching and seepage water sampling and analysis for nutrients, trace elements including radionuclides and anions as cations, pH, conductivity and DO.
4.2.2 Sample pretreatment

Fresh subhydric soil samples were weighed and successively sieved through 2000 μm, 250 μm, and 45 μm mesh sieves to obtain the coarse particles (S1), fine particles (S2), and silt (S3) samples. The untreated soil samples were vacuum dried to constant weight under -50°C for further analysis. Fresh root samples were removed from the residual soil particles by hand and rinsed twice with deionized water. The clean root samples were then fragmented and fully mixed. The random portions of mixed root samples were assigned to the free Fe oxide (Fed) extraction procedure and the rest were oven dried to constant weight at 60°C.

4.2.3 Fed Extraction from rhizosphere hydric soil and root of *P. australis*

The modified dithionite-citrate-bicarbonate (DCB) extraction method was conducted for Fed extraction (Taylor et al., 1984). 1.0 g fresh root samples were weighed and incubated in a solution of 40 ml of 0.3 M Na₃C₆H₅O₇·2H₂O, 5 ml of 1.0 M NaHCO₃, and 1.5 g of Na₂S₂O₄ at room temperature for 16 h. The result solution was transferred and diluted to 100 ml with deionized water. Approx. 0.5 g subhydric soil samples were also treated in solution for 16 h, then centrifuged at 3000 g for 30 min. The result solution was transferred and diluted to 100 ml with deionized water. The treated root and soil samples were oven dried to constant weight at 60°C.

4.2.4 Sequential chemical extraction of U in rhizosphere subhydric soil

Approx. 0.5 g fresh subhydric soil sample in dry weight (DW) was weighed into the centrifuge tube and assigned to the modified sequential chemical extraction procedure (Tessier et al., 1979) (Table 4.1).

<table>
<thead>
<tr>
<th>Desired fraction</th>
<th>Extractive reagent</th>
<th>Reaction time and temperature</th>
<th>Reagent/sample ratio (v/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1: Exchangeable</td>
<td>1 M HN₄Ac, pH 7</td>
<td>1h, RT*</td>
<td>15:1</td>
</tr>
<tr>
<td>F2: Carbonates</td>
<td>1 M NaAc in 25% HAc, pH 4</td>
<td>2h, RT</td>
<td>15:1</td>
</tr>
<tr>
<td>F3: Fe/Mn oxides</td>
<td>0.04 M NH₂OH·HCl in 25% HNO₃, pH 2</td>
<td>5h, RT</td>
<td>15:1</td>
</tr>
<tr>
<td>F4: OM</td>
<td>30% H₂O₂</td>
<td>1h, RT</td>
<td>2:1</td>
</tr>
<tr>
<td></td>
<td>2.5% Na₂CO₃</td>
<td>1h, RT</td>
<td>15:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5h, 90°C**</td>
<td></td>
</tr>
<tr>
<td>F5: Residue</td>
<td>65% HNO₃ and 30% HCl</td>
<td>2h, 180°C</td>
<td>15:1</td>
</tr>
</tbody>
</table>

*RT: Room temperature  
**The temperature was maintained by thermostatic water bath

The result solution collected from each step was centrifuged at 3000 g for 30 min at root
temperature. The supernatant was collected and made to 100 ml with deionized water and the residue was assigned to the next step. In the end of this procedure, 5 major sequential fractions from F1 to F5 were successively obtained.

4.2.5 Total digestion

The oven dried root samples were centrifugally milled to powder at 10000 g (ZM1000, Retsch GmbH, Haan, Germany). Approx. 200 mg powdered root sample was weighed and digested with 3 ml 65% HNO$_3$ and 2 ml 30% H$_2$O$_2$ at 180℃ for 2 h (Mars 5, Fa. CEM Corp.). The result solution was made to 20 ml with deionized water. The powdered dry soil particles were digested with 4 ml 65% HNO$_3$ and 4 ml 30% HCl under the same condition. The result solution was made to 50 ml with deionized water. All solutions were stored cool (4-6℃) before further analysis.

4.2.6 Elemental analysis

The fresh interstitial water samples along with the result solutions were analyzed on the inductively coupled plasma mass spectrometry (ICP-MS; PQ 2+; Thermo Elemental, USA) to determine the concentrations of Fe, U and other trace elements. The measurement was processed according to DIN-EN-ISO-17294-2 (Nassour et al., 2015). Fe was additionally measured with atomic absorption spectrometer (SOLAAR M6, Unicam Ltd., Cambridge, UK). TN and TC concentrations in subhydric soil were analyzed with Elemental C/N analyzer (Vario EL Elementar Analyses System GmbH, Hanau, Germany).

4.2.7 FT-IR analysis

The humic acid (HA) in rhizosphere subhydric soil was extracted following the IHSS method (Watanabe et al., 1994). Approx. 20 g soil sample was weighed into centrifuge tube with 200 ml of 0.1 M NaOH. The tube was shaken for 12 h with N$_2$ gas filled inside. The supernatant was collected after 10 min centrifugation at 3000 g. Acidify the supernatant with 6 M HCl with constant stirring to pH=1.0 and stood for 12 h. The supernatant was discarded after 10 min centrifugation at 3000 g and the precipitate (HA) was obtained. HA was suspended in 0.1 M HCl/0.3 M HF solution and shaken for 24h. The precipitate HA was dialyzed by against distilled water to remove Cl$^-$ and obtain the purified HA. KBr pellets method was conducted on Fourier-transform infrared (FT-IR) analysis of vacuum dried HA sample. The analysis was processed on the Spectrometer (Thermo/Nicolet Nexus 470, USA) in the range 400-4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$. Each sample was performed with 32 scans.

4.2.8 Statistical analysis

All statistical analysis of data was performed with SPSS statistics software (IBM SPSS,
4.3 Results and discussion

4.3.1 Water chemistry

The aquatic U and other co-existing metals/metalloids were detected in water collected from PO and PR (Table 4.2).

<table>
<thead>
<tr>
<th></th>
<th>Ca [mg L⁻¹]</th>
<th>Mg [mg L⁻¹]</th>
<th>Fe [μg L⁻¹]</th>
<th>Mn [μg L⁻¹]</th>
<th>As [μg L⁻¹]</th>
<th>U [μg L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>142.5±2.9</td>
<td>46.6±0.6</td>
<td>581.0±59.4</td>
<td>130.0±13.0</td>
<td>67.4±1.0</td>
<td>141.9±8.3</td>
</tr>
<tr>
<td>PR</td>
<td>122.4±5.5</td>
<td>54.2±4.3</td>
<td>560.0±30.1</td>
<td>26.2±10.5</td>
<td>188.4±13.4</td>
<td>106.6±1.7</td>
</tr>
</tbody>
</table>

The average aquatic U concentrations decreased by 24.9% from PO to PR. It suggested the aquatic U attenuation along with the water pathway, which was confirmed by the long term water chemistry monitoring data. The average aquatic U concentration from 2006-2012 measured near PO was 208±47.1 μg L⁻¹ and decreased to 72.3 ±20.3 μg L⁻¹ in tailing pond near PR (outlet downstream). The diminished aquatic U content was possibly retained by the mediators (minerals, plant litter and bacteria etc.) in subhydric soil and inhabited P. australis. Furthermore, it was also contributed by changed U oxidation states in rhizosphere under different biogeochemical conditions (Table 4.3a).

<table>
<thead>
<tr>
<th>pH</th>
<th>Eh [mV]</th>
<th>Conductivity [µS·cm⁻¹]</th>
<th>DO [mg L⁻¹]</th>
<th>HCO₃⁻ [mg L⁻¹]</th>
<th>SO₄²⁻ [mg L⁻¹]</th>
<th>o-PO₄₃⁻·P [mg L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>6.9±0.1</td>
<td>302±32</td>
<td>151±269</td>
<td>7.9±3.1</td>
<td>424.3±79.8</td>
<td>210.0±30.0</td>
</tr>
<tr>
<td>PR</td>
<td>6.8±0.1</td>
<td>54±27</td>
<td>576±156</td>
<td>0.3±0.2</td>
<td>308.3±7.5</td>
<td>*</td>
</tr>
</tbody>
</table>

* Concentration below the detection limit

The long-term monitoring data indicated the similar slightly acid to neutral pH conditions near two sites. It was beneficial for the stable presence of aquatic U(VI) in form of soluble uranyl compounds (Zhou and Gu, 2005). On the contrary, the certain Eh values in rhizosphere water were significantly decreased by 82.1% from PO to PR. The oxidizing rhizosphere in PO was shifted to relatively reductive condition in PR. The redox state gradient indicated the changed U oxidation states coupled with discrepant abiotic and biotic redox processes in rhizosphere of two sites. The high concentration of DO in PO was capable to oxidize the sparingly soluble U(IV) minerals to aquatic U(VI) compounds (Table 4.3a). However, the DO
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

depended U(IV) oxidation was possibly inhibited in anoxic PR. Moreover, the NO$_3^-$ was detected as the dominant aquatic N species in PO (Table 4.3b).

