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Influence of heavy metals in root chemistry of *Cyperus vaginatus* R.Br: A study through optical spectroscopy

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ABSTRACT

Phyto-sequestration of heavy metals originating from urban runoff entering into wetland systems is one of the ways to mitigate the pollution. Due to the chemical environment of runoff wetlands, the plants and especially their roots are often stressed. While growing on contaminated environment, wetland plants undergo a number of physio-chemical changes in their roots, stems and leaves in the form of ecological adaptations. In this study, the three category samples of the macrophyte *Cyperus vaginatus* R.Br. growing in a wetland, near to the wetland (<20 m) and far from the wetland (>100 m) were collected to determine heavy metals and organic matter status in roots. This study revealed hyperaccumulation of metals in plants growing in the wetland compared to nearby and far from the wetland. Statistical analysis showed metals accumulating pattern to be distinct for each sampling category based on location. The UV spectra of *C. vaginatus* root produced a distinct shoulder peak at 324 nm whereas the plants growing far from the wetland showed a spectroscopic shoulder at 267 nm. The plant root growing near to wetland showed both shoulders at 324 nm and 267 nm but with reduced peak height. Statistical analysis of UV spectra also followed location specificity pattern. UV spectral result was further validated by FTIR analysis where plants growing in wetland and near to wetland possessed spectra similar to UV spectral results. This observation leads us to conclude that *C. vaginatus* R.Br. exhibited changes in root chemical composition to tolerate metal toxicity and perform phytoaccumulation.

1. Introduction

Urban runoff is an important source of both inorganic and organic pollutants (Chong et al., 2011; Sidhu et al., 2013). Among these runoff pollutants, metals are considered to be potentially hazardous to plants and animals especially in urban wetlands (Obarska-Pempkowiak and Klimkowska, 1999; Otte, 2001; Cheng et al., 2002). Due to their persistency, metals often accumulate in runoff-receiving water bodies such as wetland well beyond the safety level (Nzengy'A and Wishitemi, 2000). However, metals such as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn)) are essential for specific metabolic responses in plant cells, where the elevated concentration of metals in soils and sediments cause hyperaccumulation in plants and sequestration of heavy metals into their biomass (Otte, 2001; Sabale et al., 2012).

Remediation of heavy metals using macrophytes in engineered wetlands has been proven to be an effective tool for reducing a range of pollutants that are often found in urban runoff (Obarska-Pempkowiak and Klimkowska, 1999; Karpiscak et al., 2001; Cheng et al., 2002; Vymazal and Březinová, 2015). Moreover, in terms of treating water quality, vegetated systems over wetlands are preferred over complex engineering solutions (Michailides et al., 2013; Sultana et al., 2015; Zhao et al., 2016). Selection of plant species for engineered wetland comprises the basis of healthy and meaningful wetland ecosystems (Davis and Froend, 1999). Several studies in metal polluted areas have demonstrated that vegetated wetlands can be successful in removing metals (Salt et al., 1995; Khan et al., 2009). As such studies indicate that metal uptake by plants usually decreases in the order roots > stems > leaves (Nirola et al., 2016) to lead us to investigate the root chemistry.

Uptake of metals by plants faster than they are metabolised or

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excreted creates stress that produce reactive oxygen species (ROS) such as $O_2^{\bullet -}$, $HO^{\bullet}$ and H_2O_2 (Desikan et al., 2004; Gratão et al., 2005; Sabale et al., 2012), which are potentially toxic to the plants and may change chemical composition within them to reduce plant stress level. (Adriano, 2001; Liu et al., 2007). For instance, Cruz-Orea et al. (2004) observed changes in UV shoulders as an indication of abiotic stress on *Cyperus hermaphroditus* when exposed to petroleum contaminated waste.

Our current study focuses on macrophyte *Cyperus vaginatus* R.Br. which is a common wetland species in South Australia. This species is well known for its resilience to overcome biotic and abiotic stress (Deegan et al., 2007). This genera has been well studied earlier in terms of its biomass, growth and wetland habitation (Chandra and Yadav, 2011; Akinbile et al., 2012; White and Lewis, 2012; Hou et al., 2016).

Optical spectroscopy such as Ultraviolet (UV) and fourier transform infrared (FTIR) are tools used to investigate the root chemistry to study optically active organic molecules. Several authors have reported the importance of UV absorption spectra as a qualitative tool to diagnose and discriminate natural organic substances (Vaillant et al., 2002; Kotti et al., 2007; Chang et al., 2009; Tsoumanis et al., 2010). The energies associated with UV (300–600 kJ/Mole) are sufficient to excite a molecular electron of organics to a higher energy orbital (McDonald, 2003). Fig. 1 shows various kinds of electronic excitation that may occur in organic molecules. Of the six transitions outlined, only the two lowest energy ones ($n \rightarrow \pi^*$ and$\pi \rightarrow \pi^*$) are achieved by energies available in the 190–400 nm spectra. Different organic molecules absorb photon energy at different wavelengths and are defined by the presence of certain functional groups called *chromophores*. FTIR spectroscopy is a commonly used technique to characterise principal chemical classes in plants and soil through the vibrational characteristics of their structural chemical bonds (Barber et al., 2001; Rusmin et al., 2015). Energy of mid infrared region 400–4000 cm^{-1} (2.5–50 μm) is sufficient to vibrate chemical bonds (stretching and bending) in molecule and generate unique fingerprint (Stuart, 2005).

