Heavy metal fate in pilot-scale sludge drying reed beds under various design and operation conditions

Alexandros I. Stefanakis, Vassilios A. Tsihrintzis*

Laboratory of Ecological Engineering and Technology, Department of Environmental Engineering, School of Engineering, Democritus University of Thrace, 67100 Xanthi, Greece

ABSTRACT

Thirteen pilot-scale sludge drying reed bed (SDRB) units have been constructed and operated under various settings. The beds included a cobbles lower layer, where perforated PVC aeration tubes were placed, and two gravel layers on top. The setup included planted beds with common reeds and control units. Three sludge loading rates (SLR) were examined: 30, 60 and 75 kg dm/m²/yr. Heavy metal (HM) accumulation in the residual sludge layer was negligible or low, and was found to increase with sludge layer depth. Plant uptake was low; the belowground biomass accumulated significantly more HMs compared to the aboveground biomass. Less than 16% of the influent HM left the bed through drainage. HM accumulation in the gravel layer was the major metal sink in the mass balance. On the whole, the HM content of the residual sludge was below the legal limits proposed by the EU for land application.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The operation of wastewater treatment plants (WWTPs) produces significant amounts of sludge, which contains a large number of valuable constituents, such as nutrients and organic matter, when disposed of on agricultural land. However, it may also contain undesired pollutants, such as heavy metals (HMs), synthetic organics and pathogenic microorganisms, suggesting that its final disposal may have adverse environmental and/or health impacts. Therefore, sludge treatment and disposal is of major concern around the world, and depends on various economic, environmental and legal parameters. Particularly in Greece, sludge utilization in agriculture is very limited; the majority (>90%) of produced sludge is sent to landfills, while the respective mean value in EU countries is only 27% [1].

The main EU Directives dealing with sludge management are 86/278/EEC [2] and 91/271/EEC [3]. The first one describes the necessary measures and precautions for the safe sludge use in agriculture, and defines limiting values for HMs in soil and sludge. The 3rd Draft EC Working Document on Sludge of 2000 [4] recommends stricter HM limit values for land application. According

to a European Commission Report of 2001 [5], many EU countries have adopted comparable HM limit values to those of the EU Directives (e.g., Greece, Italy, Spain, the UK), while other countries adopted stricter limits (e.g., Denmark, Finland, the Netherlands). An EC Working Document on Sludge and Bio-waste of 2010 [6] proposes even stricter limit values to incorporate bio-waste. In Greece, the EU Directive 86/278/EEC [2] was adopted by Ministerial Decision 80568/4225/91 in 1991, without any alterations, except of the addition of the following chromium limits: 500 mg/(kg dm) for Cr(III) and 10 mg/(kg dm) for Cr(VI).

Sludge drying reed beds (SDRBs) or sludge treatment wetlands have been successfully used over the last two decades [7]. They appear as an effective, safe, environmentally friendly, and economical alternative technology (low investment, operation and maintenance cost, low energy consumption) [8,9], especially appropriate for small and medium size communities or remote areas. This technology results in high water volume reduction and good sludge stabilization [10]. SDRBs seem quite effective in sludge dewatering, mainly through evapotranspiration and draining, and also in sludge mineralization [9,11–14]. Residual sludge from these facilities is a well-composted and beneficial product, which can possibly be used as an organic fertilizer or for land application [14]. The key parameter in SDRBs is the presence of reeds (*Phragmites australis*). These plants possess a high transpiration capacity and are quite tolerant to wet/dry conditions [8,13]. They contribute to the creation of aerobic microzones around their extensive root system, while they provide the necessary attachment area for microbes [15].

* Corresponding author. Tel.: +30 25410 79393; fax: +30 25410 79393; mobile: +30 6974 993867.
E-mail addresses: astefan@env.duth.gr (A.I. Stefanakis), tsihrin@otenet.gr, tsihrin@env.duth.gr (V.A. Tsihrintzis).

Although metal concentrations have been briefly reported in limited SDRB studies [11,16–18], the fate of metals allocated in various SDRB components has not been well presented. The objective of this study is to evaluate heavy metal fate in various pilot-scale SDRBs with various design configurations under Mediterranean conditions, and quantify the effect of design and operational parameters in order to evaluate sludge suitability for land application.

2. Methods and materials

2.1. Experimental setup

Thirteen similar pilot-scale SDRB units (S1–S13) were constructed and operated in an open-air laboratory (41°08′47″N, 24°55′09″E). Each unit was a plastic cylindrical tank of height 1.5 m and diameter 0.82 m. Units S1–S11 were constructed and planted in early June 2007 [13], and units S12 and S13 started operation one year earlier (May 2006) [13,19,20]. Table 1 summarizes the unit characteristics; Fig. 1 presents a vertical section of the pilot-scale units, and also a picture. Briefly, all units contained from bottom to top three substrate layers: a lower drainage 15-cm thick layer made of cobbles ($d_{50} = 90.0$ mm, range 30–180 mm), a 15-cm thick layer of medium gravel ($d_{50} = 15.0$ mm, range 4–25 mm), and a top 15-cm thick layer of fine gravel ($d_{50} = 6.0$ mm, range 1–8 mm). Aeration PVC tubes (50 mm in diameter), perforated at the bottom, were placed within the cobbles layer. One unit had no aeration tubes, while in another unit the cobbles layer was extended (25 cm thick; no fine gravel layer). Two porous media were used: one obtained from a river bed (igneous material) and one from a quarry (carbonate material). Most of the beds were planted with common reeds (*P. australis*) and one with cattails (*Typha latifolia*). One bed was kept unplanted. Three different nominal sludge loading rates (SLR) were originally planned: low, medium and high SLR (30, 60 and 75 kg dm/m^2/yr, respectively; Table 1). Slight differentiations from these nominal rates appeared during the 2.5-year loading phase, due to mechanical problems in the wastewater treatment plant that provided the sludge, resulting in slight deviations in the feeding schedule. The total sludge volumes are provided later in the text. In three units with different SLR, additional chromium concentration was added in the SAS, with a final concentration of 5002 mg Cr/(kg dm), in order to simulate the Cr content of tannery wastewater sludge. The compounds added were $CrCl_3$ and $K_2Cr_2O_7$ at a 50–50% ratio. With this setup, the effect of design (i.e., porous media size and thickness, vegetation, aeration, etc.) and operation (i.e., resting time, SLR) parameters could be tested [19,20].