Table 4.3b Water chemistry of rhizosphere - mean content of nitrogen and carbon, n=7, the error means the ±SD.
The value was collected from long term monitoring data in the input and output zone where two sampling site located from 2006 to 2012. The error means the ±SD

<table>
<thead>
<tr>
<th></th>
<th>NO$_3^-$ [mg·L$^-1$]</th>
<th>NO$_2^-$ [mg·L$^-1$]</th>
<th>NH$_4^+$-N [mg·L$^-1$]</th>
<th>DOC [mg·L$^-1$]</th>
<th>TOC [mg·L$^-1$]</th>
<th>TC [mg·L$^-1$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>4.1±2.2</td>
<td>0.3±0.4</td>
<td>6.2±2.2</td>
<td>6.4±2.2</td>
<td>94.3±18.6</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>0.01±0.01</td>
<td>0.03±0.01</td>
<td>1.8±0.4</td>
<td>3.2±2.8</td>
<td>69.2±3.9</td>
<td></td>
</tr>
</tbody>
</table>

* Concentration below the detection limit

The high content of aquatic NO$_3^-$ was capable to assist the U(IV) oxidation as electron acceptor via related biotic redox processes (Moreels et al., 2008). However, the aquatic NO$_3^-$ availability was diminished via anaerobic denitrification and assimilation of plants and microbes in PR. The remained aquatic NH$_4^+$ in PR was oppositely adverse for U(IV) minerals dissolution, but beneficial for aquatic U(VI) bioreduction (Gilson et al., 2015). The relatively higher N supply and related redox state in PO also benefited the biomass productivity of P. australis. It potentially encouraged the OMs accumulation in both subhydric soil and water pathway through the above and below ground litters of P. australis. The HCO$_3^-$ concentration in water was decreased by 27.3% from PO to PR (Table 4.3a). The higher HCO$_3^-$ availability was crucial for sustaining the aquatic U(VI) at a relatively high level in form of uranyl carbonates (Grenthe et al., 1984).

The aquatic Fe was a major heavy metal in rhizosphere water of two sites (Table 4.2). It was capable to retain U via different redox processes. The aquatic U can be rapidly precipitated with Fe(III) oxides by nitrate dependent Fe(II) oxidizing bacteria species when NO$_3^-$ is available (Senko et al., 2005; Table 4.3b). On the other hand, the U(VI) bioreduction was possibly promoted by Fe(III) reducing bacteria respiration in reductive environment (Liu et al., 2005). The diminished aquatic SO$_4^{2-}$ availability in PR indicated the enhanced respiration of anaerobic sulphate reducing bacteria species in this site (Table 4.2). They possibly mediated the anaerobic degradation of OMs to CO$_2$ and H$_2$S as final products. The aquatic U(VI) could also be reduced to U(VI) by them along for OMs degradation (Martins et al., 2010). Furthermore, the aquatic H$_2$S also improved the stability of Fe(II) ions by forming the ferrous sulfide (FeS) (Butler et al., 2005). The U(IV) could also be oxidized by redox cycling of aquatic Mn(II) and related Mn(II) oxidizing bacteria activity (Chinni et al., 2008). The Mn(II) also possibly prevented U(IV) from contacting with oxidizing agents by precipitating on surface of U(IV) minerals as MnCO$_3$ (Wang et al., 2014).

The aquatic As was highly affinity to hydrated Fe(III) oxides by forming the stable complexes...
4.3 Results and discussion

(Fritzsche et al., 2006; Kumar et al., 2016). The increased aquatic As content (As/U ratio: 0.5 in PO and 1.8 in PR) potentially competed with U retention within hydrated Fe(III) oxides (Wang et al., 2015). The high aquatic Ca availability in both sites was beneficial for improving the solubility of U(VI) by forming the soluble Ca-U-carbonate complexes (Bernhard et al., 2001). It also caused the competitive effect to hydrated Fe(III) oxides on U retention (Massey et al., 2014). For this reason, high Ca concentration was adverse for aquatic U removal in both sites. Despite an iron oversupply, the free phosphate was also detectable. The aquatic phosphate concentrations in PO and PR had indicated the eutrophic conditions of both sites. The high content of phosphates was beneficial for aquatic U precipitation (Mehta et al., 2014). Moreover, the aquatic phosphate also potentially improved the aquatic U retention within hydrated Fe(III) oxides in rhizosphere (Chang et al., 2014).

4.3.2 C/N accumulation in the rhizosphere subhydric soil

The C/N ratios were 12.0 in PO and increased by 33.3% in PR, with discrepant TN and TC concentrations in rhizosphere subhydric soil collected from two sites (Fig 4.2).

Fig 4.2 Mean total concentrations of C and N in rhizosphere subhydric soil, n=5, the error bar means the ±SD.

The subhydric soil C/N ratio was particularly low with higher TN concentrations in PO around 8.3±0.7 g·kg⁻¹. The TN and TC concentrations in PR were only respectively accounted for 18.5% and 25.6% than the corresponding values in PO. The high N retention in forms of nitrate and non-protein N associated with complex carbon compounds potentially resulted the lower C/N ratio (Odum et al., 1979). The possible soil to water nutrition translocation in PO benefited the rhizosphere water providing sufficient aquatic N for P. australis and other
organisms (e.g. algae) uptake (Table 4.3b). The N surplus thusly increased the biomass productivities of these organisms and related content of dead organisms (e.g. litters of *P. australis*) supplied to rhizosphere. The degradation of dead organisms further released the organic/inorganic C (e.g. CO\(_2\) depended (bi)carbonate) and N (e.g. amides and ammonium) to rhizosphere subhydric soil and water pathway. The increased C and N accumulation in this way consequently resulted in the organic rich subhydric soil layer formed in PO. The certain U occurrence in rhizosphere subhydric soil was then affected by the redox state related different nutrition (C/N) and OMs levels in rhizosphere subhydric soil of two sites.

4.3.3 Chemical speciation of U in rhizosphere subhydric soil

The discrepant chemical speciation of U was observed in rhizosphere subhydric soil of two sites (Fig 4.3).
The organic bound U accounted for 68.0% of soil retained U in PO and was decreased by 68.7% in PR. The total proportions of highly labile (exchangeable and carbonate) U fractions were similar in PO (17.4%) and PR (18.6%). However, the share of U in exchangeable fraction (8.1%) in PR increased by 8.0 times than PO, while the carbonate fraction (10.5%) in PR was decreased by 35.8%. The total proportion of U within Fe/Mn oxides and residual minerals was only around 14.6% in PO, but increased to 60.0% in PR. Meanwhile, the
proportions of U in Fe/Mn oxides and residue fractions were also respectively increased by 6.3 and 4.0 times from PO to PR.

The results suggested that the highly labile U in exchangeable and carbonate soil fractions only took the small share in both sites. U was mainly retained in relatively stable soil fractions (OMs, Fe/Mn oxides and other sparingly soluble minerals). However, the partition of these fractions was differently changed from PO to PR. The OMs took an outstanding position for U retention in rhizosphere subhydric soil. The high nutrition (e.g., high aquatic NO$_3^-$ and soil TN content) condition in oxidizing PO was capable to increase the biomass productivity of *P. australis* and related litters accumulation. The mobilized U was effectively absorbed by litter fragments and other OMs as organouranium in rhizosphere subhydric soil (Bednar *et al*., 2007). It caused the potential competitive effect to some inorganic mediators for U retention. The increased content of OMs also absorbed higher proportion of organic bound Fe in PO by 2.2 times than in PR (Fig 4.3). The increased content of Fe within OMs potentially diminished its capacity retaining U in form of inorganic Fe minerals (U/Fe partition ratios in Fe/Mn oxides fraction: 0.2 in PO and 1.6 in PR). However, the stability of organic bound U was possibly affected by the OMs degradation, especially in the relatively oxidizing environment. The strong oxidation ability in rhizosphere enhanced the aerobic decomposition of OMs to CO$_2$ dissolved in water as (bi)carbonates (Table 4.3b). The U within OMs was also remobilized along with the degradation and incorporated with (bi)carbonates and other soluble organic acids.

The decreased N nutrition and strong (sulfate) reducing conditions in PR might restrict the OMs accumulation and its U retention capacity. It further caused the adverse impact on the CO$_2$ and U release from OMs and related formation of uranyl carbonates. In comparison, the U retention capacity of inorganic minerals was then oppositely enhanced through related biotic and abiotic redox processes. The low OMs availability was beneficial for U(VI) absorption by Fe/Mn oxides (Du *et al*., 2016). The U(VI)-Fe(III) oxides complexes could be further desorbed by the activity of Fe(III) and sulphate reducing bacteria species and released the soluble Fe(II) (Martins *et al*., 2010; Wilkins *et al*., 2006). In aquatic environment, Fe(II) could be oxidized to suspended hydrated Fe(III) oxides in exchangeable form (Manchanda *et al*., 2006). They had the strong U absorption ability and thusly increased the labile U retention in exchangeable complexes (U/Fe partition ratios in exchangeable soil fraction: 3.0 in PO and 45.8 in PR) (Du *et al*., 2016; Fig 4.3).

In conclusion, the OM was presumed the crucial mediator affecting the speciation and stability of U retained in subhydric soil. High OM availability resulted in the dominant organic U retention than other mediators in subhydric soil. The organic bound U was still possibly released as soluble uranyl carbonates, due to the OMs degradation and CO$_2$
production. The decreased OMs content in subhydric soil relatively improved U retention capacity of Fe/Mn oxides and other minerals. It also limited the formation of uranyl carbonates and encouraged the absorption of U with hydrated oxides. In this case, a relatively firm U retention in rhizosphere subhydric soil was supposed to be obtained with the limited OMs supply.

4.3.4 Chemical composition of HAs in rhizosphere subhydric soil

As the major component in humus, the discrepant chemical composition of HAs was observed in rhizosphere subhydric soil in PO and PR (Fig 4.4).

![FT-IR spectra of organic functional groups formed in rhizosphere humic acids](image)

The spectra of HAs collected from PO and PR had the similar positions of main absorption bands. However, some differences were still observed in specific bands with discrepant absorption intensities (Fig 4.4; Table 4.4).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Band [cm$^{-1}$]</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib</td>
<td>3693, 3620</td>
<td>free hydroxyl (·OH)</td>
</tr>
<tr>
<td>II$^{a,b}$</td>
<td>3000-3600</td>
<td>O-H stretching vibration in diametric and polymeric H bridges</td>
</tr>
<tr>
<td>III$^{a,b}$</td>
<td>2950±20</td>
<td>C-H stretching vibration of aliphatic groups</td>
</tr>
<tr>
<td>IV$^{a,b}$</td>
<td>1632±2</td>
<td>C=O stretching vibration of amide, possible contributed by nitrates (R-O-NO$_2$) and nitrites (R-O-NO) groups</td>
</tr>
<tr>
<td>V$^{a,b}$</td>
<td>1435±10</td>
<td>C=N of primary amides (O=CH-NH$_2$)</td>
</tr>
<tr>
<td>VI$^{a,b}$</td>
<td>1031±3</td>
<td>C-O stretching vibration of polysaccharides or similar substances (S=O, P-O-Alkyl and =C-O-C)</td>
</tr>
<tr>
<td>VII$^b$</td>
<td>829</td>
<td>1,4-disubstitution or 1,3,4-trisubstitution benzene rings and R$_2$C=CRH groups</td>
</tr>
<tr>
<td>VIII$^b$</td>
<td>770,755</td>
<td>mono-substituted aromatic or 1,2-disubstitution benzene rings</td>
</tr>
<tr>
<td>IX$^b$</td>
<td>693</td>
<td>ring deformation vibration of mono-substituted aromatic groups</td>
</tr>
</tbody>
</table>

As a major organic group of HAs, the signal of oxhydryl (O-H) was indicated by a relatively
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a *Phragmites australis* Trin ex Steud. dominated mine tailing wetland

stronger and broader band (3000-3600 cm\(^{-1}\)) in PO. It suggested the enhanced microbial decomposition of dead organism (*e.g.* litter of *P. australis*) in humus, due to the strong oxidation ability from high DO and NO\(_3^-\) availability. The stronger signal of alkynyl (C-H) in aliphatic groups was also detected in PO. The signal of alkynyl was also detected in PR, but was indicated as the aromatic groups. The subhydric soil with sufficient alkynyl carbon was relatively active and rich in labile compounds like carbohydrates and peptides (Pedersen *et al.*, 2011). However, the presence of aromatic carbon (alkynyl) in PR indicated the possible formation of some recalcitrant OMs with relatively low bioavailability. Furthermore, the U sedimentation in rhizosphere was possibly enhanced by some recalcitrant OMs (Macdonald *et al.*, 2008). The amides groups were detected in both sites. The signal of amide groups in PO was relatively strong around 1632 cm\(^{-1}\). The amide groups in this range was mainly contributed by the NO\(_3^-/NO_2^-\) groups. The amide groups in PR were mainly existed in the primary amides (signal around 1432 cm\(^{-1}\)) contributed by NH\(_2\)\(^+\) (Hesse, 2005). It suggested that the oxidation state of OMs was restricted in reductive environment, where available NO\(_3^-/NO_2^-\) was depleted and replaced by NH\(_4^+\) from anaerobic organic N decomposition (Table 4.3b). The strong signal around 1031 cm\(^{-1}\) in PR indicated the presence of sulphinyl (S=O) (Hesse, 2005). The organic S together with primary amides in humus were possibly the products of anaerobic proteins degradations. They also suggested the potential H\(_2\)S and CO\(_2\) generation from the anaerobic sulfurous OMs decomposition. The signal in this range also indicated the possible presence of polysaccharides from bacteria and plant (root) cells in two sites (Hesse, 2005). Some polysaccharides were capable to support the soil to root translocation of U (*e.g.* exopolysaccharides (EPS) in root exudates), or oppositely promote the biosedimentation of aquatic U in subhydric soil (Jroundi *et al.*, 2007). The detailed speciation of polysaccharides in two site was not clearly showed in FT-IR data. However, the stronger and broader band in PR still suggested the potential difference on the quality (even quantity) of polysaccharides in two sites. It was possibly caused by the different aerobic/anaerobic cellular metabolism paths under changed biogeochemical conditions. The discrepant speciation of polysaccharides further caused the potential influence on the U incorporation.

In conclusion, the occurrence of organic bound U was not only depended on the quantity variation of OMs in rhizosphere subhydric soil. It was also potentially determined by the different constitutions of OMs formed under different environmental conditions and related microbial decomposition processes.

4.3.5 Fe/Fed retention in rhizosphere subhydric soil and root of *P. australis*

The Fe retention and related Fed formation was observed in rhizosphere subhydric soil and root of *P. australis* of both sites (Fig 4.5).
4.3 Results and discussion

**Fig 4.5** Mean concentrations of Fe and Fed retained in rhizosphere subhydric soil and root of *P. australis* (TS: total Fe content in rhizosphere subhydric soil, FS: Fed content in rhizosphere subhydric soil, TR: total Fe content in root of *P. australis*, FR: Fed content extracted from root surface.), n=3, the error bar means the ±SD.

The concentrations of soil retained Fe were only slightly increased by 10.4% from PO to PR. However, the Fed distribution was significantly different in two sites. The Fed concentration in PO only accounted for 45.0% of that in PR. The proportion of Fed in soil retained Fe also increased from 17.6% in PO to 35.3% in PR. The root retained Fe concentration in PO only accounted for 21.6% of that in PR. The proportions of Fed in root retained Fe were similar in two sites, which were respectively 91.9% in PO and 93.7% in PR.

The Fe concentrations showed the similar Fe retention capacities of rhizosphere subhydric soil in different biogeochemical conditions. However, the Fe in rhizosphere subhydric soil of two sites was possibly contributed by the different Fe constitutions. The increased Fed concentration in PR indicated the possibly enhanced decomposition of primary Fe(III) minerals. The limited DO and nitrate availability restricted the oxidation ability in PR. It oppositely encouraged the Fe(III) minerals reduction to soluble Fe(II) oxides via related redox processes (*e.g.* bioreduction by anaerobic Fe(III) reducing bacteria). The released Fe(II) oxides were then oxidized to suspended hydrated Fe(III) oxides particles contributing the exchangeable soil faction (Baken *et al.*, 2013). The aquatic Fe(II) also benefited the Fe uptake by root of *P. australis*. On the other hand, the root aeration made the root zone an important position for the related hydrated Fe(III) oxides formation (Tian *et al.*, 2015). The Fe(II) in root zone could be oxidized to Fed (*e.g.* ferricydrite) by root derived DO and other oxidizers (*e.g.* NO$_3^-$ and Fe(II) oxidizing bacteria) related redox processes. However, the Fed generation
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

was significantly restricted in highly oxidizing and organic rich rhizosphere of PO. The strong oxidation ability of this site improved the stability of Fe(III) minerals and restricted the Fe(II) re-solution. High content of OMs in this site also diminished the Fed availability, by absorbing Fe as organic amorphous Fe oxides (Fig 4.3; Saha et al., 1999).

In conclusion, the Fe in rhizosphere subhydric soil was firmly stabilized as Fe(III) minerals and organo-minerals with strong oxidation ability and OMs availability. The Fe(II) and related Fed generation was restricted in this environment. On the contrary, the Fed availability was significantly enhanced in reductive rhizosphere, where the stability of Fe(III) minerals was impaired by related Fe(II) generation redox processes.

4.3.6 U retention within Fed in rhizosphere subhydric soil and root of P. australis

The U concentrations in rhizosphere subhydric soil of two sites were also only slightly decreased by 6.0% from PO to PR (Table 4.5).

Table 4.5 Mean concentrations of elements within Fed (TS: total Fe content in rhizosphere subhydric soil, FS: Fed content in rhizosphere subhydric soil, TR: total Fe content in root of P. australis, FR: Fed content extracted from root surface.), n=3, the error means ±SD

<table>
<thead>
<tr>
<th></th>
<th>PO</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS (mg·kg⁻¹)</td>
<td>FS</td>
</tr>
<tr>
<td>P</td>
<td>1942.7±45.3</td>
<td>N/A*</td>
</tr>
<tr>
<td>Ca</td>
<td>11262.6±246.9</td>
<td>-**</td>
</tr>
<tr>
<td>As</td>
<td>1835.4±54.9</td>
<td>255.9±7.7</td>
</tr>
<tr>
<td>U</td>
<td>51.6±0.9</td>
<td>4.8±0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>FR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>1340.5±232.1</td>
<td>826.2±125.9</td>
</tr>
<tr>
<td>FS</td>
<td>4018.2±673.5</td>
<td>11100.9±195.2</td>
</tr>
<tr>
<td>TR</td>
<td>190.3±7.6</td>
<td>126.6±7.6</td>
</tr>
<tr>
<td>FR</td>
<td>47.7±2.2</td>
<td>9.7±0.4</td>
</tr>
</tbody>
</table>

* Element not tested.

** Content of heavy metal lower than the detection limit.

Around 9.3% of U in rhizosphere subhydric soil was retained within Fed in PO, while it was significantly increased to 20.2% in PR. Meanwhile, the concentration of U within Fed was lower in PO and only accounted for 62.9% of that in PR. The concentrations of U retained by root and Fed on root surface were respectively increased by 64.0% and 50.7% from PO to PR.

The results indicated the effective Fed assisted U retention in subhydric soil and root of P. australis, especially in the reductive rhizosphere. It was benefited by both the effective components of Fed and the related redox processes for its formation. The hydrated Fe(III) oxides in Fed had the strong ability to absorb the mobilized U in rhizosphere with them (Singh et al., 2012; Stewart et al., 2009). The U-hydrated Fe(III) oxide complexes were possibly in forms of labile exchangeable soil fraction or suspended particles in water pathway (Fig 4.3). It encouraged the soil to root U translocation along with the Fed formation in root zone. The Fed and related biofilm on root surface also formed the complex as IP to retain the root uptake U within its structure (Wang et al., 2015).
The U retention within Fed was restricted by the strong oxidation ability in rhizosphere of PO. The enhanced OMs accumulation in this site also competed with Fed by absorbing more available U and Fe as organo-minerals. It resulted in the diminished U availability in exchangeable form with hydrated Fe(III) oxides (Fig 4.3). The formation of IP on root surface and its U retention capacity were also limited, due to the decreased U and Fe availability for root uptake.

