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Metal spatial distribution assessment in *Phragmites sp.* treating pyrite mining acid drainage: X-ray Micro-CT, SEM-EDS and ICP-AES study

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Acid mine drainage (AMD) containing sulphate and heavy metals (HM), e.g., Fe, Mn, Cu and Zn in large quantities along with a high acidity and very low pH is a significant environmental problem throughout the world. The phytoremediation by constructed wetlands (CW) is being increasingly used to treat such contaminating discharges [1]. The present study was conducted to investigate HM removal efficiency from AMD of a pilot CW planted with *Phragmites sp*. in light expanded clay aggregates (Leca®). AMD was collected at Aljustrel pyrite mine (south Portugal - Iberian Pyrite Belt) retention basin. Role of vegetation in the system was assessed by HM concentration and distribution in the *Phragmites* tissues. Three main techniques were selected to analyse metal distribution: ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectroscopy), µCT (X-Ray Computed Micro-Tomography) and SEM-EDS (Scanning Electron Microscopy – Energy Dispersive Spectroscopy).

A pilot CW ($0.64m^2$ x 0.8m) was fed in a continuous vertical flow (VF) mode with AMD during a two years period under different operating conditions. Table 1 resumes inlet and outlet HM content of AMD fed to the VFCW in two selected periods. Inlet hydraulic loads of $50Lm^{-2}d^{-1}$ (Trial 8) and $100Lm^{-2}d^{-1}$ (Trial 10) were considered. Plant tissues' were sampled immediately after the trials. Plant tissues' from a VFCW fed with tap water was considered as control.HM were quantified by ICP-AES. The high Fe removal efficiencies along with a rocket increase of Fe content observed in the roots (Table 1) was intriguing, as plants did not show any sign of toxicity.

Tomographic perspectives of control and selected contaminated *Phragmites* roots' and rhizomes are show in Figure 1. Some opaque regions (minute white spots) are seen in the contaminated plants. The SEM-EDS results suggest the existence of local HM concentration, especially Fe, related to the sulphate acidic water. Control samples do not show the presence of the contamination metals (Fe, Mn, Zn, Cu). There are also differences in the metal distribution between different parts of the plant, as concluded by ICP-AES.

Fe plaques in the roots and nodular bodies inside of the xylem are seen in restricted regions (Fig. 2) [2][3]. Leafs are depleted in that metals (Fig. 2). Cu and Zn can also be accumulated in the roots but a more random distribution pattern is found. Combining both µCT and SEM-EDS results, it is possible to conclude that the present methodology is adequate to perform studies concerning the phytoremediation studies using hyper accumulative plants, as is the case of the *Phragmites* sp..

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Table 1. Performance of the VFCW planted with *Phragmites sp.* in Leca® treating an acid mine drainage under aerobic conditions. HM distribution in *Phragmites* leaf and root tissues. SO_4^{2-} correspond to soluble sulphates.

	Trial 8		Trial 10		Control		Trial 8		Trial 10	
	In	Out	In	Out	Root	Leaf	Root	Leaf	Root	Leaf
	mg/L		mg/L		mg/kg d.w.		mg/kg d.w.		mg/kg d.w.	
	260	84	230	47	2290	72	3372	203	9788	232
Mn	4.5	4.3	4.8	4.9	59.3	67.5	106	118	58.8	42.1
Cu	15.4	13.3	15.3	13.9	47.4	13.3	62.3	4.7	72.5	4.1
Zn	25.7	23.8	26.2	25.3	156	19.1	171	112	157	42.1
SO_4^{2-}	2107	1693	2776	2180	4370	7710	5300	26900	6500	3400
pH	2.6	2.8	2.5	2.8						

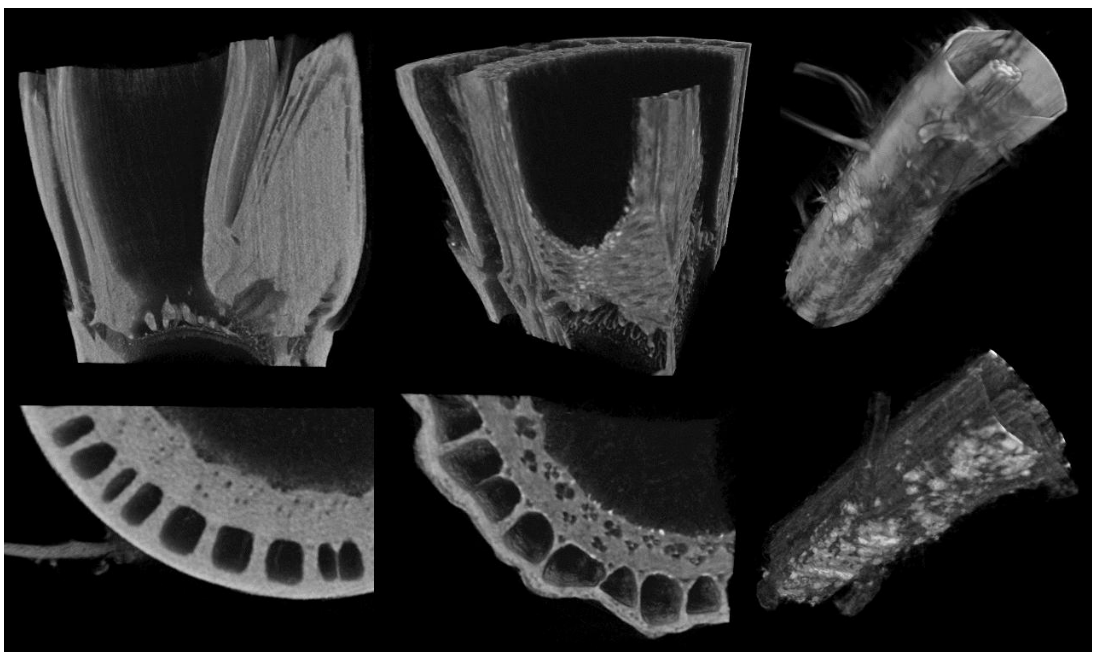

Figure 1 – Tomographic 3D models: control (left images) and trial 8 contaminated rhizomes (central images); control (top right image) and trial 8 contaminated root (bottom right image).

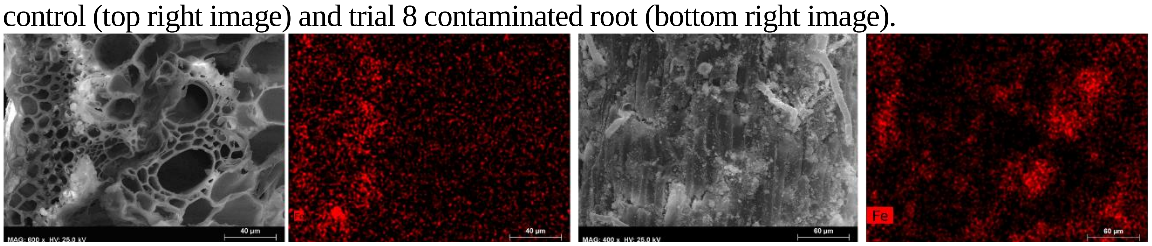

Figure 2 – SEM-EDS images: intra-rhizome Fe concentration (left) and Fe rich external plaques in the roots (right) from trial 10.

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