The surplus activated sludge (SAS) was produced and transported every time from the WWTP of the municipality of Komotini, Rhodope Province, Greece, which is an extended aeration facility, with a retention time in the aeration basin of 20 days (SAS age). The feeding was carried out manually, using a device which flooded uniformly the entire bed surface. SAS was introduced to the units in loading cycles: a feeding period of 7 days in daily equal portions, followed by a resting period of 1–3 weeks. Shorter resting periods (1–2 weeks) were used at higher temperatures and intensive evapotranspiration in the Mediterranean climate. The influent SAS had a mean TS content of 3.1%, mean VS 73.7% TS, mean TKN 54.8 mg/g dm, mean $NO_3{}^-$–N 0.69 mg/g dm and mean TP 12.3 mg/g dm [19,20]. The total sludge loads applied to the SDRB units (from October 2007 to April 2010) were 67.7, 135.1 and 168.9 kg dm/m^2 for the low, medium and high SLR, respectively. Unit S12 (low SLR), which operated for 3.5 years, received 105.8 kg dm/m^2, and unit S13 (high SLR), which operated for 1.5 year, received 135.8 kg dm/m^2.

The present study presents the results for the common operation period (October 2007–October 2010) of units S1–S12. Sludge loadings stopped in April 2010, when the resting phase started, and lasted for another 6 months (till October 2010). The loading of unit S13 stopped in June 2008, but sludge sampling continued (about 2.5 years of resting). This study also presents the results from the extended resting phase (June 2008–October 2010) of this unit.

2.2. Sampling and analytical methods

Residual sludge samples were collected at the end of each resting period using a core sampler, in order to sample the entire sludge layer depth. To obtain a more representative residual sludge sample, all samples were taken from a minimum of two different points from each bed surface. From winter 2009, samples taken were split in a top and a lower part, in order to test the differences in the upper and lower sludge layers. Water samples for the characterization of the drained water were also taken at various time-points (i.e., 10 min, 2 h and 1 d) after sludge application.

Plants were harvested each winter and the total produced aboveground dry biomass was weighted. Harvested biomass samples were analyzed to examine the accumulation of HMs, while for four units, separate analyses were carried out in leaves and stems. Analyses were also performed in root samples (belowground biomass). Aboveground biomass samples were not washed before analyzing. Moisture measurements on biomass samples always showed a moisture content around 5%.

All heavy metal analyses (Cr, Cd, Pb, Cu, Ni, Mn, Zn, Fe) were carried out by acid digestion with conc. HNO_3 and conc. HCl (1:3, v:v), followed by a flame atomic absorption spectrometer (VarianR model SpectrAA). For metal standards production, commercially available solutions (initial concentration of 1000 ppm) were used. Before each measurement series, a 4 point-calibration curve was prepared using the standard solutions, while the precision of the instrument was tested every 10 measurements by testing one of the standard solutions. For a deviation above 5% of the nominal standard solution concentration, a new calibration curve was prepared. The limit of detection (LOD) was calculated for each metal as the concentration that would give a signal greater than 3.89 standard deviations above the mean noise level (based on $n = 7$ and at a 95% confidence level), according to APHA [21].

Meteorological data (air temperature, precipitation depth, atmospheric pressure, air humidity, and wind velocity and direction) were recorded on site for the entire operation period, at a 5-min time interval, using an ELE MM900/950 station.

2.3. Statistical analyses

Paired *t*-test instead of simple *t*-test (95% confidence interval of the difference) was used to examine the differences of the upper and lower half of the residual sludge layer in all units, as also the differences between the HM content of pairs of units with different design and operation characteristics. These paired *t*-tests calculate the confidence interval of the mean of the differences of the parameters measured during the same sampling time at a time period; therefore, results are not affected by the variability of a measurement versus time.

Additionally, two-way ANOVA at the 95% significance level was used in order to examine the time effect on the system performance. In the comparison of pair of units with a different characteristic, this specific characteristic and the year of operation after the unit establishment were used as independent variables. Homogeneity of variance tests (Levene) were bypassed, since the number of data points for each group was the same (parallel experiments). Post hoc pair comparisons were also performed to test equal variations, using Tukey honestly significant difference test. All tests were carried out using SPSS for Windows Release 17.0.

Table 1
Pilot-scale SDRB unit construction and operation characteristics [13,19,20].

Unit	Meso-porous media		Plant species	Aeration tubes	Cr added	SLR (kg dm/m^2/yr)
	Origin[a]	Size				
S1	R	Fine	Reed	Yes	No	75
S2	Q	Fine	Reed	Yes	No	75
S3	R	Fine	Cattail[b]	Yes	No	75
S4	R	Fine	No	Yes	No	75
S5	R	Fine	Reed	Yes	No	30
S6	R	Fine	Reed	Yes	No	60
S7	R	Fine	Reed	Yes	Yes	75
S8	R	Fine	Reed	Yes	Yes	30
S9	R	Fine	Reed	Yes	Yes	60
S10	R	Fine	Reed[b]	No	No	75
S11	R	Coarse	Reed	Yes	No	75
S12	R	Fine	Reed	Yes	No	30
S13	R	Fine	Reed	Yes	No	75

[a] R: river bed and Q: quarry.
[b] Initially planted; after first year plants dried up.

3. Results and discussion

3.1. Meteorological conditions

The average annual temperature value for the study period (three years) was 16.5 °C (26.3 °C and 6.7 °C for the warm and cold seasons, respectively). The mean annual precipitation depth was 678 mm (256.5 and 421.7 mm for the warm and cold seasons, respectively), with a mean annual rain frequency of 115 days/year. These values are typical of North Mediterranean climate [19].

3.2. Heavy metal concentration

3.2.1. Residual sludge

Table 2 contains the mean and standard deviation of HM content in the residual sludge for each unit during the loading and the resting phases; Fig. 2 presents time series charts for all metals for the entire period of operation. The influent SAS treated contained low to medium concentrations of these metals, compared to the limit values [2]. The decreasing order of influent sludge contents was Fe > Zn > Cr > Mn > Pb > Cu > Ni > Cd. As shown, the mean content of most metals in the residual sludge is lower compared to the influent SAS (Table 2). A slight increase occurred in Mn, possibly due to the slower precipitation of Mn oxides and also inhibition by the presence of Fe [22]. Fig. 2 shows that there is HM accumulation in the residual sludge. Accumulation proceeds slowly for Cr, Cd, Pb and Ni, which showed a slight decrease with time, and faster for Cu, Mn, Zn and Fe. It is noticeable that the contents of Cd (Fig. 2e) and Ni (Fig. 2d) present a reduction with time, which coincided with respective decrease of influent concentrations. These two metals (Cd and Ni) present relatively high mobility and bioavailability in sediments [5]. Similar variations (low accumulation rates) have been reported by Uggetti et al. [17] for comparable SLRs (51–55 kg dm/m^2/yr), while Uggetti et al. [23] report negligible accumulation at even higher SLRs (125–175 kg dm/m^2/yr). Concerning the three units receiving high Cr concentration (S7–S9; Fig. 2c), the accumulation rate was higher and the Cr content in the residual sludge exceeded the influent SAS content after the second year of operation, as a result of the high influent concentration. This also seems to be the case for Mn, whose influent concentrations were increased during the second year of operation, resulting in higher Mn accumulation in residual sludge.

Slight seasonal variations occurred in HM content. A small increase was observed during summer, when higher temperatures enhanced the dewatering process, thus, decreasing the volume of the sludge layer. The application of the resting phase resulted in an increase of Cu, Mn, and Fe, possibly due to limited oxidation (limited oxygen transfer by plant roots due to gradual plant dryness), while Cr, Cd, Zn, Pb and Ni content was decreased (Table 2), as the lack of loadings assisted in downward metal leaching and draining. The three units with additional Cr (S7, S8 and S9) showed a high Cr increase during the resting phase, since the sludge volume reduction resulted in the increase of the already highly accumulated Cr content.

On the whole, the concentrations of all HMs were below the legal limits of the Sludge Directive 86/278/EEC [2], as also the 3rd Draft EU Working Document on Sludge [4] for land application.

3.2.2. Upper–lower residual sludge layer

The mean HM content of the upper and lower half of the residual sludge layer for the entire operation period is presented in Fig. 3. Slightly increased concentrations with depth were found for most metals (Cr, Cd, Pb, Cu, Ni, Zn, Fe); however, these differences were not statistically significant (paired t-tests: $p < 0.05$), as also reported elsewhere [16,24], with the exception of Cr content in units S7 and S9, which received additional Cr (see Supplemental online material (SM); Table SM-1). The lower half of the residual sludge layer contains sludge from older applications. The dewatering process reduces the sludge layer volume during the resting periods, and through downward flow (drainage) a slightly increased metal content is seen in the lower half.

3.2.3. Drained water

Table 3 presents the HM concentrations in the influent sludge and the drained water for all SDRBs, and the respective percentage of the influent concentration that drains at three different time points after sludge application. Mean HM concentrations in the drained water were quite lower compared to the influent SAS, as also reported elsewhere [11,24], and the differences were found to be statistically significant in all cases (paired t-tests: $p < 0.05$; Table SM-1). Standard deviation values were low (i.e., 0.11 for Cr, 0.15 for Cu, 0.03 for Cd, 0.13 for Ni, 0.17 for Pb, 0.17 for Mn, 0.09 for Zn and 0.21 for Fe), especially, after the first year of operation, when the pollutant concentrations in the drained water gradually increased with time. Lower concentrations were observed for Fe and Zn, due to the precipitation of formed metal oxides, and the lowest for Ni with more than 31% of the influent concentration lost through draining. Furthermore, the measurements at different time points show that HM concentration tends to decrease with time, and after 1 day, the mean HM concentration in the drained water of the SDRB units corresponds to less than 10% of the influent concentration. In the three units with additional Cr, the Cr concentration in the drained water was higher. This means that more Cr leaves the bed through drainage. On the whole, about 16.3% on the

Table 2

Mean and standard deviation of heavy metal content (mg/(kg dm)) during the loading (L-phase: October 2007–April 2010) and resting (R-phase: April 2010–October 2010) phases for all units; and limit values, according to various standards.

	Cr	Cd	Pb	Cu	Ni	Mn	Zn	Fe
Directive 86/271 [2]	–	20–40	750–1200	1000–1750	300–400	–	2500–4000	–
3rd Draft 2000 [4]	1000	10	750	1000	300	–	2500	–
MD 80568/4225/91	500/10[a]	20–40	750–1200	1000–1750	300–400	–	2500–4000	–
SAS	275.8 ± 71.6 (5002 ± 1494[b])	9.6 ± 2.7	117.5 ± 38.0	94.7 ± 49.4	83.9 ± 42.7	140.7 ± 133.5	422.9 ± 223.8	3354 ± 2158
S1								
L-phase	144.0 ± 117.1	6.8 ± 4.4	58.1 ± 23.1	80.8 ± 53.2	70.9 ± 33.1	159.6 ± 139.7	420.5 ± 264.9	3087.0 ± 2051.0
R-phase	61.0 ± 21.9	4.7 ± 4.1	31.1 ± 16.3	115.6 ± 7.2	27.8 ± 16.1	251.0 ± 115.6	527.9 ± 147.0	4578.0 ± 79.0
S2								
L-phase	162.2 ± 164.5	8.1 ± 4.7	61.8 ± 25.8	86.6 ± 47.4	45.3 ± 16.7	141.6 ± 114.1	371.8 ± 217.5	2979.0 ± 1941.0
R-phase	38.9 ± 15.2	4.1 ± 3.4	37.2 ± 22.9	103.2 ± 18.2	29.5 ± 11.7	241.9 ± 68.0	526.8 ± 210.8	4755.0 ± 381.0
S3								
L-phase	232.6 ± 177.9	9.8 ± 5.5	90.4 ± 32.5	91.5 ± 66.2	57.9 ± 28.5	141.8 ± 108.4	432.2 ± 253.0	3052.0 ± 2144.0
R-phase	35.0 ± 13.2	4.8 ± 2.9	36.4 ± 16.2	124.9 ± 13.5	23.7 ± 29.5	208.1 ± 15.8	415.8 ± 191.6	4459.0 ± 897.0
S4								
L-phase	197.6 ± 173.8	10.4 ± 6.4	89.1 ± 29.1	89.6 ± 43.0	59.8 ± 27.1	157.2 ± 109.7	413.1 ± 259.5	3104.0 ± 2188.0
R-phase	53.7 ± 34.4	4.5 ± 3.8	38.7 ± 26.3	115.2 ± 5.6	23.6 ± 27.1	205.0 ± 8.5	462.3 ± 218.3	4394.0 ± 698.0
S5								
L-phase	142.6 ± 134.3	5.5 ± 3.7	46.2 ± 22.7	86.4 ± 56.6	45.6 ± 17.1	145.7 ± 87.3	383.5 ± 161.3	2632.0 ± 1746.0
R-phase	295.8 ± 28.2	2.3 ± 2.7	19.7 ± 12.2	126.5 ± 13.3	23.3 ± 34.7	229.8 ± 73.1	397.5 ± 128.0	4255.0 ± 759.0
S6								
L-phase	182.6 ± 118.5	6.0 ± 3.3	57.0 ± 23.3	89.6 ± 52.7	47.9 ± 29.5	144.5 ± 108.1	395.6 ± 203.3	3068.0 ± 1946.0
R-phase	110.1 ± 66.9	4.3 ± 6.6	26.0 ± 19.1	102.4 ± 10.8	34.1 ± 30.0	171.5 ± 25.0	364.1 ± 147.2	4158.0 ± 367.0
S7[b]								
L-phase	3462.8 ± 2060.3	7.5 ± 4.8	67.3 ± 30.1	90.6 ± 45.9	59.2 ± 20.0	149.3 ± 126.2	395.4 ± 238.9	2590.0 ± 1697.0
R-phase	8604.6 ± 2952.4	2.5 ± 3.6	39.8 ± 21.5	94.1 ± 12.9	43.0 ± 1.0	169.8 ± 54.2	377.3 ± 116.4	3970.0 ± 335.0
S8[b]								
L-phase	3322.0 ± 2078.1	6.1 ± 3.7	64.9 ± 26.7	80.6 ± 55.1	55.2 ± 24.9	125.6 ± 111.9	369.9 ± 230.5	2644.0 ± 1843.0
R-phase	7686.4 ± 1221.9	3.7 ± 5.4	37.5 ± 30.2	112.8 ± 6.8	45.8 ± 7.8	168.3 ± 15.5	418.3 ± 207.1	4166.0 ± 755.0
S9[b]								
L-phase	3624.6 ± 2229.8	7.2 ± 4.9	64.4 ± 23.2	87.8 ± 44.3	57.6 ± 24.7	137.9 ± 109.8	402.8 ± 225.5	2815.0 ± 2005.0
R-phase	8041.5 ± 1972.1	2.9 ± 4.1	49.9 ± 24.1	95.2 ± 18.4	42.3 ± 14.6	139.7 ± 82.6	362.6 ± 143.0	4107.0 ± 361.0
S10								
L-phase	304.2 ± 237.2	9.0 ± 4.5	90.5 ± 33.1	95.5 ± 58.7	63.9 ± 37.4	173.7 ± 141.3	404.2 ± 191.0	2880.0 ± 1862.0
R-phase	188.5 ± 103	5.2 ± 7.5	51.6 ± 4.7	127.7 ± 19.1	30.3 ± 11.9	149.5 ± 82.8	401.0 ± 123.2	4539.0 ± 232.0
S11								
L-phase	223.5 ± 212.6	8.8 ± 5.8	66.3 ± 20.8	83.5 ± 48.0	43.9 ± 17.2	148.5 ± 110.7	380.6 ± 197.5	2539.0 ± 1729.0
R-phase	146.6 ± 61.7	5.0 ± 7.3	36.6 ± 18.3	146.9 ± 34.2	31.1 ± 7.5	222.9 ± 144.8	660.7 ± 328.3	4366.0 ± 237.0
S12[c]								
L-phase	106.9 ± 107.7	6.3 ± 3.1	50.5 ± 17.9	72.4 ± 43.4	43.8 ± 30.1	122.2 ± 82.0	316.1 ± 169.0	2316.0 ± 1596.0
R-phase	116.5 ± 131.8	5.3 ± 2.4	34.0 ± 9.5	112.1 ± 21.0	34.1 ± 24.4	194.0 ± 155.9	568.5 ± 164.1	4236.0 ± 538.0
S13[d]								
R-phase	106.5 ± 78.4	5.4 ± 2.7	36.3 ± 15.2	94.8 ± 54.2	37.6 ± 13.4	168.7 ± 74.2	385.5 ± 188.1	3716.0 ± 1368.0

[a] Cr(III) = 500 mg/(kg dm) and Cr(VI) = 10 mg/(kg dm).

[b] Cr added.

[c] Loading of unit S12 started in October 2006. Data presented here are for the period October 2007–October 2010, as for units S1–S11.

[d] For S13 unit, only data from its extended resting phase are presented (June 2008–October 2010).
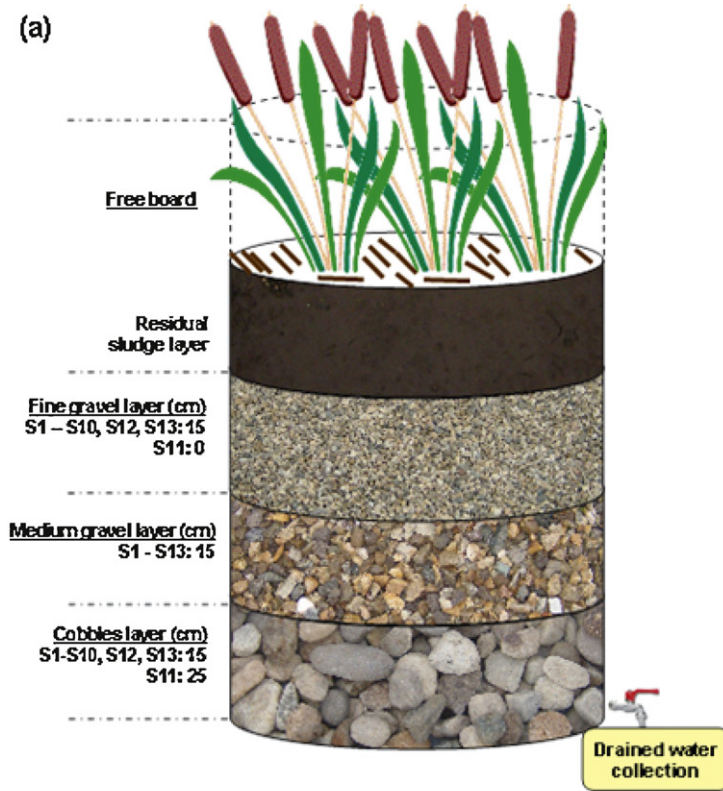
Fig. 1. (a) Schematic vertical section of the pilot-scale SDRB units [19,20] and (b) view of some pilot-scale units.

average of the influent HM concentration is lost through draining, a value comparable to that (13.3%) reported by Begg et al. [11].

3.2.4. Plant uptake

Accumulation of metals in plants is another factor of HM removal [22,25]. Table 4 presents the produced aboveground biomass densities for each annual harvesting campaign, as also metal analyses in above- and belowground biomass. Biomass values significantly increased year-by-year as the reeds grew and

matured, especially after the second year of operation, when the produced biomass values were 2–4 times higher than the respective values of the first year. Common reeds appeared to be quite tolerant of HM uptake [25], since even units S7, S8 and S9 with high Cr content did not show any obvious toxicity signs. Cattails in unit S3 and reeds in unit S10 (no aeration tubes) dried during the first summer of operation (2008). The HM concentrations in plant biomass were, in decreasing order, Cr > Fe > Zn > Mn > Cu > Pb > Ni > Cd. Uptake values significantly increased year-by-year for most metals, reaching
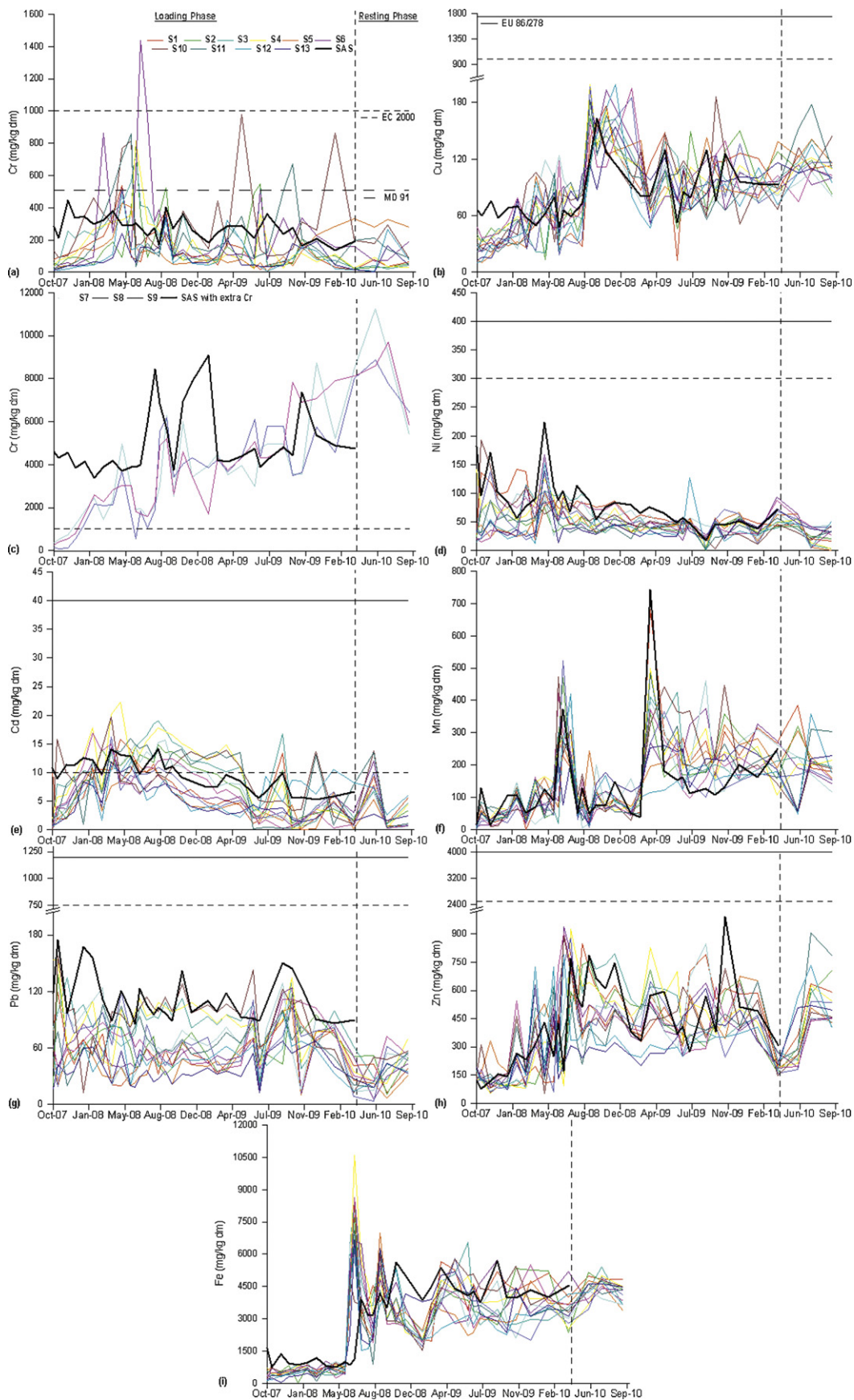
Fig. 2. Timeseries charts of heavy metal content in the residual sludge: (a) Cr, (b) Cu, (c) Cr in units with extra Cr addition; (d) Ni, (e) Cd, (f) Mn, (g) Pb, (h) Zn, and (i) Fe.

Table 3
Mean concentration of heavy metals in the influent SAS and the drained water for all SDRB units at various time points, and respective percentage of the influent concentration that drains at each time point in all units.

	Cr		Cd		Pb		Cu		Ni		Mn		Zn		Fe	
	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%
IN (SAS)	2.78 (24.8[a])		0.10		1.18		0.95		0.84		1.41		4.23		33.54	
S1																
10 min	0.75	27.0	0.02	21.1	0.50	42.6	0.14	14.8	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
2 h	0.32	11.5	0.15	23.4	0.15	12.8	0.22	22.7	0.34	40.6	0.51	36.0	0.19	4.4	0.59	1.8
1 d	0.05	1.8	0.04	1.1	0.04	3.4	0.11	11.6	0.14	16.7	0.48	34.1	0.00	0.0	1.16	3.5
S2																
10 min	0.63	22.7	0.00	0.0	0.41	34.9	0.31	32.7	0.27	32.2	0.00	0.0	0.00	0.0	0.00	0.0
2 h	0.33	11.9	0.06	57.9	0.22	18.7	0.21	22.2	0.18	21.5	0.57	40.5	0.46	10.9	2.33	6.9
1 d	0.03	1.1	0.00	0.0	0.00	0.0	0.05	5.3	0.00	0.0	0.00	0.0	0.29	6.8	0.52	1.6
S3																
10 min	0.64	23.0	0.04	42.1	0.00	0.0	0.46	48.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
2 h	0.15	5.4	0.03	33.4	0.75	63.8	0.29	30.5	0.66	78.7	0.19	13.9	0.32	7.5	1.01	3.0
1 d	0.02	0.7	0.00	0.0	0.00	0.0	0.22	23.2	0.68	81.0	0.00	0.0	0.18	4.2	0.00	0.0
S4																
10 min	0.45	16.2	0.00	0.0	0.20	17.0	0.59	62.3	0.54	64.4	0.00	0.0	0.00	0.0	0.00	0.0
2 h	0.31	11.2	0.04	44.2	0.51	43.4	0.22	22.9	0.39	47.4	0.06	4.5	0.37	8.7	0.97	2.9
1 d	0.01	0.4	0.00	0.0	0.00	0.0	0.19	20.1	0.22	26.2	0.00	0.0	0.00	0.0	0.2	0.6
S5																
10 min	0.81	29.1	0.00	0.0	0.00	0.0	0.58	61.2	0.20	23.8	0.00	0.0	0.00	0.0	0.00	0.0
2 h	0.52	18.7	0.01	11.8	0.50	42.7	0.10	10.8	0.19	22.5	0.40	28.3	0.09	2.0	0.75	2.2
1 d	0.08	2.9	0.01	6.3	0.11	9.4	0.08	8.4	0.00	0.0	0.00	0.0	0.00	1.5	1.52	4.5
S6																
10 min	0.88	3.17	0.00	0.0	0.42	35.7	0.61	64.4	0.42	50.1	0.18	12.8	0.00	0.0	0.00	0.0
2 h	0.30	10.8	0.003	28.3	0.18	14.9	0.07	7.8	0.18	21.4	0.12	8.4	0.08	1.9	0.03	0.1
1 d	0.13	4.7	0.02	21.1	0.00	0.0	0.08	8.4	0.00	0.0	0.00	0.0	0.00	0.0	0.06	1.8
S7[a]																
10 min	17.22	69.4	0.01	12.6	0.22	18.7	0.55	58.1	0.52	62.0	0.00	0.0	0.00	0.0	0.00	0.0
2 h	11.33	45.7	0.02	21.1	0.35	29.8	0.13	13.2	0.23	27.4	0.38	27.0	0.31	7.3	1.10	3.3
1 d	8.11	32.7	0.00	3.4	0.24	20.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	2.2	6.6
S8[a]																
10 min	15.55	62.7	0.07	68.4	0.54	46.0	0.50	52.7	0.31	36.9	0.10	7.1	0.00	0.0	0.00	0.0
2 h	10.10	40.7	0.03	32.6	0.20	17.1	0.11	11.4	0.22	26.2	0.12	8.8	0.26	6.2	0.40	1.2
1 d	7.5	30.2	0.00	0.0	0.08	6.8	0.06	6.3	0.05	6.0	0.00	0.0	0.00	0.0	0.20	0.6
S9[a]																
10 min	16.16	65.2	0.08	84.2	0.65	55.3	0.50	52.8	0.56	66.7	0.00	0.0	0.00	0.0	0.00	0.0
2 h	12.22	49.3	0.01	12.6	0.21	17.9	0.11	11.5	0.09	10.3	0.18	13.1	0.12	2.9	0.00	0.0
1 d	7.76	31.3	0.00	0.0	0.11	9.4	0.10	10.6	0.03	3.8	0.00	0.0	0.00	0.0	3.88	11.6
S10																
10 min	0.42	15.1	0.00	0.0	0.50	42.6	0.24	25.3	0.72	85.8	0.76	54.0	0.00	0.0	0.08	0.2
2 h	0.16	5.8	0.03	31.1	0.16	13.6	0.09	9.1	0.36	43.4	0.27	19.2	0.19	4.6	0.23	0.7
1 d	0.09	3.2	0.00	0.0	0.05	4.3	0.24	25.3	0.56	66.7	0.52	36.7	0.00	0.0	3.16	9.4
S11																
10 min	1.05	37.8	0.06	63.2	0.52	44.3	0.10	10.6	0.58	69.1	0.65	46.2	0.00	0.0	0.50	1.5
2 h	0.71	25.5	0.01	11.2	0.31	26.4	0.22	22.8	0.22	25.6	0.22	15.6	0.15	3.6	0.00	0.0
1 d	0.25	9.0	0.02	25.3	0.15	12.8	0.00	0.0	0.20	23.8	0.08	5.7	0.00	0.0	0.00	0.0
S12																
10 min	0.42	15.1	0.00	0.0	0.42	35.7	0.17	18.0	0.28	33.4	0.06	4.3	0.00	0.0	0.00	0.0
2 h	0.23	8.3	0.01	9.5	0.14	11.9	0.09	9.3	0.13	15.5	0.16	11.4	0.09	2.1	0.52	1.6
1 d	0.02	0.7	0.01	10.5	0.04	3.4	0.03	2.6	0.00	0.0	0.00	0.0	0.20	4.7	1.33	4.0

[a] Chromium addition.

higher values during the second year or operation. Lower concentrations at the last harvesting are rational, since the loading during the last year lasted for only 3 months (till April 2010). Increased uptake rates are linked to higher sludge loads. The maximum HM concentration was found in the roots, followed by leaves and stems, as also found by Cheng et al. [26] and Yadav et al. [27].