On the other hand, the U retention within Fed was also potentially affected by other co-existing elements. The P in rhizosphere potentially contributed the U retention within Fed through related P-U-Fe interaction paths. The P (possible in form of phosphate) in rhizosphere had the potential to form the sparingly soluble uranyl-phosphate complexes with aquatic U(VI). The phosphate was capable to mediate the co-precipitation of U(VI) and hydrated Fe(III) oxides, by absorbing uranyl in poorly crystalline nanosized iron phosphates (Seder-Colomina et al., 2015). The root uptake P was observed mainly precipitated on root surface within Fed (IP) in both sites (Table 4.5). It was beneficial for the aquatic U(VI) retention within IP associated by P in the complex (Chang et al., 2014). The P in rhizosphere also precipitated with Ca and U in form of Ca-uranyl-phosphate minerals (e.g. autunite (Ca(UO$_2$)$_2$(PO$_4$)$_2$·10H$_2$O)) (Ohnuki et al., 2004). However, the Ca in rhizosphere mainly incorporated with U by forming the soluble Ca-uranyl-carbonate complexes with the high content of (bi)carbonate (Tabel 4.2 and Table 4.3a). The competitive U absorption effect of Ca to Fe oxides in rhizosphere was also enhanced by carbonates formation from OMs degradation (Stewart et al., 2007). It might exclude Ca from the U-Fed complexes. However, Ca was still detected together with Fed on root surface (Table 4.5). It could be a result of IP derived elements retention effect. The aquatic Ca was also be retained within IP together with other elements (Fe, U and P etc.) in this process (Jiang et al., 2009). The microbial respiration in IP also caused generation of CO$_2$ and related (bi)carbonates on root surface. It benefitted the formation of Ca-uranyl-carbonates within the IP structure. However, Ca was presumed to be only temporarily retained in IP and highly potential to translocate to other plant tissues (Mortvedt 1994). It thusly resulted in the U translocation from IP to inner root tissue in form of Ca-uranyl-carbonates (Table 5). As was highly affinity to the hydrated Fe(III) oxides (e.g. ferrihydrite) by forming the stable Fe-As complexes (Essilfie-Dughan et al., 2013). The increased aquatic As availability from PO to PR encouraged the absorption of this metalloid within Fed (Table 4.2 and Table 4.5). It caused the potential competitive absorption effect to U by consuming the available capacity of Fed (Wang et al., 2015).

In conclusion, Fed was an effective mediator contributing the Fe and U interaction in rhizosphere, especially in reductive environment with high Fed availability. It could relatively enhanced the mobility of U in rhizosphere subhydric soil (e.g. in form of suspended
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

U-hydrated Fe(III) oxides particles). It further benefited the soil to root U translocation by forming the U-Fed complex within the IP on root surface. The retention of U and Fe within high content of OMs was adverse for Fed assisted U retention. Some other elements (e.g. As and Ca) might also impaired the U retention capacity of Fed through the related competitive absorption effect. However, it was also possibly assisted by some impurities (e.g. P) co-existing within Fed.

4.3.7 Particle size related U distribution in rhizosphere subhydric soil.

The particle size related U distribution in rhizosphere subhydric soil followed the discrepant patterns in PO and PR, regardless of the similar concentrations of soil retained U in two sites (Fig 4.6; Table 4.5).

![Graph showing U and Fe distribution in S1, S2, and S3 fractions in PO and PR.](image)

**Fig 4.6** Particle size related U and Fe distribution in the rhizosphere subhydric soil (S1: coarse subhydric soil particle; S2: fine subhydric soil particle; S3: silt particle), n=3, the error bar means ±SD

The highest U concentration in PO was detected in S1 and only slightly decreased by 0.8% in S2. The U concentration in S3 was lower than other two fractions and decreased by 16.4% than in S1. On the contrary, the U concentration in S3 was increased in PR. It was respectively 23.9% and 2.9% higher than in S1 and S2. The soil retained Fe was also distributed in all fractions of both sites. The Fe concentration in S2 was higher than other fractions in PO. It was respectively decreased by 9.6% in S1 and 49.7% in S3. The Fe concentration in S3 collected from PR was relatively 53.5% and 21.9% higher than the corresponding values in S1 and S2 from the same site. The particle size related Fe and U distribution was significantly correlated in both sites ($R^2=0.9488$ in PO and 0.8449 in PR). It indicated the positive role of Fe (hydr)oxides assisting U retention in rhizosphere subhydric
4.4 Conclusions

The result suggested that the large (coarse and fine) subhydric soil particles had the higher U retention efficiency than silt particles in PO. The accumulation of OMs from plant litters could be a major cause for this result. The increased content of litters fragments had the ability to absorb, complex and incorporate more aquatic U in rhizosphere (Dienemann et al., 2006). The microbial decomposition of litters also stimulated the formation of large size organo-mineral, associated by coarse particulate OMs, primary minerals and microbial biomass with them (Zeller and Dambrine 2011). Hence, the high content of organic mediators contributed the U retention in large size organic subhydric soil particles (Fig 4.3). The higher concentrations of Fe in coarse and fine subhydric soil particles also possibly contributed by the Fe (hydr)oxides in form of primary minerals and organo-minerals with OMs (Fig 4.3). The interaction of U and Fe was thusly processed with these agents in the coarse and fine subhydric soil particles. The lower Fe and U concentrations in silt particles in PO also supported the assumption, which suggested the U retention within small size active mediators (e.g. hydrate Fe(III) oxides) was restricted when high contents of OMs and primary minerals was available (Fig 4.3 and Fig 4.5). Consequently, the mobilized (soluble and suspended) U availability was limited by large particle size preferred U partition in the organic rich and relatively oxidizing rhizosphere.

It was conversed in the rhizosphere with lower nutrient condition and oxidation ability. The decreased OMs accumulation might diminish the contribution of large size subhydric soil particles on U retention (Fig 4.3 and Fig 4.6). On the other hand, the U retention within primary minerals was also impaired due to the enhanced microbial hydrolysis effect in the reductive envrionment. The primary minerals hydrolysis (e.g. Fe(II) generation) further benefited the U absorption by the increased content of dissociative/suspensible oxides (e.g. Fed) in silt (Fig 4.5).

It was presumed that the particle size related states of organic/inorganic mediators influenced the specific U distribution in different subhydric soil particles. However the difference are relativley small. and the uranium retention in the coarse fraction is unexpectedly large.

4.4 Conclusions

The results of this study had indicated the discrepant U retention patterns in rhizosphere subhydric soil, when redox state related biogeochemical conditions were changed. The U within highly labile (exchangeable and carbonate) soil fractions was only took small share of its total content in rhizosphere subhydric soil. Most of U was firmly retained in subhydric soil, but mainly contributed by different fractions under different biogeochemical conditions. The organic rich subhydric soil showed the relatively higher efficiency retaining U and some
4. Rhizosphere subhydric soil associated uranium retention along a redox state gradient in a
Phragmites australis Trin ex Steud. dominated mine tailing wetland

crucial elements (e.g. Fe) with OMs. It diminished the U retention capacity of inorganic
minerals. However, the stability of organic bound U was possibly impaired by OMs
degradation. The strong oxidation ability (e.g. high DO and nitrate availability) in rhizosphere
encouraged the aerobic microbial decomposition of OMs to CO₂ and related organic bound U
remobilization. The dissolution of U(IV) to uranyl carbonate was encouraged by high nitrate
and CO₂ derived (bi)carbonates availability. The share of organic bound U was restricted
when nutrition level and related OMs accumulation were limited in rhizosphere subhydric soil.
It benefited the U retention within other inorganic mediators (e.g. Fed). The different
constitutions of OMs also indicated the changed microbial respiration paths under different
redox state condition. The U(VI) bioreduction was potentially mediated by the anaerobic
bacteria species (e.g. sulphate reducing bacteria and Fe(III) reducing bacteria) in reductive
rhizosphere. It promoted the precipitation of aquatic U(VI) to sparingly soluble U(IV)
minerals. The anaerobic bacteria respiration also resulted in the Fe(III) minerals derived Fe(II)
generation and further formation of Fed. The Fed was capable to improve the mobility of U
by forming the complexes in the silt/suspended range subhydric soil particles. It also
mediated the soil to root U translocation by forming the Fe-bacteria complex as IP on root.
The Fed assisted U retention was partially restricted by high OMs availability in rhizosphere.
The high Ca availability increased the aquatic U(VI) mobility and also caused the potential
competitive U retention effect to Fe (hydr)oxides. The aquatic As in rhizosphere also
potentially competed with U retained within Fed. These elements had the adverse effect on
the retention of U in Fed and should be controlled in the practical application for aquatic U
immobilization. On the contrary, the P assisted the both the precipitation of U in rhizosphere
and also the U retention within Fed. In general, the accumulation of OMs was crucial and
competitive for U retention in subhydric soil. The inorganic mediators (e.g. Fed) was also the
effective involved in the related redox processes. There were still some aspects need to be
further studied. The detailed specification on the constitution of OMs could help to
understand the mechanisms of their specific interaction with U. The Fed showed the positive
role on the U retention in subhydric soil and related soil to root U translocation. Despite of the
OMs and some metals/metalloids species, the possible influence of other environmental
factors (e.g. N species) on the interaction of Fed and U was also valuable to determined.

4.5 References

Characterisation of hydrous ferric oxides derived from iron-rich groundwaters and
their contribution to the suspended sediment of streams. Applied Geochemistry 39,
59-68.

Bayliss P, van Dam RA, Bartolo RE (2012): Quantitative ecological risk assessment of the
Magela Creek floodplain in Kakadu national park, Australia: Comparing point source risks from the ranger uranium mine to diffuse landscape-scale risks. Human and Ecological Risk Assessment 18, 115-151.


4. Rhizosphere subhydric soil soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland


4.5 References


4. Rhizosphere subhydric soil soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland


Tebo BM, Obraztsova AY (1998): Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. Fems Microbiology Letters 162, 193-198.


4. Rhizosphere subhydric soil soil associated uranium retention along a redox state gradient in a Phragmites australis Trin ex Steud. dominated mine tailing wetland

5. Transpiration effect on the uranium retention via *Phragmites australis* Trin. ex Steud. in a U tailing site based wetland

Weiqing Wang, Matthias Webering, E. Gert Dudel
*Institute of Plant and Wood Chemistry, Dresden University of Technology, Pienner Strasse 19, D-01737 Tharandt, Germany*

Manuscript prepared for submission

**Abstract** Studies have suggested the positive role of transpiration increasing the aquatic metals/metalloids removal via vascular plant derived phytoremediation. However, the potential effect of transpiration on the aquatic U uptake by plant has not been fully revealed yet. Hence, a field investigation was conducted in a section of preferential U contaminated water pathway across a U mine tailing wetland. The section of water pathway was only slightly different in biogeochmical characteristics but partially shielded by *Salix* canopy. The transpiration rate (TR) of *Phragmites australis* Trin. ex Steud. was calculated from the continuously measured meteorological parameters and canopy data in the course of vegetation season. Two sites with different TR conditions (PS: 3.3±1.2 mm·d^{-1} vs. PL: 1.1±0.5 mm·d^{-1}) were respectively determined. The U contrations were measured in root, stem and leaves dry matter of *P. australis* collected in early developmental stage (DS1) in late of May and mature stage (DS2) in August. Relatively higher U concentrations in root were observed under higher TR condition in DS1 (PS: 12.9±1.7 mg·kg^{-1} vs. PL: 8.4±1.6 mg·kg^{-1}) and DS2 (PS: 21.8±3.1 mg·kg^{-1} vs. PL: 10.5±1.6 mg·kg^{-1}). The leaves was more preferential for U retention in above ground biomass of *P. australis* than stem. The U concentrations in leaves and stem were also increased under higher TR condition, expecially for young leaves in DS1 (high TR: 0.13±0.01 mg·kg^{-1} vs. low TR: 0.06±0.02 mg·kg^{-1}). However, they only accounted for less than 1% of corresponding U concentration in root, regradless of the TR variation. The results suggested the improved aquatic U retention efficiency in both above and below ground tissues of *P. australis* under higher TR condition. The different TR conditions did not changed the fact that the root of *P. australis* was dominant tissue contributing the aquatic U removal by *P. australis* via rhizofiltration. Furthermore, the comparative adventage of aquatic U rhizofiltration was extended along with the maturation of *P. australis* in the late of season.

**Keywords** Aquatic uranium, Transpiration, Rhizofiltration, Phytoextraction, Bio-concentration

**Acknowledgement** The authors would like to thank the support provided by Technical University of Dresden and China Scholarship Council for this study (Grant No. 2011671061). The authors also appreciate the assistance of the colleagues (particularly Dr. Arndt Weiske,
The Uranium (U) mining industry is currently developed worldwide to support the increased demand of purified U based nuclear power application. The U mining and ore processing usually produced considerable amount of wastes containing residual U and other contaminants in the running U mines and the tailing sites formed after the decommissioning of these mines. The buried and exposed U in these deposit sites continually contribute the high radiation dosage to surroundings (Carvalho et al., 2007; Lottermoser and Ashley 2006). Moreover, the chemical toxicity of U is also critical to ecosystem, especially to the human health (Domingo 2001). Comparing with the other radionuclides (e.g. Ra), the U is relatively mobilized in the environment (Nassour et al., 2015). The environmental risk of U can be further exported and expanded through the migration of aquatic U from its deposit site (e.g. tailing site) to surroundings along with the surface and underground water pathway (Lienert et al., 1994; Sobakin et al., 2015). It results in the higher content of U in water than the recommended level, even by hundreds of times in some cases (Kurtio et al., 2002). The high content of U in water, especially the drinking water source, is harmful to surrounding population and necessary to be diminished to the safety level. (Orloff et al., 2004).

The phytoremediation has been considered a possible option to effectively and economically control the aquatic U contamination. It is a passive and environment-friendly biotechnology contributed by different sub-technologies, including phytoextraction, phytovolatilization, phytostabilisation, phytodegradation and rhizofiltration (Dushenkov 2003). The aquatic contaminants (e.g. metals/metalloids) can be uptake and retained within the plant tissues (root, shoot and leaves etc.) through these sub-technologies. The phytoremediation technology is also confirmed feasible for the aquatic U removal in both terrestrial and aquatic environments by various plant species. Study on the terrestrial Armoracia rusticana showed that it retained 86-98% of aquatic U from solution (Soudek et al., 2011). Favas et al. (2014) have indicated that sorts of submerged, free-floating and rooted emergent plants are capable to remove high content of aquatic U from rhizosphere. The sub-technologies of phytoremediation are possibly have the discrepant effect on aquatic U removal coupled with different plant species and environments. Studies on some terrestrial plant species (e.g. Helianthus annuus and Brassica juncea) showed high aquatic U retention capacity of their aboveground biomass through phytoextraction (Chang et al., 2005; Mihalik et al., 2010). Some plant species (e.g. Rorippa sylvestris) possibly have the strong aquatic U phytoextraction ability (Cordeiro et al., 2016). However, the phytoextraction dominated aquatic U removal is not common in aquatic environment. Instead, the rhizofiltration has been considered as a major sub-technology for
many aquatic plant species to retain the aquatic U (Vymazal et al., 2007). Pratas et al., (2014) have found that 56-85% of aquatic U could be retained by the root of some aquatic plant species (*Callitriche stagnalis*, *Potamogeton natans* and *Potamogeton pectinatus*) through rhizofiltration. Cordeiro et al. (2016) have also confirmed the higher aquatic U retention capacity for root of *Nasturtium officinale* than its other tissues. The root of some large emergent macrophytes (e.g. *Phragmites australis*) also capable to retain higher concentration of U than other tissues (Vymazal et al., 2007). However, it has not made the root of aquatic plant species the only tissue for aquatic U retention. The aquatic U is also retained in other tissues (Vymazal et al., 2007). It is possibly a result of root to stem translocation of U (Straczek et al., 2010).

The transpiration of plant has been considered as a crucial physiological mechanism regulating the aquatic elements root uptake and the related root to stem translocation. The transpiration derived water loss from the leaves further produced high demand for root water uptake (Green et al., 2006). It is beneficial for the rhizofiltration of aquatic contaminants (Cd, Zn and Cu etc.) by sorts of plant species (e.g. *Triticum aestivum* and *Fagopyrum esculentum*) (Salah and Barrington 2006; Tani and Barrington 2005). Furthermore, the transpiration derived water loss also increase the hydraulic gradient and the related root to stem water redistribution inside the plant (Green et al., 2006). It also possibly benefited the root to stem translocation of aquatic metals/metalloids. Lai (2015) has found that the aquatic Cd accumulation in stem of *Impatiens walleriana* was positively increased under high transpiration rate (TR) condition. However, the most of current evidences are still from the studies on the terrestrial plant in natural and lab (e.g. hydroponics) conditions. The potential effect of transpiration on the phytoremediation of aquatic plant species, especially on the emergent macrophytes, is still largely unknown. Furthermore, the aquatic U removal via phytoremediation technologies under the influence of transpiration has also not been paid enough concern. Aranjuelo et al. (2014) have studied the aquatic U uptake by plant coupled with the changed TR conditions and indicated the positive correlation between the leaf U content and leaf transpiration. However, this investigation has been conducted on the terrestrial *Arabidopsis* cultured by the solution of uranyl nitrate under the controlled extreme laboratory condition. The evidence from this study was not enough to understand the real performance of aquatic plant species, especially in natural environment.

For now, the performance of transpiration assisting the aquatic U translocation from rhizosphere to plant and the related phytoremediation efficiency (e.g. phytoextraction and rhizofiltration) is still larger unknown. It was hypothesized that the different TR conditions have the potential to affect the rhizofiltration derived aquatic U retention. It further affect the root to stem U translocation and related phytoextraction efficiency. To verify these hypotheses,
5. Transpiration effect on the uranium retention via Phragmites australis Trin ex Steud. in a U tailing site based wetland

A field investigation was conducted in a Phragmites australis wetland based on a U mine tailing site. The certain U retention in roots and above ground biomass was characterized under different TR conditions.

5.2 Material and methods

5.2.1 Study site and sampling

The field investigation was conducted in a U tailing site located in the decommissioned U mine and ore processing site in Mechelgrün-Zobes, near village Neuensalz, Germany. A wetland dominated by P. australis was gradually formed since the late 1970s (Fig 5.1).

![Figure 5.1](image1.png)

**Fig 5.1** Description of sampling sites in the reed bad wetland in Neuensalz-Zobes, Western Ore Mountains

The wetland is continuously fed by the U contaminated leachate water from flooded mine in the eastern part. It was collected by the preferential surface water pathway in the northern part of wetland and transported to the western tailing pond. The wetland was hydrologically decoupled from the surrounding landscape with stabilized water level by the bypass channel since 2002/2003. The P. australis stand in northwestern wetland was shaded by the canopy of Salix species in this region. Two sampling sites (completed open PS and shaded PL) were assigned aside the edge of the shadowed area in a distance of approx. 20 m. The daily temperature and humidity for TR calculation were automatically recorded in situ. The complete meteorological data set for the vegetation period was provided by the nearby measuring station of the German Weather Service in Plauen-Ost. The interstitial water in rhizosphere was biweekly collected through 0.45µm syringe filters (Roth GmbH, Germany) and stored in the PE vessels. The pH and redox state values in water were determined by the pH/ORP meter (TM-39 meter, Fa. Sensortechnik, Germany). The DO concentration and conductivity was determined by the oximeter (LF95 bzw. Oxymeter, Fa. WTW, Germany). In order to avoid the interference from water quality change during the transportation, all parameters were directly measured in situ using the glass electrode detector before interstitial water sampling and the plant biomass harvest was carried out. The P. australis stand was
harvested in two selected developmental stages in 2013. The first stage (DS1) was in the end of May, when the *P. australis* was at the early stage of development without the significant difference in two sites (average stalk length 1.4 cm in PS and 1.2 cm in PL). The second stage (DS2) was in the beginning of August, when the *P. australis* was about to fully mature in different levels (average stalk length 3.6 m in PS and 2.4 m in PL). Five culms of *P. australis* were randomly collected in 1 m$^2$ zone with the intact above and below ground tissues. The water and intact plant samples were transferred to lab and stored cool (4-6°C) for further analysis.

5.2.2 TR calculation

TR was calculated according to the Priestley-Taylor formula as Eq. (5.1), which is proved effective in former practices (Lenters et al., 2011; Rosenberry et al., 2004). By this formula, the evaporation is calculated as a potential transpiration. However, the proportion of this part is negligible and can be set to the actual transpiration based on the permanent water-saturated condition (Sanchez-Carrillo et al., 2001).

$$TR = \zeta \frac{s}{s+\gamma} \left( R_n - G/L \right) 10000$$ (5.1)

Where, $\zeta$ is the Priestley-Taylor constant equal to 1.26. $s$ is the slope of the saturation vapor pressure curve. $\gamma$ is the psychrometer constant equal to 0.66 hPa·K$^{-1}$. $R_n$ [J·cm$^{-2}$·d$^{-1}$] is the net radiation. $G$ [J·cm$^{-2}$·d$^{-1}$] is the soil heat flux. $L$ [MJ·kg$^{-1}$] is the Evaporation heat of water.

The Saturated vapor pressure ($e_s$) was calculated by Magnus Formula as Eq. (5.2) (Lawrence 2005).

$$e_s = 6.11 hPa \cdot e^{\frac{17.62T}{243.12+T}}$$ (5.2)

Where, $T$ [°C] is the mean value of daily temperature recorded by the temperature-humidity logger installed 1m above ground at PS and PL.

The $R_n$ was obtained from the balance of the shortwave and long-wave solar terrestrial radiation as Eq. (5.3).

$$R_n = (1-\alpha)R_G - R_{nl}$$ (5.3)

Where, $\alpha$ is the albedo equal to 0.21 in this study. $R_G$ [J·cm$^{-2}$·d$^{-1}$] is the global radiation. $R_{nl}$ [J·cm$^{-2}$·d$^{-1}$] is the long-wave net radiation.

The $R_G$ was calculated based on the sunshine duration in sampling sites (recorded by the nearby weather station) as Eq. (5.4) (Allen et al., 1998).
5. Transpiration effect on the uranium retention via *Phragmites australis* Trin ex Steud. in a U
tailing site based wetland

\[
R_d = (0.19 + 0.55 \frac{S_d}{S_0}) R_{ex} \quad (5.4)
\]

Where, \(S_d \) [h] is the sunshine hours. \(S_0 \) [h] is the astronomically possible duration of sunshine. \(R_{ex} \) [J·cm\(^{-2}\)·d\(^{-1}\)] is the extraterrestrial radiation.

Two different formulas were applied for \(R_{nl} \) calculation. The \(R_{nl} \) of PS was calculated by FAO Standard formula as Eq. (5.5) (Allen 2000).

\[
R_{nl} = \sigma \left[ \frac{T_{max}^4 + T_{min}^4}{2} \right] (0.34 - 0.14 \sqrt{e_a}) \left( 1.35 \frac{R_{G0}}{R_{G0}} - 0.35 \right) \quad (5.5)
\]

Where, \(\sigma \) is the Stefan-Boltzmann constant equal to \(4.9 \times 10^{-7} \) J·cm\(^{-2}\)·d\(^{-1}\)·K\(^{-4}\). \(T_{max} \) [K] is the maximum daily temperature. \(T_{min} \) [K] is the minimum daily temperature. \(e_a \) [hPa] is the vapor pressure. \(R_{G0} \) [J·cm\(^{-2}\)·d\(^{-1}\)] is the shortwave radiation.

However, Eq. (5.5) was not suitable for the \(R_{nl} \) of PL calculation, because of the canopy absorption effect from the trees. In this case, Eq. (5.6) was chosen to get the reliable value (Black *et al.*, 1991).

\[
R_{nl} = \left[ (1 - \beta) \left( \frac{R_{G0}^2}{R_{G0}^2} + \left( 1 - \frac{R_{G0}^2}{R_{G0}^2} \right) \right) + (\beta - 1) \right] \sigma T_{K}^4 \quad (5.6)
\]

Where, \(e_a \) is the emissivity of the atmosphere. \(B \) is the ratio of the shortwave radiation above and below the canopy using a canopy analyzer system (Sunscan SS1-COM, Delta T devices, Cambridge, UK).

5.2.3 Total digestion and chemical analysis

The harvested *P. australis* was separated to root, stem and leaves blade. Each part of samples were rinsed with deionized water to remove the residual subhydric soil particles on their surface. They were then respectively divided to suitable portions and fully mixed. The prepared samples of each part were oven dried to constant weight at 60℃. The dried leaves, stem and root samples were powered in the centrifugal grinder (ZM1000, Retsch GmbH, Haan, Germany) at 10000 g. Approx. 200 mg stem and leaves samples and 100 mg root samples were respectively weighed into the tube and digested with 3 ml 65% (w/v) HNO\(_3\) and 2 ml 30% (w/v) H\(_2\)O\(_2\) at 180℃ for 2 h (Mars 5, Fa. CEM Corp.). The result solution was made to 20 ml with deionized water. The fresh water and digested solutions were analyzed on the inductively coupled plasma mass spectrometry (ICP-MS, PQ 2+, Thermo Elemental, USA)
5.3 Results

to determine the concentration of U and other elements.

5.2.4 Statistical analysis

All statistical analysis of data was performed with SPSS statistics software (IBM SPSS, Chicago, IL, USA) and Excel 2010 software. All concentrations in solid samples were based on the dry mass.

5.3 Results

5.3.1 Water chemistry

The similar biogeochemistry characteristics were observed in PS and PL along with the preferential surface water pathway (Table 5.1).

Table 5.1 Mean values of biogeochemical conditions in water pathway. The monitoring data was collected from May to August, n=6, the error means ±SD

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>DO [mg·L⁻¹]</th>
<th>Redox state [mV]</th>
<th>Conductivity [μs·cm⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>7.8±0.2</td>
<td>6.9±1.1</td>
<td>247.5±32.4</td>
<td>1009.2±316.8</td>
</tr>
<tr>
<td>PL</td>
<td>7.6±0.2</td>
<td>6.2±0.9</td>
<td>226.5±34.4</td>
<td>1245.7±528.5</td>
</tr>
</tbody>
</table>

The aquatic pH values maintained the neutral to slightly alkaline levels in both sites. The DO concentrations in both sites were at the oxic levels with small difference. The average DO concentration in PS was only slightly increased by 10.1% than the value in PL. Similar like the DO concentration variation, the average Eh value in PS was also slightly increased by 9.3% in PL. The average conductivity value in PS was surprisingly decreased by 19.0% than that in PL.

The aquatic U concentrations in both PS and PL were also fluctuated along with the time course during the investigation (Fig 5.2).
5. Transpiration effect on the uranium retention via Phragmites australis Trin ex Steud. in a U tailing site based wetland

![Graphs showing the concentration of U, Mg, Ca, and As over time in two sites from May to August.](image)

**Fig 5.2** Mean concentrations of aquatic U, Mg, Ca and As in water pathway of two sites from May to August, n=3, the error bar means ±SD

The average aquatic U concentration in PS during the whole investigation period was around 111.6±74.7 μg·l⁻¹ and 27.0% higher than the value in PL. The uneven distribution of aquatic U were observed in each site along with the time course. The initial aquatic U concentrations of two sites were similar (51.5±0.8 μg·l⁻¹ in PS and 50.0±1.0 μg·l⁻¹ in PL) and relatively lower in late of May (DS1), which were gradually increased in following days. The maximum aquatic U concentrations were observed in both sites around 50 days and respectively 4.7 times in PS and 3.3 times in PL higher than the initial values. Furthermore, the maximum aquatic U concentration in PS was significantly increased by 48.0% than in PL. The aquatic U concentrations in two site were diminished to lower level at the end of investigation in August (DS2) and similar to the initial values.

The change of water hardness (represented by aquatic Mg/Ca concentrations) showed the similar pattern comparing with U concentration variation in each site (Fig 5.2). The aquatic Ca and U concentrations were positively correlated in both sites ($R^2=0.8406$ in PS and 0.8382 in PL) during whole investigation. The similar positive correlation ($R^2=0.9956$ in PS and 0.9882 in PL) was also observed between the aquatic Mg and U concentrations in two sites during the same period. Aquatic As was also a major contaminant in both site. It maintained the high level during the most time of investigation and only experienced a short term decrease around 20 days.
5.3.2 Diurnal TR variation in shaded and complete open area

The transpiration of *P. australis* shared the similar fluctuation patterns along with the time course in PS and PL during the whole investigation (Fig 5.3).

![Graph showing diurnal TR variation](image)

**Fig 5.3** Daily transpiration of reed with and without the shield of canopy (PS: completely open area with strong sunlight; PL: shaded area with low sunlight)

The average daily TRs in two sites were relatively lower in the initial 10 days at the end of May and respectively around 1.8±0.6 mm·d\(^{-1}\) in PS and 0.5±0.2 mm·d\(^{-1}\) in PL. They were gradually increased in the following days and reached to peaks (5.7 mm·d\(^{-1}\) in PS and 1.8 mm·d\(^{-1}\) in PL) in two sites around 30 days at the end of June. Since then the daily TRs variation had maintained a fluctuating but relatively stable state in each site till the end of investigation. The daily TR of *P. australis* in PS maintain the relatively higher level during the whole investigation. The mean daily TR was around 3.3±1.2 mm·d\(^{-1}\) in PS and 3.0 times higher than that in PL. The total cumulative transpiration derived water loss (represented by water level) in PS was 234.6 mm during whole investigation and also 3.1 times higher than that in PL.

5.3.3 U retention in above ground tissues of *P. australis* under different TR conditions

The different U concentrations were observed in stem and leaves of *P. australis* collected from two sites in both developmental stages (Fig 5.4).
The U concentration in leaves of *P. australis* collected from PS were respectively 2.1 (DS1) and 1.8 (DS2) times higher than the leaves from PL at the same stage. The negligible difference on the stem retained U concentrations was observed between two sites at both stages. The U concentrations in stem from PS were slightly increased by 1.2% (DS1) and 24.5% (DS2) than stem from PL. Both the U concentrations in leaves and stem from two sites were decreased at the end of investigation. They were respectively decreased by for 74.7% (leaves) and 70.9% (stem) in PS from DS1 to DS2, while the ratios were 70.2% (leaves) and 76.4% (stem) in PL. However, the leaves from both sites shared the higher U concentrations than stem in both stages. The U concentrations in leaves from PS were respectively 3.5 (DS1) and 3.0 (DS2) times higher than in the corresponding stems. The U concentrations in leaves from PL were also respectively 1.7(DS1) and 2.1(DS2) times higher than in stems.

The difference on U retention ability in above ground tissues of *P. australis* also showed on the certain bio-concentration factors (BCFs) calculated by U concentrations in water (in mg·l⁻¹) and plant (in mg·kg⁻¹). The BCFs of leaves in PS were 2.2 (DS1) and 0.6 (DS2) in two stages. They were respectively 2.3 and 1.5 times higher than the corresponding BCFs of leaves in PL in each stage. The BCFs of stem in PS were lower than leaves, which were only 0.9 (DS1) and 0.4 (DS2) at two stages. They were also 1.7 and 2.4 times higher than the corresponding BCFs of stem in PL.
5.3.4 U retention in root of *P. australis* under different TR conditions

The roots of *P. australis* from both sites were also observed retaining U with the discrepant concentrations in both stages (Fig 5.5).

![Graph showing U concentration in roots harvested in two developmental stages of *P. australis*, n=3, the error means ±SD (PS-R: root harvested in completely open area; PL-R: root harvested in shaded area).]

The U concentrations in roots collected from PS were respectively 1.5 (DS1) and 2.1 (DS2) times higher comparing than the roots from PL in each stage. The root retained U concentrations in both sites were relatively lower in DS1. They respectively accounted for 59.3% (PS) and 80.0% (PL) of the U concentrations in roots from same site in DS2. The aquatic U BCF of root in PS was 214.8 in DS1 and 1.7 times higher than the corresponding value of root in PL. It was increased by 72.6% in PS from DS1 to DS2 and also still 1.8 times higher than that in PL in DS2. The root/leaves U concentration ratios in PS and PL were respectively 99.4 and 135.1 in DS1, then significantly increased to 662.9 and 567.4 in DS2. The root/stem U concentration ratio showed the similar pattern. They were respectively 349.9 (PS) and 229.8 (PL) in DS1, then increased to 2028.2 (PS) and 1215.1 (PL) in DS2.

5.4 Discussion

The results have suggested that the water pathway across two sampling sites maintained the natural to alkaline and highly aerated conditions (Table 5.1). The natural to alkaline water was beneficial to sustain the stable presence of soluble U(VI) compounds (*e.g.* uranyl carbonate) (Banyai *et al.*, 1995). The strong oxidation ability derived from high concentrations of DO and other possible oxidizers (*e.g.* nitrate) further promoted the biotic and abiotic oxidation of...
U(IV) to soluble U(VI) compounds. The uranyl carbonates in natural to alkaline water were highly possible to incorporate with Ca and Mg as aquatic Ca-U-CO$_3$ and Mg-U-CO$_3$ complexes (Bernhard et al., 2001; Endrizzi and Rao 2014). The aquatic U availability in form of these complexes was then positively encouraged by the increased water hardness related Mg and Ca contents (Fig 5.2). Consequently, the certain biogeochemical characteristics ensured the reliable aquatic U availability for plant uptake under the different transpiration conditions.

The results have indicated the discrepant transpiration efficiencies of *P. australis* between the *Salix* species shaded area and completed open area. The solar radiation was an effective mediator regulating the leaf transpiration (Fang et al., 2016). The canopy of *Salix* species had the potential to reduce the solar radiation dosage reached to the lower *P. australis* layer. The diminished solar radiation absorbed by *P. australis* possibly decreased its leaf hydraulic conductance and was adverse for the transpiration efficiency improvement on leaf surface (McJannet et al., 2007; Scoffoni et al., 2008). It consequently resulted in the relatively lower TR and related water loss from leaves of *P. australis* in *Salix* species partially shaded area than in the complete open area (Fig 5.3). The weak transpiration derived water loss diminished the force for the passive root water uptake and root to stem water redistribution (Green et al., 2006; Renkema et al., 2012). It further caused the potential adverse effect to the aquatic U uptake and retention in different tissues of *P. australis* along with the mass flow.

Aquatic U was retained in both stem and leaves of *P. australis*. The U concentration in leaves of *P. australis* was higher than in stem under the different TR conditions (Fig 5.4). It suggested that the leaves of *P. australis* were more efficient for aquatic U retention than stem. This result was similar to the observation on the distribution of other metals retained by *P. australis* (Vymazal et al., 2007). The leaves of *P. australis* was as a crucial (possibly terminal) storage tissue to accept the aquatic U delivered from root via vascular bundle. In this case, the change of TR conditions was possible to affect the certain U retention efficiency in leaves and also in stem of *P. australis*. The high TR condition resulted in the strong water loss from the leaves of *P. australis* from May to August (Fig 5.3). The transpiration derived dehydration in leaves potentially formed the leaves-stem-root hydraulic gradient and related transpirational pull (Howard et al., 2009; Levitt 1956). The transpirational pull was a crucial force passively redistributing water with aquatic elements (nutrients and non-essential elements) from root to above ground tissues. The high TR condition thusly enhanced the transpirational pull force and related mass flow delivery efficiency in the vascular system of *P. australis*. It benefited the aquatic U translocation from root to stem and eventually to leaves of *P. australis* together with the water redistribution. The enhanced transpirational pull under high TR condition thusly improved aquatic U bio-concentration efficiency of leaves.
The negligible difference on the U concentrations was observed between stems of *P. australis* developed under different TR conditions, especially in their early stage (Fig 5.4). However, the BCF results from both developmental stages still suggested that the aquatic U bio-concentration ability of stem was improved under the high TR condition. It could also be the result of enhanced U upward translocation from root through the high TR derived strong transpirational pull. However, the certain BCF of stem was still lower than the corresponding values of leaves, regardless of TR variation. It further confirmed that the leaves of *P. australis* were more effective retaining the aquatic U than the stem. In conclusion, the strong transpiration had the potential to improve the aquatic U retention through the *P. australis* derived phytoextraction in both leaves and stem. Furthermore, the leaves of *P. australis* were supposed to be more effective for phytoextraction of aquatic U than stem, regardless the different TR conditions and developmental stages.

The aquatic U retention via rhizofiltration of *P. australis* was also effective under different TR conditions and developmental stages (Fig 5.5). However, the certain rhizofiltration efficiency was discrepant under the different TR conditions. The stronger transpirational pull was capable to improve the passive root water uptake efficiency (Green *et al.*, 2006). Meanwhile, it also benefited the aquatic (essential and non-essential) elements translocation from rhizosphere to root along with the mass flow (Kashem and Singh 2002). By this means, the aquatic U uptake and retention by root was encouraged by the higher TR of *P. australis*. It consequently enhanced the aquatic U bio-concentration ability of root. On the other hand, the U concentrations in leaves and stem were only accounted for less than 1% than in root, regardless of the TR variation (Fig 5.4 and Fig 5.5). It suggested that the root to stem U translocation was detectable but weak for *P. australis*, even under higher TR condition.

The stronger solar radiation derived high TR of *P. australis* was coupled with an enhanced photosynthesis rate (PR) of leaves (Tuzet *et al.*, 2003). High PR improved the root aeration derived radial oxygen loss (Lai *et al.*, 2012). It thusly increased the oxygen supply and related oxidation ability in root zone, which was crucial for the formation of some aquatic U affinitive complexation mediators (*e.g.* Fe plaque) (Tian *et al.*, 2015). The coupled high TR and PR also improved the root to leaves CO₂ translocation and related organic carbon (OC) assimilation in leaves (Tuzet *et al.*, 2003). The high productivity of OC in leaves further improved the C supply for root/bacterial respiration in rhizosphere. It was possibly beneficial for the bacteria (*e.g.* Fe(II) oxidizing bacteria) driven aquatic U retention on root surface (Yoshida *et al.*, 2008). The possible complexation mediators (*e.g.* Fe plaque) then retained considerable amount of aquatic U (even most of it in some cases) on the root surface of *P. australis* (Chang *et al.*, 2014; Wang *et al.*, 2015). They were crucial for the aquatic U rhizofiltration, but by retaining it outside the real root tissue. This part of U was not available
for root to stem delivery. The aquatic U retention in above ground stem and leaves was then consequently limited for this reason.

The rhizofiltration efficiency was further enhanced from the early developmental stage in June to the nearly mature stage in August (Fig 5.5). It was possibly caused by continuously uptake of the aquatic U in rhizosphere (Fig 5.2). On the other hand, the fully developed suberized and lignified root biomass of mature \textit{P. australis} also improved its capacity to retaining more aquatic U from the rhizosphere. The potentially extended root (mainly newly developed young root) surface area of \textit{P. australis} was also capable retaining more aquatic U within high contents of complexation mediators (Jiang \textit{et al.}, 2009). The increased aquatic U content retained by mature root, especially by the mediators on root surface, further limited its upward translocation to above ground tissues. Meanwhile, the maturation of above ground tissues was also accompanied with the increased biomass accumulation. It was presumed that the limited aquatic U supply and increased dry matter assimilation in matured leaves and stem possibly decreased the proportions of U in these tissues.

In conclusion, the aquatic U uptake and retention by root of \textit{P. australis} was also enhanced when TR was increased. Furthermore, the root was “technically” the major tissue supporting the aquatic U removal by \textit{P. australis} via rhizofiltration. It was also a crucial barrier keeping the aquatic U phytoextraction in significantly lower level.

5.5 Conclusions

The results of this investigation revealed the positive effect of transpiration on the aquatic U removal via \textit{P. australis} derived phytoremediation. The aquatic U retention in root, stem and leaves of \textit{P. australis} was both enhanced under high TR condition. The root was the major and crucial tissue contributing the aquatic U retention by \textit{P. australis}, regardless of the changed transpiration condition. It was an effective barrier resulted in the relatively negligible aquatic U bio-concentration efficiency for above ground leaves and stem of \textit{P. australis}. The comparative advantage of rhizofiltration was further extended when \textit{P. australis} was nearly mature in August. The fully developed root in mature \textit{P. australis} possibly increased the aquatic U bio-concentration capacity via rhizofiltration. The limited root to stem translocation of U might not match to the biomass accumulation during the maturation of leaves and stem. However, the aquatic U bio-concentration in leaves and stem of \textit{P. australis} was still benefited from the high TR condition. It allowed more aquatic U upward transferred from root via vascular bundle of \textit{P. australis}. Furthermore, the upward transferred U was preferentially retained in leaves of \textit{P. australis} with relatively higher bio-concentration ability than stem. It made the leaves of \textit{P. australis} the crucial and more effective tissue contributing the aquatic U phytoextraction. However, there were still some certain topics from this study needed to be
further researched: The concentration and related BCF values represented the aquatic U retention efficiency via different tissues of *P. australis*. However, it might not enough to estimate the real gross capacity of *P. australis* retaining the aquatic U when the biomass production was not involved. The biomass accumulation of *P. australis* was possibly affected by the availability of different nutrients (e.g. N inform of nitrate or ammonium) under changed TR conditions. Furthermore, the certain effect of transpiration on the aquatic U retention with some mediators (e.g. Fe plaque) was also not detailed showed in this field study. In this case, it was necessary to study on the coupled influence of nutrition condition and transpiration on the biomass related U removal by *P. australis*, especially by rhizofiltration in aquatic environment under more strength experimental controlled conditions.

5.6 References


5. Transpiration effect on the uranium retention via Phragmites australis Trin ex Steud. in a U
 tailing site based wetland


5. Transpiration effect on the uranium retention via *Phragmites australis* Trin ex Steud. in a U tailing site based wetland


Weiqing Wang, E. Gert Dudel

*Institute of Plant and Wood Chemistry, Dresden University of Technology, Pienner Strasse 19, D-01737 Tharandt, Germany*

Published in: *Journal of Environmental Radioactivity*, 2018, 181C: 138-146.

Article link address: [https://doi.org/10.1016/j.jenvrad.2017.11.011](https://doi.org/10.1016/j.jenvrad.2017.11.011)
7. Summary and conclusion

The macrophytes have the ability accumulating multiple metals/metalloids species from the terrestrial and aquatic environments. The environmental-friendly phytoremediation technologies via these plant species have been applied for non-degradable pollutants removal. The macrophytes derived rhizofiltration is a major and efficient technology for metals/metalloids removal, especially in aquatic environments (e.g. wetland).

Comparing with the common metals/metalloids often studied, aquatic U rhizofiltration via macrophytes has been just considered recently. In this study, the field investigation in a U tailing basin wetland showed that the rhizofiltration was crucial for aquatic U retention via *Phragmites australis* Trin ex Steud. (water to root bioconcentration factor (BCF): 670 to 1556). The aquatic U retention efficiency in aboveground biomass of *P. australis* was insufficient (BCF: 0.4 to 5.3), comparing with the rhizofiltration. However, the high productivity (1.2 to 1.9 kg·m\(^{-2}\) per growing season) of *P. australis* still resulted in a notable yearly U accumulation in the areal total aboveground biomass (0.04 to 0.35 mg·m\(^{-2}\) per growing season). It was potentially promoted by the enhanced aquatic U rhizofiltration. The U within aboveground biomass could be released to submerse soil with the degradable or recalcitrant fallen litters. It enhanced the organic carbon supply in rhizosphere together with the root litter, and potential water to root U translocation within mobilized organic compounds. Hence the rhizofiltration stood in the crucial position of the plant-litter-water-soil U recycling in aquatic environment.

The results from field investigation and mesocosm experiment further suggested that the Fe plaque (IP) on root surface was crucial for aquatic U rhizofiltration. The IP contained most of root retained U in both environments (proportion of U within IP: 55.8 to 82.6% in field and 66.7 to 86.0% in mesocosm). However, the efficiency of IP assisted aquatic U rhizofiltration was affected by the redox state gradient (-179 to 220 mV) related redox processes. Field investigation suggested that high content of dissolved oxygen (up to 8.2 mg·l\(^{-1}\)) was capable to rapidly oxidize soluble Fe(II) as sparingly soluble Fe(III) oxides precipitated in subhydric soil. It consequently limited the aquatic Fe availability for root uptake and precipitation as IP. However, the strong oxidation ability also relatively increased aquatic U(VI) availability incorporated with inorganics and degradable organic matters. It was adverse for controlling the aquatic U concentration (66.7 to 92.0 μg·l\(^{-1}\) in field). On the other hand, it also benefited the U uptake by inner root tissue and upward translocation to aboveground biomass of *P. australis*.

The different inorganic N species also significantly influenced IP assisted aquatic U
rhizofiltration. The aquatic NH$_4^+$ sustained the reduction and acidification (via nitrification) potential for Fe(III) and U(VI) bioreduction in rhizosphere (-87 to 21 mV in NH$_4^+$ cultured mesocosm pots). It improved the root uptake (mainly within IP) of Fe and U (2992.9 to 5010.7 mg·kg$^{-1}$ Fe and 45.7 to 62.8 mg·kg$^{-1}$ U in NH$_4^+$ cultured root). On the contrary, the NO$_3^-$ depended strong oxidation ability (23 to 224 mV in NO$_3^-$ cultured mesocosm pots) inhibited the IP formation and the related aquatic U rhizofiltration efficiency (1568.5 to 2569.5 mg·kg$^{-1}$ Fe and 26.2 to 49.6 mg·kg$^{-1}$ U in NO$_3^-$ cultured root). The aquatic U availability in rhizosphere was also increased via NO$_3^-$ depended oxidation processes (aquatic U concentration in mesocosm: 1.6 to 589.3 μg·L$^{-1}$ (NO$_3^-$) vs. 1.4 to 58.2 μg·L$^{-1}$ (NH$_4^+$)). The sufficient nitrogen supply is also a significant driving force for high biomass productivity of _P. australis_. The higher biomass of _P. australis_ increased the U accumulation capacity for root and aboveground tissues. The nitrogen related high biomass accumulation of _P. australis_ also potentially enhanced the share of organic bound U in subhydric soil via plant litters supply.

The IP assisted aquatic U rhizofiltration was also affected by the co-existing metals/metalloids in rhizosphere. The field investigation indicated that high As availability (aquatic As/U ratio: 0.7 to 1.6) inhibited the U retention within IP through the competitive absorption, due to its high affinity to IP. The Ca improved the aquatic U(VI) availability by forming the soluble Ca-uranyl-carbonate compounds. The Ca also potentially competed with hydrated Fe(III) oxides within IP by incorporating with U and encourage the U retention within inner root tissue. The P was beneficial for U retention within IP possibly in form of U-Fe-phosphate complexes. However, it was still need to be proofed in further studies.

Despite of the biogeochemical conditions in rhizosphere, the aboveground transpiration of _P. australis_ also affected the IP formation and related aquatic U rhizofiltration. The higher transpiration rate (TR) of _P. australis_ (3.3±1.2 mm·d$^{-1}$ in field, 4.5±2.0 mm·d$^{-1}$ (NH$_4^+$)/5.0±2.2 mm·d$^{-1}$(NO$_3^-$) in mesocosm) increased the aquatic nutrient/non-essential elements availability for root uptake. For this reason, the aquatic U rhizofiltration of _P. australis_ (21.8±3.1 mg·kg$^{-1}$ in field, 62.1±1.0 mg·kg$^{-1}$ (NH$_4^+$)/47.6±1.8 mg·kg$^{-1}$ (NO$_3^-$) in mesocosm) was enhanced under higher TR. The higher TR also promoted the formation of IP and its U retention capacity. Furthermore, the U translocation from root to above ground biomass (mainly in leaves) of _P. australis_ was also enhanced under higher TR. It was potentially benefited by the increased transpirational pull and root uptake of other active mediator (e.g. Ca). The effect of transpiration was also coupled with the different N species on IP assisted aquatic U rhizofiltration. The higher TR depended strong root uptake and assimilation of N increased the biomass accumulation of _P. australis_. Furthermore, the higher TR also potentially increased the share of root in biomass partition of _P. australis_. Consequently, the stronger transpiration resulted in the higher aquatic U accumulation in area
related root biomass (up to 84.0±3.6 mg·m$^{-2}$ (NH$_4^+$) and 86.4±5.8 mg·m$^{-2}$ (NO$_3^-$) U per season in mesocosm).

In conclusion, it was possible for eutrophic *P. australis* stands to retain the aquatic U via rhizofiltration. The IP on root surface was a crucial mediator contributing the aquatic U rhizofiltration, especially in iron rich milieu. The efficiency of IP assisted aquatic U rhizofiltration could be further improved under suitable environmental conditions. In this study, these conditions might include: i) reductive rhizosphere environment with active reducers (*e.g.* NH$_4^+$) encouraging Fe(II) generation for IP formation and U retention within it; ii) limited competitive elements (*e.g.* As and Ca) co-existed with Fe and U in rhizosphere; iii) sufficient nutrients (*e.g.* N) supply and related high biomass productivity of plant; iv) strong transpiration effect improved the nutrient assimilation of root and also the aquatic U/Fe availability for root uptake. By adjusting these conditions (also include other potential factors not discussed in this study), an effective rhizofiltration technology was supposed to be applied for aquatic U removal.
Erklärung


Dresden, 02.12.2017                                                                 Weiqing Wang
Ort, Datum                                                                                   Unterschrift