This paper investigates the influence of metals in root chemistry of wetland plant *C. vaginatus* within and at various distances away from the wetland. The pH and physical property of soils including the total metal analysis of samples from wetland sediment as well as near to the wetlands are characterised to relate to the root behaviour pattern of *C. vaginatus*. A careful interpretation of UV and FTIR spectroscopy was conducted to study the peak levels to determine the impact of metals on organic compound behaviour.

2. Materials and methods

2.1. Site characteristics

Samples of the macrophyte *C. vaginatus* were collected from a wetland and its surrounding environment located in Mawson Lakes, Adelaide (34° 48′ 38″ S and 138° 37′ 22″ E), as shown in Fig. 2. Six samples were collected from the *wetland*, *near to the wetland* (<20 m away from the wetland) and *away from the wetland* (>100 m from the wetland) to represent different degrees of contamination in the collected samples. The wetland receives stormwater runoff from a nearby carpark, a road, and pavement areas as well as from surrounding vegetated areas. The area 20 m away from the wetland receives stormwater occasionally, especially during heavy rainfall periods. The third sampling location (>100 m from the wetland) does not receive any stormwater runoff.

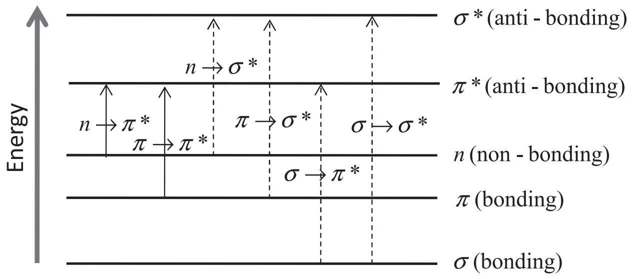

Fig. 1. Possible electronic transition.

2.2. Metals analysis

The root sections of the *C. vaginatus* were uprooted using plastic handy shovel and were placed in polyvinyl carbon bag. In the laboratory, the fresh root samples washed 10 times with Milli-Q water, then dried in an oven at 85 °C for 24 h and placed in a desiccator for 48 h. A 0.5 g of dry root mass was treated with 10 mL concentrated nitric acid (Ajax Finechem, Australia, 70% w/w) and then transferred in a Teflon vessel before digesting in a microwave (CEM-MAR6, USA) at 180 °C for 45 min. The digested extracts were filtered and diluted to make below 2% nitric acid level for analysis. The analysis was carried out using Inductively-coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7500ce, Agilent Technologies, Japan).

Soil/sediment samples collected from the sampling sites (using plastic shovel) were dried in oven at 105 °C for 24 h 0.5 g of soil/sediment sample was digested with concentrated nitric acid using microwave digester and diluted in a similar way mentioned above. Metals in the soil/sediment were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 5300V, USA). Nitric acid blank was prepared and measured in similar manner mentioned above for blank correction. The standard mixture of metals (ICP-AM-3, High Purity Standards, USA) was used for calibration. Blank controls were run after every 10 samples.

Soil/sediment pH was measured by submerging pH electrode (Eutech instruments PC510, Singapore) into the soil water suspension (prepared by adding 1.0 g soil in 10 mL distilled water and was shaken overnight and then centrifuged at 4000 rpm for10 min).

2.3. Optical spectra analysis

2.3.1. UV analysis

Dry root sample (0.1 g) was crushed and transferred to pyrex bottle containing 50 ml of 0.1 M NaOH solution (Aryal et al., 2014). The bottle was placed in an orbital shaker for 24 h and filtered through a 0.45 μm filter. The filtrate was analyzed using a UV spectrometer (MTech, China). The UV instrument was operated at a bandwidth of 1 nm with a quartz cell of 10 mm path length from 200 to 500 nm. The scanning speed was 190 nm/min (slow). The photometric accuracy was 0.004 Abs at 1.0 Abs.

As the absorbance of a sample is proportional to the number of absorbing molecules in the spectrometer light beam (e.g. their molar concentration in the sample tube), it is necessary to correct the absorbance value for this and other operational factors if the spectra of different compounds are to be compared in a meaningful way (Vaillant et al., 2002; Panda and Datta, 2008). To minimise the influence of concentration, the UV spectra were normalised as suggested by Vaillant et al. (2002). This was done by attributing the same area (norm) to all spectra for the chosen wavelength (190–400 nm). For a given spectrum and small acquisition step (1 nm), the area is: