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Polishing of synthetic electroplating wastewater in microcosm upflow constructed wetlands: Metals removal mechanisms

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Abstract This paper presents the study of the metals removal mechanisms in microcosm upflow constructed wetlands used for polishing of synthetic electroplating wastewater. Four types of columns were used: with and without plants and with different bed media (peat or gravel). The main design goal of the columns with peat was to promote precipitation of metals with biogenic sulfides produced by sulfate-reducing bacteria. The feed of the system contained mainly Cu, Ni, Pb, Zn, sulfates and cyanides. The substrate from the microcosm was sampled after cessation of the experiment (53 weeks) from the bottom and top layers of selected columns and was analyzed using sequential extraction procedure and scanning electron microscopy-energy-dispersive X-ray spectroscopy method. Additionally, solids retained on filter surface after filtering water samples withdrawn from the water layer covering the bed media in selected columns were analyzed by the latter method. The obtained results showed that the intended process for metals removal, which was precipitation of metal sulfides, was responsible for binding only a minor fraction of metals. The major portion of metals was present as exchangeable and reducible fraction. The presented study suggests that the onset of the conditions favorable for precipitation of sulfides may not occur during short-term experiment. It may stem from the specific characteristics of the experiment. It is suggested that short-term studies should not be extrapolated to predict long-term behavior of the system.

Keywords electroplating wastewater, metals, cyanides, constructed wetlands, upflow, mechanism

Abbreviations

bot	bottom
BSE	backscattered electron (detector)
CW	constructed wetland
DW	dry weight
EDS	energy-dispersive X-ray spectroscopy
HRT	hydraulic retention time
SEM	scanning electron microscopy
SEP	sequential extraction procedure
SRB	sulfate-reducing bacteria
UF	upflow
UF-GP	vegetated upflow column with gravel
UF-GU	unvegetated upflow column with gravel
UF-PP	vegetated upflow column with peat
UF-PU	unvegetated upflow column with peat

1 Introduction

The concept of using constructed wetlands (CWs) for cost- and energy-efficient treatment of industrial wastewaters laden with metals has been demonstrated with a high degree of success in the world [1,2]. The application of CWs for the treatment of electroplating wastewater was reported scarcely in the literature. On the Web Of Knowledge [3] a query including ‘electroplating’ and ‘wetland*’ (as topics) yielded only 6 results. Five of which dealt with interactions between plants and metals, and one regarding mobility of Cr in natural wetlands. Noteworthy, none of these articles reported application of CWs for the treatment of electroplating wastewater. The challenge of treating or polishing electroplating wastewater in CWs is that it contains high concentrations of metals and cyanides (often on the order of hundreds of mg/L) and is deficient in organic matter [4]. Indeed, the treatment of raw electroplating wastewater appears unfeasible to the toxic effect on the plants and microorganisms and the necessity of frequent exchange of the bed medium due to exhaustion of its sorption properties [5]. Constructed wetlands can be used for the treatment (polishing) of pretreated electroplating wastewater, in which relatively low concentrations of metals and cyanides are present. Metals because of their nature, unlike cyanides or numerous organic compounds, cannot be degraded in CWs to simpler substances. For this reason, the retention mechanism of metallic pollutants is crucial as it determines their mobility in the system [6,2]. The removal of metals in subsurface-flow CWs is dominated by four mechanisms: adsorption,

precipitation of metal sulfides or (oxy)hydroxides, and deposition of suspended solids. It should be noted that a minor fraction of metals is removed by hydrophytes, which unlike terrestrial plants, cannot hyperaccumulate metals [7]. Precipitation of metals with biogenic sulfides is the crucial removal process because metal sulfides can remain in the sediments as long as anoxic conditions occur and solubility and mobility is very low as compared to other retention mechanisms [2]. This process is mediated by sulfate-reducing bacteria (SRB), whose growth requires: anoxic conditions, presence of simple organic compounds (as electron donors), sulfates (as electron acceptors) [8,9]. Considering the polishing of electroplating wastewater in CWs it can be stated that the required availability of sulfates can be easily met because this type of wastewater contains elevated concentrations of sulfates (added as sulfuric acid to adjust pH) [10]. Anoxic conditions can occur in the CWs with saturated bed media as horizontal-flow systems or vertical-flow system with saturated bed (e.g. upflow (UF) systems). A proper carbon source should be provided to cause other microorganisms to remove the oxygen from the environment but also to stimulate the growth of SRB [2]. The aforementioned carbon deficiency of electroplating wastewater necessitates the use of organic carbon sources for SRB when this wastewater is to be treated in CWs. Carbon source for SRB can be of internal or external origin. The latter can be added as aqueous solution to the wastewater and the former is present in the system as a solid form. Both types of carbon source can be used in CWs [11]. Constructed wetlands were found to efficiently remove metals by their precipitation with sulphides, but this process is dependent on numerous factors such as: type of wastewater, type of carbon source, retention time, maturity of the system etc. [5,12-14]. The predominant removal mechanisms should be evaluated for a specific case.

The efficiency of CWs for wastewater treatment is often based on influent-effluent comparison, in which the treatment system is regarded as a "black box" [2]. The knowledge of the major removal mechanisms allows prediction of the future performance of a system and its impact on the environment after its operation is terminated. Importantly, the information furnished by the small-scale studies could enhance the design of large-scale system by emphasizing those features of a system that promote the dominance of the most advantageous mechanism of removal.

The goal of the experiment presented in this paper was to study the removal mechanisms of metallic pollutants in microcosm upflow CWs polishing synthetic wastewater. The obtained results were also discussed in light of the treatment efficiency of the microcosm system

presented in [15]. The detailed description of the experimental system and the performance results were presented in [15].

2 Methods

2.1 Microcosm constructed wetlands

The experimental system included four types of microcosm upflow CWs depending on the type of bed media and the presence of vegetation: vegetated columns with an equivolume mixture of peat and gravel (hereafter 'peat') (UF-PP columns), unvegetated columns with peat (UF-PU), vegetated columns with gravel (UF-GP) and unvegetated columns with gravel (UF-GU). Each type of column was duplicated, therefore 8 columns were used in total. The goal of the columns with peat (UF-PP and UF-PU) was the removal of metals by their precipitation with biogenic sulfides produced by SRB. Peat was used to serve as a carbon source for SRB on the assumption of its hydrolysis and fermentation to produce compounds utilized by SRB (e.g. acetates or lactates). The columns with gravel were used as reference systems, to which no carbon was added, neither as solid material (e.g. peat) nor dissolved substances present in the feed, before week 44 of the experiment. In week 44 lactates were added to stimulate SRB in the gravel columns and to compare the effect of internal (peat) and external (lactates) carbon source on the performance of the microcosm CWs. The feed with lactates were added only to the gravel columns. The electron acceptor for SRB was sulfates present in the feed. The columns were fed for 53 weeks (the whole experiment) with synthetic electroplating wastewater. In the end of the experiment the substrate from the columns was analyzed to elucidate metal removal mechanisms. The composition of the feed was modified during the experiment and was presented in detail in [15]. The median composition of wastewater (with minimum - maximum values in the brackets) for the whole experiment (53 weeks) was as follows:

- For the feed without lactates: Cu 1.23 mg/L (0.16 – 20.58 mg/L), Ni 5.00 mg/L (0.99 – 6.75 mg/L), Pb 0.67 mg/L (0.28 – 3.95 mg/L), Zn 3.65 mg/L (1.20 – 16.80 mg/L), cyanide 1.04 mg/L (0.425 – 5.99 mg/L), sulfate 850 mg/L (720 – 855 mg/L);
- For the feed with lactates: Cu 2.65 mg/L (0.98 – 4.86 mg/L), Ni 5.83 mg/L (4.77 – 6.58 mg/L), Pb 0.78 mg/L (0.50 – 1.29 mg/L), Zn 6.27 mg/L (5.38 – 7.26 mg/L), cyanide 1.28 mg/L (0.40 – 5.69 mg/L), sulfate 855 mg/L (700 – 935 mg/L).