The mobility of metals within the reeds was tested by calculating the translocation factors (TF) of all the HMs in plants [26,28]. Five TF expressions were used (Table 5): the ratio of above- to belowground biomass content (TF_1), the ratio of sludge content to root content (TF_2), the ratio of leaf to stem content (TF_3), the ratio of leaf to root content (TF_4) and the ratio of stem to root content (TF_5). TF1 values

showed that roots accumulated in some cases nearly the double amount of metals compared to the aboveground biomass, with the exception of Zn which showed a higher mobility, while the lower TF_1 values were observed for Cu, as also reported by Cheng et al. [26]. Despite the high HM content in the roots, TF_2 values show that HM accumulation in the residual sludge is higher for most metals. TF_3 for all HMs was above 2, which means that the HM content in leaves is 2–5 times higher than that in stems, but lower compared to the HM content in the roots (TF_4), except of Zn and Mn which present a higher mobility. Finally, TF_5 values show than the HM content in stems is less than half of that in roots. On the whole, it can be stated that HM accumulation is higher in the belowground

Table 4
Harvested biomass densities and heavy metal contents in various plant parts.

	S1	S2	S3	S5	S6	S7	S8	S9	S10	S11	S12
Density (g/m²)											
Jan 2008	119.3	100.0	121.1	112.3	71.9	200.0	257.9	152.6	149.1	101.8	445.9
Jan 2009	363.2	466.7	–	377.2	419.3	398.2	198.2	326.3	–	452.6	770.8
Jan 2010	1496.5	1078.9	–	680.7	721.1	1354.4	400.0	886.0	–	1152.6	857.9
Jan 2011	701.8	736.8	–	438.6	526.3	1403.5	614.0	626.3	–	1052.6	621.0

|	S1		S2	S3	S5	S6	S7		S8		S9		S10	S11	S12
	L[a]	S[a]	L+S	L+S	L+S	L+S	L	S	L	S	L	S	L+S	L+S	L+S
Cr (mg/(kg dm))															
Jan 2008	113.1		102.3	60.0	78.9	240.0	717.0		211.5		1563.4		33.0	27.5	75.3
Jan 2009	55.6	30.3	99.8	–	46.6	129.3	846.7	74.1	3027.6	507.0	819.6	97.5	–	86.6	52.2
Jan 2010	75.9	58.4	117.4	–	86.6	154.8	1770.9	351.8	4242.9	793.7	2117.7	703.6	–	97.5	56.7
Jan 2011	8.8	4.0	15.0	–	18.7	43.5	38.7	23.1	28.0	17.5	16.2	14.5	–	36.0	23.8
Roots	115.9		173.0	–	169.5	180.8	4876.7		4627.7		2456.6		–	143.1	281.2
Cd (mg/(kg dm))															
Jan 2008	0.18		0.26	0.66	0.17	0.42	0.12		0.11		0.15		0.18	0.06	0.12
Jan 2009	1.62	1.59	0.54	–	0.69	0.67	1.34	0.47	0.18	0.09	0.39	0.39	–	0.100	0.19
Jan 2010	0.92	0.42	0.38	–	0.41	0.28	0.89	0.58	0.69	0.31	1.04	0.74	–	0.33	0.15
Jan 2011	0.41	0.38	1.33	–	1.07	0.25	1.86	0.59	0.50	0.23	0.12	0.09	–	0.64	0.11
Roots	1.60		1.21	–	1.44	0.86	0.81		1.24		0.79		–	0.34	0.45
Pb (mg/(kg dm))															
Jan 2008	11.4		11.2	18.5	7.0	22.5	6.4		5.1		7.1		9.8	6.3	8.2
Jan 2009	16.2	9.2	9.9	–	8.6	13.5	12.2	7.1	26.7	5.9	9.6	7.3	–	12.4	20.0
Jan 2010	28.6	8.4	10.4	–	10.4	13.3	21.5	14.1	43.7	17.1	39.2	23.9	–	24.1	29.5
Jan 2011	30.7	13.3	19.0	–	15.2	15.3	18.3	14.1	18.4	9.1	14.7	6.4	–	18.3	19.3
Roots	101.0		69.5	–	63.5	60.9	60.8		80.8		32.2		–	34.1	34.4
Cu (mg/(kg dm))															
Jan 2008	3.4		6.3	16.9	3.0	18.7	7.3		5.5		5.5		11.2	6.8	4.4
Jan 2009	24.7	7.9	13.8	–	14.2	15.5	24.9	15.9	33.6	13.0	12.9	8.2	–	9.6	16.1
Jan 2010	49.9	28.5	21.5	–	23.7	23.1	35.2	19.3	44.4	13.3	31.8	12.8	–	26.2	22.4
Jan 2011	18.0	8.3	11.4	–	14.7	14.8	13.9	8.7	14.1	11.2	13.6	12.1	–	16.2	14.8
Roots	194.6		65.9	–	108.9	74.2	111.6		117.8		48.8		–	40.4	51.5
Ni (mg/(kg dm))															
Jan 2008	0.71		1.02	0.36	0.45	1.58	0.90		0.69		0.89		1.05	1.25	0.92
Jan 2009	18.1	12.0	5.5	–	11.4	10.6	5.4	2.0	13.4	3.9	13.6	6.4	–	4.7	10.9
Jan 2010	16.5	16.3	10.3	–	10.5	11.5	22.1	14.0	12.7	7.3	6.2	1.7	–	4.8	8.7
Jan 2011	5.5	3.1	6.1	–	4.5	6.0	8.3	4.3	8.5	2.4	5.5	2.3	–	1.9	4.6
Roots	35.4		26.3	–	23.3	16.2	16.8		24.7		11.2		–	5.9	76.9
Mn (mg/(kg dm))															
Jan 2008	36.1		38.3	190.5	13.5	76.4	23.4		30.2		25.1		64.4	32.7	24.9
Jan 2009	222.3	54.8	51.9	–	160.2	80.9	282.5	154.8	210.3	53.7	250.9	46.9	–	75.3	85.9
Jan 2010	217.8	83.9	52.9	–	104.4	96.2	275.0	111.7	239.8	57.2	154.7	54.4	–	79.8	75.9
Jan 2011	110.6	34.4	22.7	–	67.2	69.1	252.9	32.1	187.6	52.4	220.4	70.9	–	53.2	45.2
Roots	240.8		142.9	–	209.2	103.7	143.0		181.6		59.7		–	85.2	119.9
Zn (mg/(kg dm))															
Jan 2008	87.4		104.4	96.2	68.4	118.2	98.4		108.0		114.3		113.7	85.0	92.1
Jan 2009	198.0	120.5	105.2	–	154.4	132.4	201.4	154.0	161.6	115.2	144.2	86.9	–	126.1	3.1
Jan 2010	218.8	216.6	164.1	–	185.1	198.5	233.4	169.7	196.3	172.1	220.7	133.0	–	187.3	188.0
Jan 2011	83.4	43.5	60.3	–	81.8	81.5	58.1	43.6	95.3	59.2	72.6	36.3	–	81.3	75.5
Roots	155.5		124.3	–	132.4	123.2	131.1		105.1		99.6		–	123.4	122.6
Fe (mg/(kg dm))															
Jan 2008	225.8		290.0	578.5	150.1	835.6	173.7		188.2		217.2		581.3	267.8	192.1
Jan 2009	722.7	95.2	188.9	–	374.7	507.8	399.3	106.2	499.1	274.4	377.0	116.3	–	263.3	252.1
Jan 2010	1499.0	559.6	453.3	–	491.3	593.0	748.8	300.9	2259.0	341.1	1211.1	374.8	–	925.2	415.2
Jan 2011	227.1	69.6	45.5	–	118.8	83.8	131.2	66.7	196.0	56.6	63.1	42.3	–	103.9	130.1
Roots	4257.0		2719.8	–	2686.7	1760.1	1419.4		1702.7		965.7		–	1535.0	1340.7