The feed without lactates was used during the whole experiment (weeks 1-53) as the influent of the columns with peat and in weeks 1-43 as the influent of the columns with gravel. In weeks 44-53 the feed with lactates was used as the influent of the columns with gravel. The results presented in this paper are for the planted columns with peat (UF-PP), planted columns with gravel (UF-GP), and to lesser extent for the unplanted columns filled with gravel (UF-GU). The UF-PU columns will not be further discussed in this article.

2.2 Sampling procedure

Substrate samples were taken from the bottom and top of columns after cessation of the experiment (53 weeks). It was assumed that these zones were represented by 150 mm layer from the bottom or top surface. The samples were then homogenized and subdivided into the subsamples for sequential extraction procedure (SEP) and scanning electron microscopy - energy-dispersive X-ray spectroscopy (SEM-EDS) analyses. The additional samples were: filters through which water layer over the bed medium of the UF-GU and UF-PP columns were filtered. To this end 1.5 L wastewater was withdrawn from the water layer of the UF-PP and UF-GU columns and was filtered through a 0.45 μm filter.

2.3 Sequential extraction procedure

The manner in which metals were bound to substrate in the experimental system was assessed by the SEP based on the BCR2 protocol [16]. This procedure consists of three steps devised to extract three operationally defined fractions. In the first step exchangeable, water- and acid-soluble fraction was targeted and 0.11 M acetic acid was used. The second step was to extract reducible phase by the use of 0.5 M hydroxylammonium hydrochloride at pH 1.5. The target phase in step 3 was reducible fraction and reagents used were 8.8 M H_2O_2 at 85°C and then 1 M ammonium acetate at pH 2.0. As an internal check on the procedure, the residue from step 3 was digested in *aqua regia* (step 4) and the amount of metals extracted (i.e., sum of step 1, step 2, step 3 and residue) was compared with that obtained by independent *aqua regia* digestion of a separate sample of the sediment. The SEP according to the BCR2 protocol is presented in Tab. 1 including operationally-defined fractions and the corresponding nominal target phases. Noteworthy, metals bound as sulfides are extracted from the analyzed substrate in step 3 and are regarded as oxidisable fraction.

Table 1 The BCR2 sequential extraction scheme (adapted from [16,17])

	Reagent	Fraction label	Nominal target phase(s)
Step 1	Acetic acid, 0.11 mol/L	Exchangeable, water and acid-soluble	Soluble and exchangeable cations and carbonates
Step 2	Hydroxylammonium hydrochloride 0.5 mol/L at pH 1.5	Reducible	Fe-Mn oxyhydroxides
Step 3	Hydrogen peroxide (85°C), 300 mg/g, 8.8 mol/L then ammonium acetate, 1.0 mol/L	Oxidisable	Organic matter and sulphides
Step 4	<i>Aqua regia</i> ¹	Residual	

¹Although not officially a step in sequential extraction, it is recommended that the residue at the end of Step 3 be digested with *aqua regia* and the sum of the four fractions be compared with the results of a separate *aqua regia* digestion of the sample

The samples were air-dried before analysis for 48 hours. The SEP was conducted with approx. 2 g of substrate accurately weighed in 150 mL polypropylene centrifuge tubes. The increased mass of samples (compared to the protocol) was taken because of their poor homogeneity due to the presence of coarse gravel. The extracting solutions were added in a volume twice as large as in the BCR2 protocol, with the exception of step 3. The *aqua regia* digestion was based on the ISO 11466:1995 norm [18] using 50 mL of the extracting solution. Each step (1–3) involved shaking the samples with extracting solution for 16 h on the end-over-end shaker. The extract from the solid residue was separated by centrifugation at 3000 g for 20 min and the supernatant was decanted into a PE container, filtered and stored at 4°C for analyses. The residue was washed by adding 20 mL of distilled water, shaking for 15 min, centrifuging for 20 min, and then discarding the supernatant. The amount of metals extracted in the independent *aqua regia* digestion was assumed to represent pseudo-total concentration of metals in substrate. The extracts were analyzed for Cu, Ni, Pb, Zn using flame atomic absorption spectroscopy (AA Scan 1 Thermo Jarrell Ash apparatus). The recoveries between the sum of steps 1-4 and single-step *aqua regia* digestion of substrate samples were: Cu 109.56%, Ni 129.92%, Pb 123.75%, Zn 101.84%.

2.4 Scanning electron microscopy-energy-dispersive X-ray spectroscopy

Surface morphology of the samples was carried out using a scanning electron microscope (SEM) HITACHI S-3400N with backscattered electron detector (BSE). The analysis of chemical composition of the samples was performed using energy-dispersive X-ray spectroscopy (EDS) Thermo Noran System (System Six). EDS analysis is a semi-quantitative method with the limit of detection of ca. 0.2 atomic % depending on an element. SEM imaging and chemical microanalysis were performed in a low vacuum (50 Pa) and at

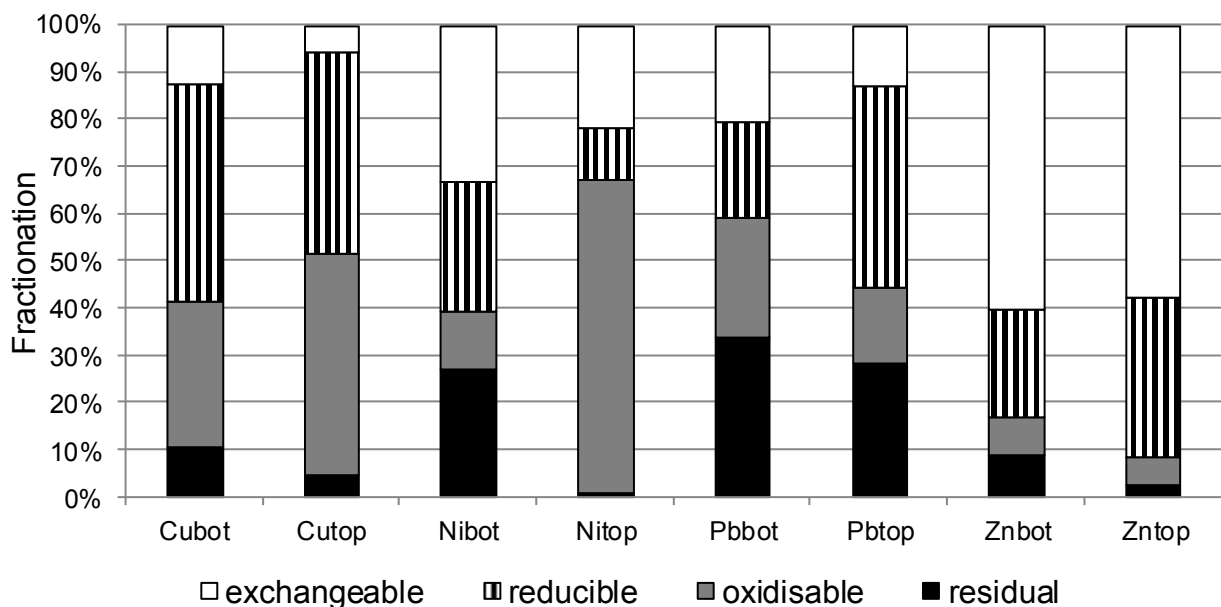
accelerating voltage of 15 kV. The analyzed samples were placed on titanium sample mounts covered with carbon tape.

3 Results and discussion

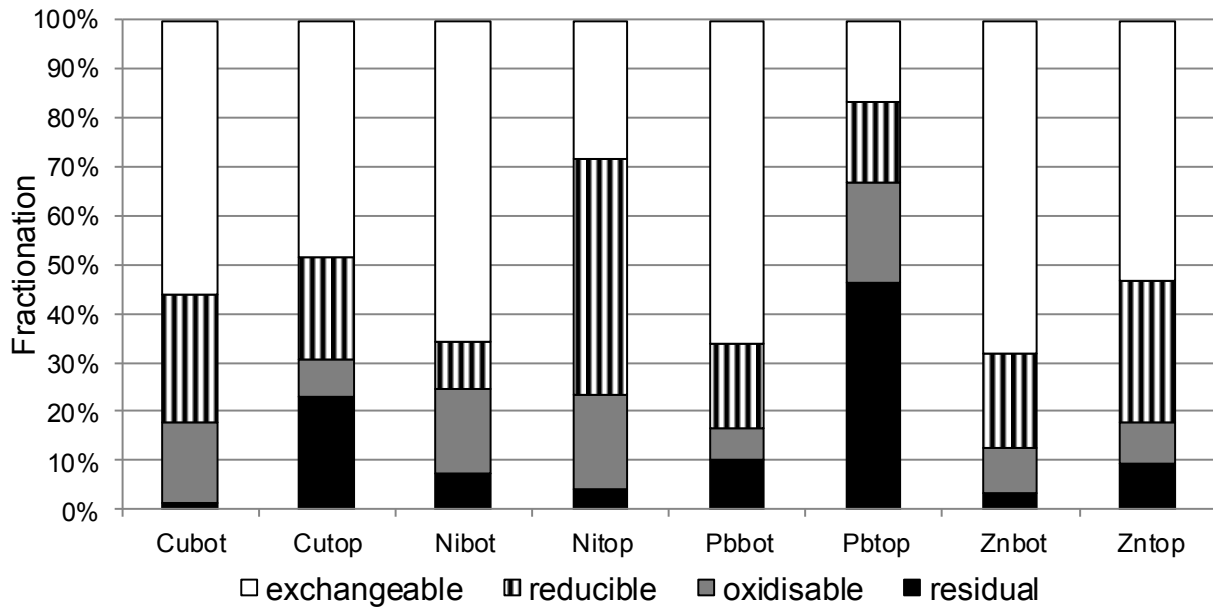
The fractionation of metals in the substrate of CWs is highly important as unstable forms of metals may be easily leached from the system and may compromise the treatment efficient in the long-term operation. The substrates from the experimental were analysed by the SEP to elucidate the fractionation of metals in the experimental systems. The SEM-EDS analysis was used to verify the results of SEP and to examine the hypothesis that elemental sulfur was precipitated in the columns of the experimental system.

3.1 Operationally-defined metal fractions in the spent substrate

The fractionation of metals in the substrate of CWs is highly important as unstable forms of metals may be easily leached from the system. The upward flow direction and constant saturation of the bed media was believed, when designing the experiment, to promote stable anoxic conditions and to remove majority of the contaminants in the bottom part of the column. To verify this hypothesis the bed media from the planted columns were analyzed by the SEP and *aqua regia* extraction. The substrates from the UF-PP and UF-GP columns were analyzed by the SEP to elucidate the fractionation of metals in the experimental system. The results for the UF-PP column are presented in Fig. 1a and for the UF-GP column in Fig. 1b.



(a)



(b)

Figure 1 Metal fractions in the bottom (bot) and top layers of the UF-PP column (a) and UF-GP column (b)

On comparing the results presented in Fig. 1 it can be observed that the proportion of various binding mechanisms varies between the columns with peat (UF-PP) and gravel (UF-GP). The exchangeable fraction is higher in the UF-GP column than in the UF-PP column for all the metals in the bottom and top layers, except for Zn in the top layer. The exchangeable fraction is 43% higher for Cu (both in top and bottom layers), 33% and 8% higher for Ni (bottom and top layers, respectively), 44% and 6% for Pb (bottom and top layers, respectively), and 8% for Zn in the bottom layer. Only the exchangeable fraction of Zn in the top layer of the UF-GP column is 5% lower than in the UF-PP column. In contrast to the observations for the exchangeable fraction, the reducible fraction is higher in the UF-PP column than in the UF-GP column with only one exception, which is Ni in the top layer of the bed media higher by 39% in the UF-GP column. In comparison to the UF-GP column the reducible fraction of Cu in the UF-PP column is 22% and 24% higher (bottom and top layers, respectively), 18% higher for Ni in the bottom layer, 3% and 28% for Pb (bottom and top layers, respectively), and 4% and 5% for Zn (bottom and top layers, respectively). As compared to the exchangeable and reducible fractions in both the UF-PP and UF-GP columns the oxidisable fraction is lower for most metals in both top or bottom layers. The oxidisable fraction is higher in the UF-PP column for Cu by 16% and 41% (bottom and top layers, respectively), 46% for Ni in the top layer, and 19% for Pb in the bottom layer. It is almost equal for Zn in either layers, as the difference is 1 – 2%. In the case of Ni in the bottom layer and Pb in the

top layer, the oxidisable fraction is higher in the UF-GP columns by 5% and 8%, respectively. It was found that Cu and Ni were bound with the less mobile fractions, and that Zn was bound mostly with the most easily leached fractions. Similar observations were presented in [13] for the surface-flow CW treating domestic wastewater.

The operationally-defined oxidisable fraction (nominal fractions: organic matter and sulfides [17]) was found to be the major fraction only for Ni (66.4%) and Cu (48.8%) in the top layers of the UF-PP columns. Interestingly, the removal of these elements was the highest in the UF-PP columns (above 90%) [15]. The obtained results indicate that desired process of metals precipitation with biogenic sulfides did not play a major role in removal of the metallic contaminants in the UF-PP columns, with the exception of Cu and Ni in the top layers of these columns. There are two groups of factors that could be responsible for this observation. The first group includes operational factors: deficiency of organic carbon available for SRB, presence of oxidized forms of Fe and Mn, brevity of the experiment or short hydraulic retention time (HRT). The second group includes analytical factors, which are related to the manner in which the samples were analyzed. The performance results indicated low or negative removal of sulfates in the peat and gravel columns, respectively. The addition of lactates allowed achieving positive removal (approx. 4%) of sulfates in the columns with gravel in the last 10 weeks of the experiment [15]. The brevity of the experiment (53 weeks) was not sufficient for establishing efficient sulfate-reducing reactors. The results presented in this article indicate that adsorption and oxic processes were predominant in the studied columns. Oxidized forms of Fe and Mn, which are more favorable in SRB metabolism than sulfates, could serve as electron acceptors for SRB until they were depleted. Also the relatively short HRT [15] could be hindering the onset of conditions conducive to sulfate reduction. Some researchers also did not find sulfides precipitation to be the major sink for metals. For example, Neculita et al. [19] found that only up to 15% of metals were removed by the precipitation with sulfides in planted passive bioreactors with HRT 7.3 d and 10 d on analyzing spent substrate withdrawn from the reactors in week 44 of the experiment.

The artifacts introduced during the analysis of the samples could have been introduced by oxidizing the reduced forms of metals and extracting oxidisable fraction by hydroxylammonium chloride, which was used in the applied SEP [20, 21]. Ngiam and Lim [20] found that anoxic sediments were oxidized despite the fact that oxygen-free atmosphere were maintained during the analysis. Peltier et al. [21] found that the application of hydroxylammonium chloride may lead to great underestimation of the sulfidic fraction in

anoxic sediments. These shortcomings practically preclude inter-experimental comparison of SEP results, however, it is possible to employ SEP to compare the fraction of metals when uniform conditions are ensured during the analysis (as in this study).

It was expected that the sulfidic fraction would not be the major sink for metals in the UF-GP columns due to the organic carbon deficiency in the bed medium and in the feed. The UF-GP columns were used as reference columns in terms of the effect of organic bed medium (peat). Noteworthy, the UF-GP columns were amended by adding lactates to the feed to stimulate the activity of SRB in the last 10 weeks of the experiment. The addition of lactates was found to cause significant increase in the removal of metals and sulfates [15], but the short period of lactate dosing did not cause the oxidisable fraction to be a major sink for any of the studied metals in any of the analysed layers.

3.2 Pseudo-total concentrations of metals in the substrate

The relative content of the metal fractions does not indicate the quantitative distribution in the columns and the concentration which in concert with speciation can furnish complete information on the toxicological risk associated with the contaminants bound in the CWs. The pseudo-total concentration of metals in the tested substrates is represented by the amount of metals extracted in the *aqua regia* digestion of the substrate (Tab. 2). The presentation of the absolute concentrations of the fractions was omitted in this article, as large difference between the concentrations of metals would diminish the clarity of the data presentation regarding the fractionation of metals.

Table 2 Pseudo-total concentrations of metals (mg/kg DW) in the bottom and top layers of the substrate sampled from the planted UF columns

Column	Sample location	Cu	Ni	Pb	Zn
UF-PP	bottom	5.61	12.10	2.40	24.20
	top	11.74	66.65	7.47	103.9
UF-GP	bottom	59.91	91.1	20.14	132.61
	top	9.00	24.13	3.54	29.89

The main difference between the UF-PP and UF-GP columns consisted in the quantitative distribution of metals. In the UF-PP columns higher concentration of all the metals was observed in the top part of the columns in contrast to the UF-GP column. It is difficult to elucidate the cause of different behavior of metals in the UF-PP and the UF-GP columns. The

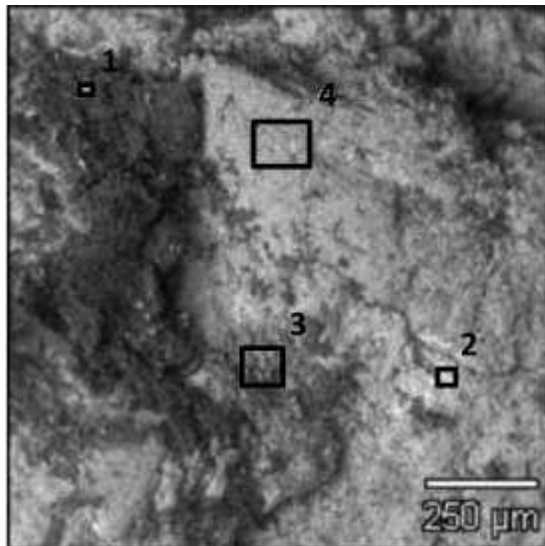
probably explanation could be the acidifying effect of peat (pH 3.5-4.5, [15]) that enhanced mobility of metals entering the system and deeper penetration into the column with subsequent binding in the top part of the column at the interface between anoxic and oxic layers where various processes could play a role in the removal. The concentration of metals in both parts of the planted columns was in the decreasing order of $Zn > Ni > Cu > Pb$.

3.3 SEM-EDS analysis of the spent substrate and filtered solids

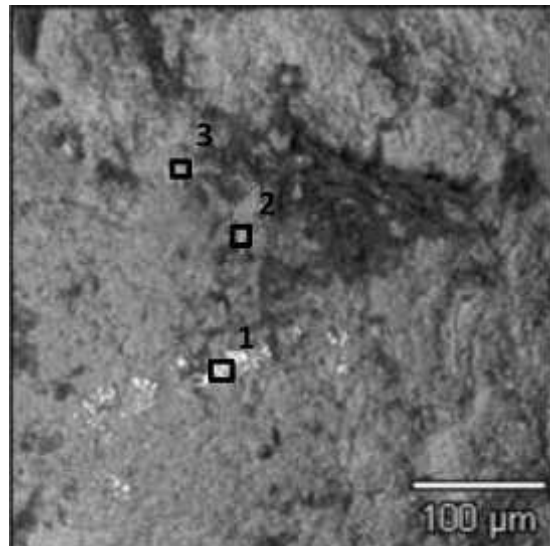
The application of SEP for anoxic sediments has been widely discussed in the literature. It was suggested that commonly applied SEPs may underestimate the amount of sulfidic fraction in those sediments. Those chemical analyses should be verified using, among other methods, X-ray absorption spectroscopy or SEM-EDS [21].

3.3.1 SEM-EDS analysis of the substrate from the UF-PP

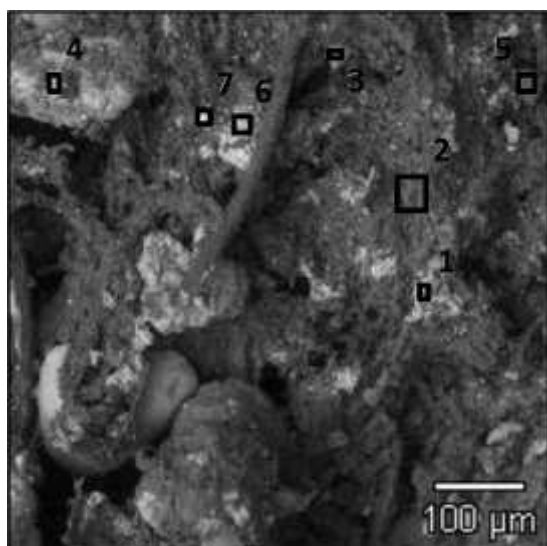
The SEM-BSE images of the samples taken from the bottom and top parts of the UF-PP column are presented in Fig. 2 and the elemental composition of the selected areas is listed in Tab. 3. The colors of the rectangles indicating the selected area may be ignored. White or black colors were used to increase visibility.



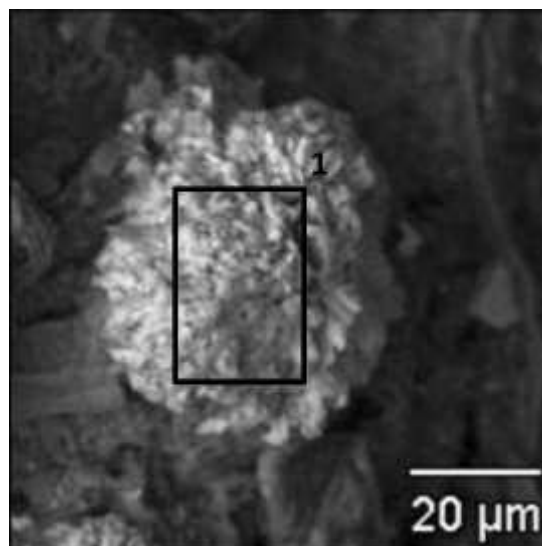
(a) Sample UF-PP-bot(1)



(b) Sample UF-PP-bot(2)



(c) Sample UF-PP-top(1)



(d) Sample UF-PP-top(2)

Figure 2 SEM-BSE images of wetland substrates sampled from the bottom (bot) and top of the UF-PP column

Table 3 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 2 determined using EDS

Sample_Area	Atomic % ¹											
	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Mn	Fe
UF-PP-bot(1)_ar1	2.9	2.5	16.2	56.7	-	2.7	-	10.2	3.4		-	5.3
UF-PP-bot(1)_ar2	3.4	1.2	14.1	59.1	-	1.5	-	3.8	1.0	0.6	-	15.3
UF-PP-bot(1)_ar3	2.4	1.7	16.6	64.2	-	2.4	-	7.5	2.4	-	-	2.7
UF-PP-bot(1)_ar4	8.6	0.8	16.6	69.7	-	-	-	2.2	0.9	-		1.2
UF-PP-bot(2)_ar1	-	-	3.9	78.8	-	-	-	1.2	0.5	-	-	-
UF-PP-bot(2)_ar2	1.0	4.5	13.9	60.7	-	0.7	-	7.5	-	1.7	-	9.8
UF-PP-bot(2)_ar3	1.1	0.6	5.6	30.9	23.0	0.5	-	2.2	35.5	-	-	0.6
UF-PP-top(1)_ar1	1.0	0.6	1.1	4.8	-	43.0	-	0.4	48.2	-	-	0.9
UF-PP-top(1)_ar2	8.6	5.4	5.9	24.4	1.1	20.6	2.7	2.8	26.0	-	-	2.6
UF-PP-top(1)_ar3	1.2	0.7	1.0	3.5	-	45.3	-	-	48.3	-	-	-
UF-PP-top(1)_ar4	1.6	0.7	1.3	9.2	-	41.6	-	-	44.6	-	-	1.0
UF-PP-top(1)_ar5	9.3	7.1	4.8	14.9	-	22.5	5.1	-	32.9	-	-	3.5
UF-PP-top(1)_ar6	1.1	0.7	1.2	4.6	-	45.9	-	0.4	46.1	-	-	-
UF-PP-top(1)_ar7	2.7	1.5	2.0	7.1	0.3	52.2	-	0.4	6.7	-	0.6	26.5
UF-PP-top(2)_ar1	1.2	0.5	0.6	3.3	0.3	45.9	-	-	47.5	-	-	0.6

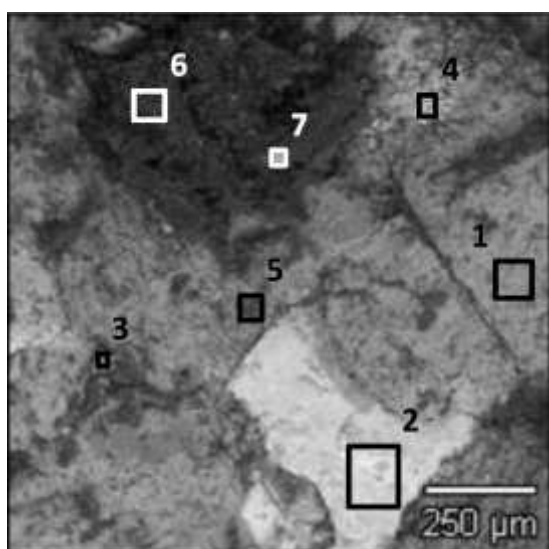
¹Compositions normalized to 100%

It can be seen that the grain of gravel had some peat attached to it, in which small bright particles were present, indicating their higher mean atom mass resulting probably from the presence of Fe. The amount of S is small suggesting that sulfides were not abundant in the top

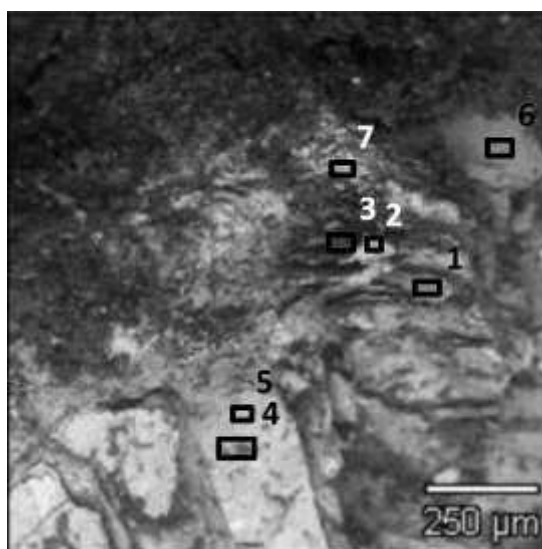
part of the UF-PP column. The UF-PP-bot(2) sample contained elevated amount of Ni but it was probably of native origin. Both UF-PP-top samples contain the amounts of Ca and S suggesting the presence of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The increased Fe content was observed in the area 7 of the UF-PP-top(1) sample. The characteristics of the adjacent areas may suggest that Fe is not present in sulfide form, but rather as oxidized species, namely (oxy)hydroxides.

3.3.2 SEM-EDS analysis of the substrate from the UF-GP

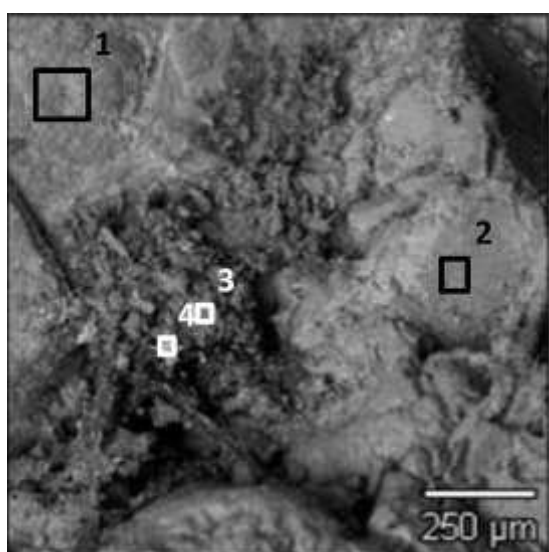
The SEM-BSE images of the samples taken from the bottom and top parts of the UF-GP column are presented in Fig. 3 and the elemental composition of the selected areas is listed in Tab. 4.



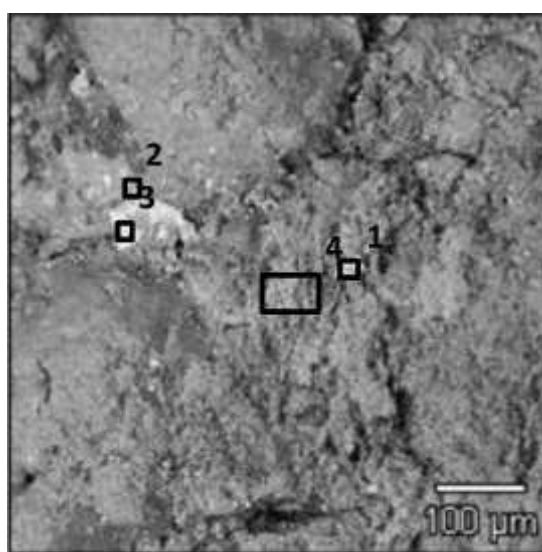
(b) Sample UF-GP-bot(1)



(b) Sample UF-GP-bot(2)



(c) Sample UF-GP-top(1)



(d) Sample UF-GP-top(2)

Figure 3 SEM-BSE images of wetland substrates sampled from the bottom (bot) and top of the UF-GP column

Table 4 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 3 determined using EDS

Sample_Area	Atomic % ¹														
	Na	Mg	Al	Si	P	S	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn
UF-GP-bot(1)_ar1	7.2	0.6	24.7	59.0	-	0.9	3.3	1.9	-	-	-	2.4	-	-	-
UF-GP-bot(1)_ar2	1.9	7.3	20.7	35.1	-	6.1	2.0	1.1	1.9	-	-	23.9	-	-	-
UF-GP-bot(1)_ar3	3.8	1.0	15.1	40.9	-	1.3	6.1	5.7	4.6	-	-	21.6	-	-	-
UF-GP-bot(1)_ar4	3.2	4.1	16.9	35.1	0.5	8.9	2.7	2.4	2.9	-	-	23.3	-	-	-
UF-GP-bot(1)_ar5	6.7	1.3	23.2	54.0	-	2.4	3.2	4.4	-	-	-	4.9	-	-	-
UF-GP-bot(1)_ar6	6.1	2.5	13.9	36.1	1.1	9.6	2.3	16.2	0.9	-	-	11.3	-	-	-
UF-GP-bot(1)_ar7	3.9	1.3	8.0	20.4	0.6	26.9	0.9	5.2	-	-	0.5	32.1	-	-	-
UF-GP-bot(2)_ar1	0.8	10.1	19.8	29.7	-	4.1	1.0	1.2	0.8	-	0.8	31.8	-	-	-
UF-GP-bot(2)_ar2	1.0	6.3	14.8	26.4	-	4.2	4.9	1.3	2.6	-	0.7	37.9	-	-	-
UF-GP-bot(2)_ar3	1.3	7.3	17.4	29.8	-	5.8	3.6	1.7	2.8	-	0.5	29.7	-	-	-
UF-GP-bot(2)_ar4	1.2	7.7	16.9	26.8	0.4	4.6	2.2	1.9	1.3	-	0.7	36.3	-	-	-
UF-GP-bot(2)_ar5	0.7	6.4	16.1	26.9	-	2.9	4.2	1.2	3.1	-	0.5	37.9	-	-	-
UF-GP-bot(2)_ar6	-	0.7	9.6	79.0	-	1.4	3.4	1.3	-	-	-	4.6	-	-	-
UF-GP-bot(2)_ar7	0.9	7.1	15.7	28.7	-	9.1	2.5	1.8	1.5	-	-	32.7	-	-	-
UF-GP-top(1)_ar1	8.4		19.1	61.3	-	2.5	3.3	2.1	-	-	-	3.3	-	-	-
UF-GP-top(1)_ar2	1.9	1.0	28.7	52.2	0.7	1.1	11.4	-	0.2	-	-	2.8	-	-	-
UF-GP-top(1)_ar3	3.4	0.4	5.8	82.1	0.8	1.7	1.5	1.2	-	-	-	2.1	-	-	-
UF-GP-top(1)_ar4	4.2	5.6	17.2	47.5	0.6	2.2	5.0	1.6	1.3	-	-	13.2	0.8	-	-
UF-GP-top(2)_ar1	3.0	0.7	8.8	23.1	-	0.6	1.9	0.7	-	10.9	0.8	44.5	5.1	-	-
UF-GP-top(2)_ar2	-	0.8	9.6	45.2	1.5	14.3	1.8	2.1	-	-	-	6.8	-	0.8	17.1
UF-GP-top(2)_ar3	2.5	3.7	16.5	51.1	0.7	1.1	7.3	1.1	1.2	-	-	14.9	-	-	-
UF-GP-top(2)_ar4	2.4	1.5	26.1	53.1	0.7	0.9	10.9	-	0.2	-	-	4.2	-	-	-

¹Compositions normalized to 100%

Both samples had similar composition characterized by elevated amounts of Fe accompanied by much lower amounts of Mn. It was also observed that the increased content of Fe corresponded to elevated amounts of S, which may indicate the presence of sulfides (e.g. area 7 of sample UF-GP-bot(1)). This may also suggest that ferrous iron competes for sulfide with other cations, which were present in the feed as was observed by Song [22]. Some of the areas observed on the analyzed material seemed to be native to the substrate, e.g. area 2 of sample UF-GP-bot(1), and areas 4-5 of sample UF-GP-bot(2). The EDS analysis of the sample from the top part of the UF-GP column indicated the presence of the metals of concern: Ni, Cu and Zn. Area 2 of sample UF-GP-top(2) contains elevated amounts of S and Zn, which may suggest the presence of sulfides. Also area 4 of the UF-GP-top(2) may contain sulfides, however, the amount of S is small. The composition of area 1 of sample UF-GP-top(2) is untypical as it contains chromium Cr, which was not added to the feed and it is likely that it was native to the substrate.

3.3.3 SEM-EDS analysis of the raw gravel

The above-presented results of the SEM-EDS analysis of the spent substrate contained several areas in which the elemental composition was much different from the other analyzed areas. In this situation it was assumed that the detected elements were of native origin. This means that they were present in the media before filling the columns. The SEM-EDS analysis was performed to furnish more information on the forms of native contaminants in the raw media.

The SEM-BSE images of the samples of the raw gravel used in the microcosm system are presented in Fig. 4 and the elemental composition of the selected areas is listed in Tab. 5.

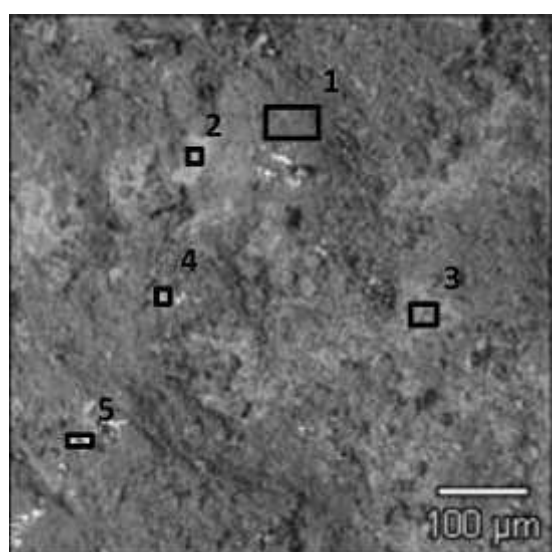


Figure 4 SEM-BSE image of the raw gravel used as a bed medium

Table 5 Elemental (atomic %) composition of the areas (ar#) indicated in the image in Fig. 4 determined using EDS

Sample_Area	Atomic % ¹									
	Na	Mg	Al	Si	K	Ca	Ti	Fe	Co	Ni
Gravel(1)_ar1	0.6	0.6	10.3	82.8	2.0	0.7	-	3.1	-	-
Gravel(1)_ar2	0.6	5.3	20.5	51.9	8.5	-	1.4	11.7	-	-
Gravel(1)_ar3	0.9	3.7	26.5	53.1	6.3	-	1.0	8.6	-	-
Gravel(1)_ar4	0.9	1.0	11.6	34.5	1.4	-	0.2	2.3	0.9	47.2
Gravel(1)_ar5	1.3	1.5	14.0	66.6	1.5	1.1	0.3	3.2	-	10.4

¹Compositions normalized to 100%

It can be observed that the surface of the gravel is rough. This property of the material could be essential as it provides advantageous substrate for bacterial attachment and biofilm formation. The elemental composition indicates that the gravel contained more impurities that could have been leached into the wastewater. Noteworthy, Fe was found in all the analyzed

areas, Ni was found in two areas, and Co was found once. It was not analyzed if the native elements could be leached from the gravel. Selection of bed medium for the full-scale system should include analysis of the material as elevated concentration of metals in the medium may pose environmental risk. Additionally, metals of the native origin may be construed as the removed from the wastewater when a spent substrate is analyzed.

3.3.4 Analysis of suspended solids retained on filters

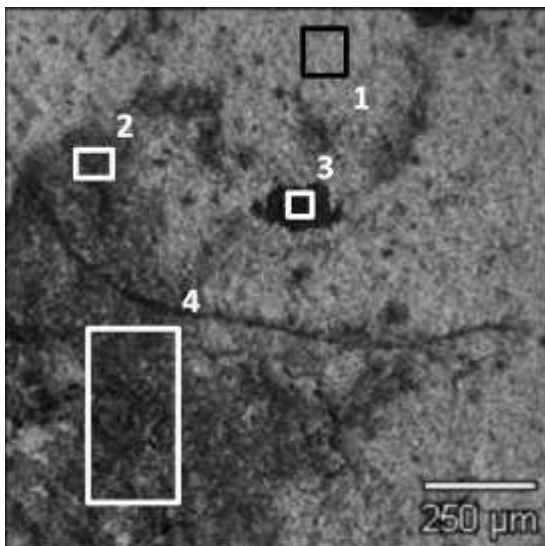
The concentration of contaminants was only analyzed in filtered samples of the influent and effluent of the experimental system [15]. This is a common practice, however, certain metals species (e.g. aggregated hydroxides or sulfides) may not pass through filter, markedly overestimating the removal efficiency of the system. This analysis is not fully representative as the sediments on the surface of the bed media in the columns were resuspended while sampling the wastewater above the surface of the bed. Resuspension of the sediments was aimed at analyzing the white precipitates easily noticeable in the UF-GU column and presumed to be elemental S. The white precipitates were only observed in the columns with gravel (UF-GP and UF-GU), which were fed with the influent containing lactates from week 44 on. Noteworthy, the white precipitates were absent in these columns before the addition of lactates to the feed. It was hypothesized that lactates stimulated bacterial production of sulfides, which subsequently were oxidized to elemental S on the anoxic-oxic interface in the columns with gravel.

The lack of peat particles in the UF-GU columns allowed observation of dilute skim milk appearance in this column, which was caused probably by elemental S (Fig. 5). It was hypothesized that the occurrence of elemental S was the effect of sulfur-oxidizing bacteria as *Beggiatoa* that incompletely oxidize sulfide to S [23].

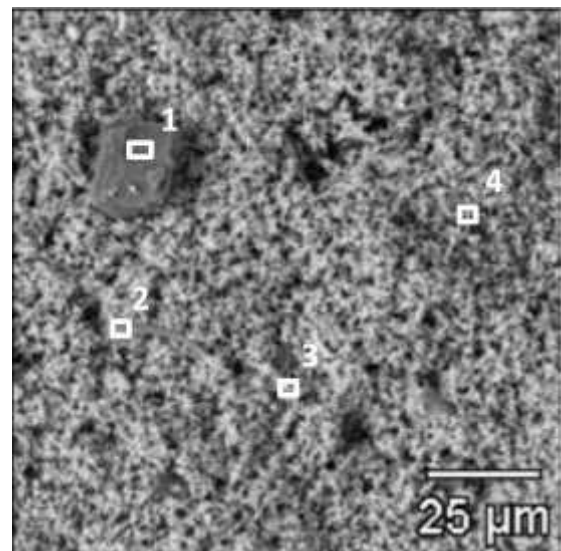


Figure 5 Outflow of the UF-GU column with visible white precipitates

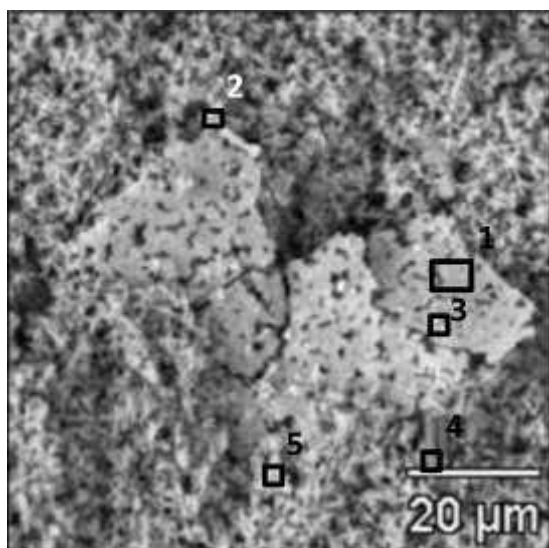
The SEM-BSE images of the filter through which the wastewater from the UF-GU column was filtered are presented in Fig. 6 and the elemental composition of the selected areas is listed in Tab. 6.



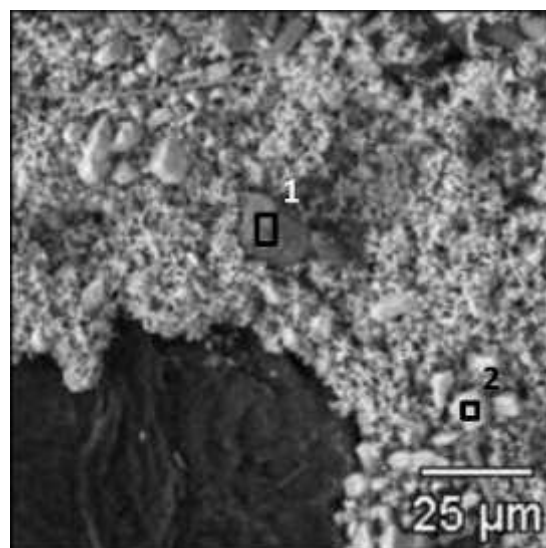
(a) Sample UF-GU-filter(1)



(b) Sample UF-GU-filter(2)



(c) Sample UF-GU-filter(3)



(d) Sample UF-GU-filter(4)

Figure 6 SEM-BSE images of the filter surface through which the effluent from the UF-GU column was passed

Table 6 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 6 determined using EDS

Sample_Area	Atomic % ¹												
	Na	Mg	Al	Si	P	S	K	Ca	Fe	Ti	Ni	Cu	Zn
UF-GU-filter(1)_ar1	-	-	-	0.9	-	99.1	-	-	-	-	-	-	-
UF-GU-filter(1)_ar2	-	-	0.7	4.2	1.3	84.8	-	3.8	1.4	-	0.8	-	3.0
UF-GU-filter(1)_ar3	0.9	-	-	2.6	-	91.7	-	2.4	-	-	-	-	2.4
UF-GU-filter(1)_ar4	-	0.1	1.0	20.5	1.6	65.1	0.4	2.9	1.2	-	0.8	1.1	5.4
UF-GU-filter(2)_ar1	1.4	-	6.3	34.7	-	52.0	4.7	-	0.9	-	-	-	-
UF-GU-filter(2)_ar2	1.5	6.0	8.1	19.4	-	50.5	-	12.1	1.8	-	-	-	0.6
UF-GU-filter(2)_ar3	-	-	-	-	-	100.0	-	-	-	-	-	-	-
UF-GU-filter(2)_ar4	-	-	-	10.7	-	88.8	-	0.5	-	-	-	-	-
UF-GU-filter(3)_pt1	-	-	-	1.3	-	85.8	-	-	-	-	0.8	5.4	6.7
UF-GU-filter(3)_pt2	-	-	-	1.9	-	83.3	-	1.5	-	-	1.1	5.9	6.4
UF-GU-filter(3)_pt3	-	-	-	1.3	-	88.0	-	0.4	-	-	-	4.2	6.2
UF-GU-filter(3)_pt4	4.1	0.3	3.0	24.9	-	62.0	1.0	1.0	-	0.7	-	-	3.1
UF-GU-filter(3)_pt5	-	-	-	0.5	-	99.5	-	-	-	-	-	-	-
UF-GU-filter(4)_pt1	0.3	-	2.3	39.9	-	53.7	0.4	0.4	1.2	-	-	-	1.7
UF-GU-filter(4)_pt2	-	-	-	1.0	-	99.0	-	-	-	-	-	-	-

¹Compositions normalized to 100%

It can be seen that all the analyzed areas contained high amounts of S. It was mostly in amorphous form but could also be present in the crystalline form (area 1 of sample UF-GU-filter(2)). Also the metals of concern including Ni, Cu and Zn were present on the filter as species that are difficult to determine, however, the presence of sulfides cannot be excluded.

In the UF-GU-filter(4) sample more crystals of sulfur were observed. The metals of concern, especially Cu and Zn were present in higher concentration than in the previous two samples.

The white tarnish was also observed on the topmost grains of gravel in the UF-GU column. This layer of gravel was collected and analyzed to confirm if the white precipitates found on the filter were also present on the gravel. The SEM-BSE images of topmost gravel layer from the UF-GU column are presented in Fig. 7 and the elemental composition of the selected areas is listed in Tab. 7.

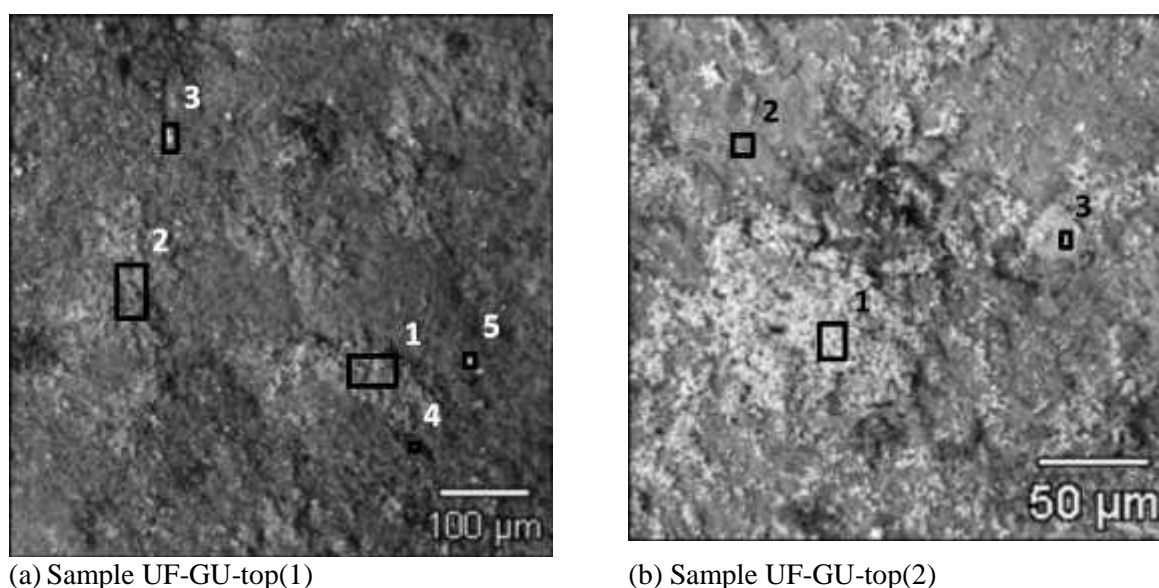


Figure 7 SEM-BSE images of wetland substrates sampled from the top of the UF-GU column

Table 7 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7 determined using EDS

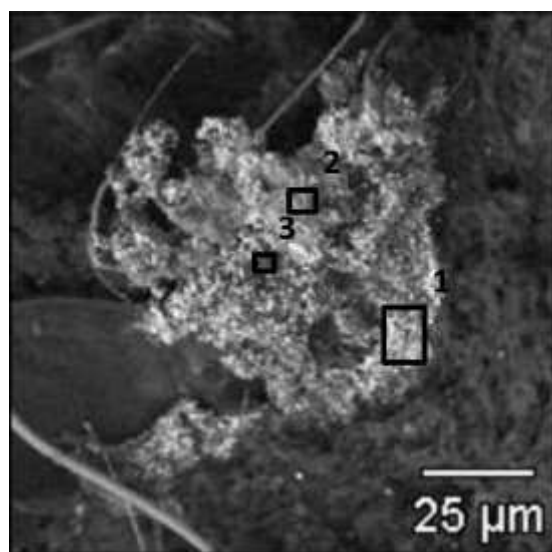
Sample_Area	Atomic % ¹										
	Na	Mg	Al	Si	P	S	K	Ca	Ti	Fe	Ni
UF-GU-top(1)_ar1	2.3	0.1	4.6	25.3	-	40.8	1.1	24.6	-	0.7	0.5
UF-GU-top(1)_ar2	2.6	-	5.0	25.8	-	36.5	0.9	28.4	-	0.9	-
UF-GU-top(1)_ar3	7.1	-	13.5	62.6	-	8.8	3.5	2.2	1.3	1.1	-
UF-GU-top(1)_ar4	5.1	0.5	12.1	59.9	0.8	12.1	3.9	3.5	-	1.3	0.7
UF-GU-top(1)_ar5	4.3	0.5	9.5	61.3	-	10.6	2.6	2.8	-	8.4	-
UF-GU-top(2)_ar1	1.2	-	2.5	15.4	-	80.0	0.5	0.4	-	-	-
UF-GU-top(2)_ar2	4.0	0.6	12.4	56.6	-	19.5	4.1	0.7	-	2.2	-
UF-GU-top(2)_ar3	2.5	3.0	12.6	44.3	-	21.3	5.0	-	1.2	10.0	-

¹Compositions normalized to 100%

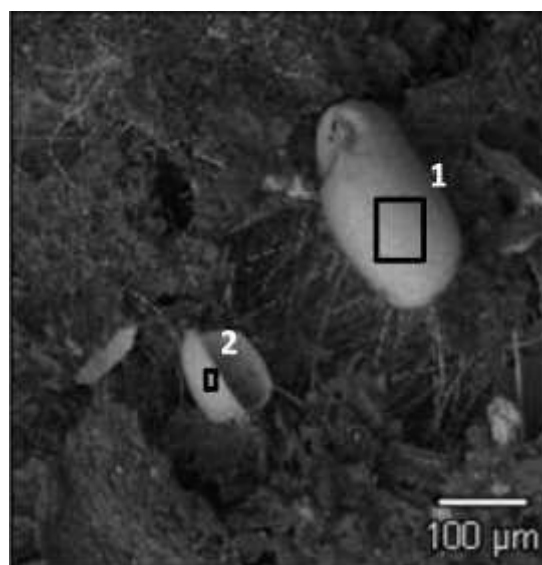
The EDS analysis of the gravel confirmed the presence of S in the UF-GU column, however, in quantities smaller than on the filter. This could be caused, amongst others, by the composition of the gravel itself and the drying of gravel, which could promote precipitation of

sulfates of, for example, Ca and Na. However, most of the forms observed on the gravel are not crystalline, whereas sulfates of Ca and Na form crystalline forms. It is noticeable that the amount of the S precipitates is smaller than on the filter and that it forms cluster of different sizes. The largest cluster of S (area 1 of sample UF-GU-top(2)) contained 80% of this element. The crystalline forms in sample UF-GU-top(1) that had the appearance of a rosette consisted mainly of S and Ca and were probably gypsum.

The precipitates occurring in the columns with gravel were not observed in the UF-PP and UF-PU columns, which could be attributed to the entrapment of the precipitates in a thick layer of sediments (originating from the peat) in these columns. The main reason might be, however, that the columns with peat (UF-PP and UF-PU) were not fed with the influent containing lactates. The SEM-BSE images of the filter through which the wastewater from the UF-PP column was filtered are presented in Fig. 8 and the elemental composition of the selected areas is listed in Tab. 8.



(a) Sample UF-PP-filter(1)



(b) Sample UF-PP-filter(2)

Figure 8 SEM-BSE images of the filter surface through which the effluent from the UF-PP column was passed

Table 8 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 8 determined using EDS

Sample_Area	Atomic % ¹												
	Na	Mg	Al	Si	P	S	K	Ca	Ti	Mn	Fe	Ni	Zn
UF-PP-filter(1)_ar1	2.2	0.8	1.7	8.1	0.5	18.1	0.6	5.5	0.2	0.8	34.6	17.8	9.1
UF-PP-filter(1)_ar2		0.1	0.7	3.4	0.4	47.1		37.8			5.0	2.1	3.4
UF-PP-filter(1)_ar3	2.1	0.9	2.0	8.5	0.9	44.4	0.7	10.8		0.4	15.4	6.6	7.3

UF-PP-filter(2)_ar1			1.0	5.7		2.4	0.4	83.7			1.3		1.3
UF-PP-filter(2)_ar2	2.4	1.1	2.1	9.4	0.7	3.9	0.6	71.6			1.5		1.5

¹Compositions normalized to 100%

The elemental composition of the solids retained on the filter indicates elevated concentration of S and two of the metals of concern: Ni and Zn (Tab. 7). The presence of elemental S cannot be excluded, however, the S species present in the sample could be rather sulfate and sulfide. Sulfate was probably present in area 2 of the UF-PP-filter(1) sample as calcium sulfate. This compound could form when the substrate was dried for the analysis. Interestingly, Cu was not detected in the two areas analyzed on the filter, as in the other samples of substrate taken from this column (Section. 3.3.1). The properties of the sediments in the UF-PP columns (peat) hinder observation and analysis of small particles such as elemental S or sulfides, however, neither naked-eye observation nor SEM-EDS analysis indicated the presence of elemental S. The hypothesis on the presence of elemental S cannot be corroborated nor rejected. The egg-shaped objects in Fig. 8b were probably cysts of *Daphnia* or *Oligochaeta*.

It should also be mentioned that the white precipitates observed in the UF-GU column cannot be regarded as S with absolute certainty. This is because some of the heavy metals have X-ray spectra that overlap the light element X-ray spectra and create difficulties with analysis. In this study it is of particular importance that the M series of Pb at 2.346 and 2.502 overlaps the K α line of S [24]. Similar appearance as is assumed to be caused by elemental sulfur can be also cause by the precipitates of lead sulfate. Hence, the hypothesized presence of sulfur should be confirmed using other analytical method.

The unsightly appearance of the dilute skim milk phenomena, whether it is elemental S or not, should be prevented in the full-scale systems.

4 Conclusions

The target process for the removal of metals in the columns filled with peat was precipitation of metals with biogenic sulfide being the product of the activity of SRB. In general, this process was found not to play a major role in the removal of metals. The major portion of metals was bound to exchangeable or reducible fractions. It allows conclusion that adsorption and oxic removal processes are mainly responsible for the removal of metals in a short-time experiment, which was described in this paper. Peat was found, however, to promote precipitation of metal sulfides to higher extent than gravel. The oxidisable fraction (including sulfidic fraction) was found to be the major sink for Cu and Ni in the top layers of substrate in

the UF columns filled with peat. Zn was bound with exchangeable fraction in both gravel and peat media and was prone to be easily leached from the system.

The commonly available tools as SEP and SEM-EDS analysis, however, tend to underestimate the contribution of the metal sulfide precipitation. According to the statement that the metal retention mechanism evolves during the life of a system [19] it cannot be ruled out that CWs can effectively remove metals from pretreated electroplating wastewater by sulfide precipitation. However, relatively brief microcosm experiment might not be suitable, therefore a pilot-scale study using real electroplating should be performed over a longer period. The presented study suggests that the onset of the conditions favorable for precipitation of sulfides may not occur after a relatively long period of operation. It may stem from the fact that peat (as internal carbon source), and all the more low carbon concentration in electroplating wastewater, are not sufficient for SRB. It should be further studied how the onset of sulfide precipitation could be accelerated. In the first part of this paper [15] lactates were added to stimulate the activity of SRB, however, dosing started only in the last 10 weeks of the experiment and did not affect the binding mode of metals in the extent that could be represented by the SEP or SEM-EDS results.

As it was reported in [15] both gravel and peat columns were efficient in removal of Cu, Ni and Zn. The presented results show, however, that the mechanisms responsible for the retention of metals vary. It is suggested that short-term studies should not be extrapolated to predict long-term behavior of the system. High efficiency of the CWs filled with gravel treating carbon-deficient wastewater under anoxic conditions in short-time experiments might be misleading as adsorption and oxic phases are responsible for the removal of metals. The former process may be terminated when the sorption capacity is exhausted. Oxic phases, on the other hand, can be dissolved on the onset of anoxic conditions e.g. when the system is amended with external carbon source.

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