[a] L: leaves and S: stems.

Table 5
Heavy metals translocation factors: ratio of above- to belowground biomass content (TF_1), sludge content to root content ratio (TF_2), leaf to stem content ratio (TF_3), leaf to root content ratio (TF_4) and stem to root content ratio (TF_5).

	Cr		Cd		Pb		Cu		Ni		Mn		Zn		Fe	
	TF1	TF2	TF1	TF2	TF1	TF2	TF1	TF2	TF1	TF2	TF1	TF2	TF1	TF2	TF1	TF2
S1	0.96	1.18	0.51	4.14	0.14	0.59	0.10	0.43	0.30	1.90	0.45	0.70	1.00	2.76	0.13	0.76
S2	0.62	0.98	0.32	6.38	0.15	1.23	0.21	1.34	0.21	1.67	0.33	1.05	1.00	3.10	0.11	1.12
S5	0.42	0.83	0.29	3.61	0.14	0.86	0.13	0.82	0.32	1.87	0.44	0.73	1.03	2.91	0.13	1.03
S6	0.97	1.47	0.53	6.82	0.27	1.07	0.26	1.22	0.49	2.89	0.81	1.42	1.22	3.19	0.37	1.80
S7	0.26	0.80	0.72	8.68	0.19	1.03	0.16	0.81	0.45	3.45	1.01	1.06	1.21	3.00	0.22	1.91
S8	0.63	0.80	0.20	4.73	0.21	0.78	0.16	0.71	0.26	2.20	0.57	0.71	1.37	3.56	0.37	1.63
S9	0.72	1.63	0.60	8.65	0.49	2.70	0.26	1.81	0.45	5.13	1.56	2.31	1.36	4.01	0.43	3.03
S11	0.49	1.52	0.47	24.70	0.42	1.44	0.35	2.20	0.61	7.26	0.74	1.82	1.08	3.28	0.32	1.76
S12	0.23	0.38	0.34	13.64	0.56	1.09	0.28	1.47	0.09	0.56	0.52	1.07	1.12	2.75	0.21	1.85
Mean	0.59	1.07	0.44	9.04	0.28	1.20	0.21	1.20	0.35	2.99	0.72	1.21	1.15	3.17	0.25	1.65

	Cr			Cd			Pb			Cu			Ni			Mn			Zn			Fe		
	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5	TF3	TF4	TF5
S1	1.78	0.40	0.27	1.43	0.61	0.50	2.49	0.25	0.10	2.35	0.16	0.08	1.43	0.38	0.30	3.29	0.76	0.24	1.52	1.07	0.82	4.51	0.19	0.06
S7	8.23	0.18	0.03	2.50	1.68	0.68	1.51	0.28	0.19	1.66	0.22	0.13	2.05	0.71	0.40	4.06	1.89	0.70	1.34	1.25	0.93	2.74	0.30	0.11
S8	4.31	0.53	0.09	2.17	0.37	0.17	3.04	0.37	0.13	2.40	0.26	0.11	2.92	0.47	0.18	3.90	1.17	0.30	1.38	1.44	1.10	3.97	0.58	0.13
S9	4.18	0.40	0.11	1.24	0.65	0.51	1.76	0.66	0.39	1.72	0.40	0.23	2.75	0.77	0.31	3.77	3.50	0.96	1.77	1.46	0.86	2.66	0.57	0.18
Mean	4.62	0.38	0.13	1.83	0.83	0.46	2.20	0.39	0.20	2.03	0.26	0.13	2.29	0.58	0.30	3.75	1.83	0.55	1.51	1.31	0.93	3.47	0.41	0.12
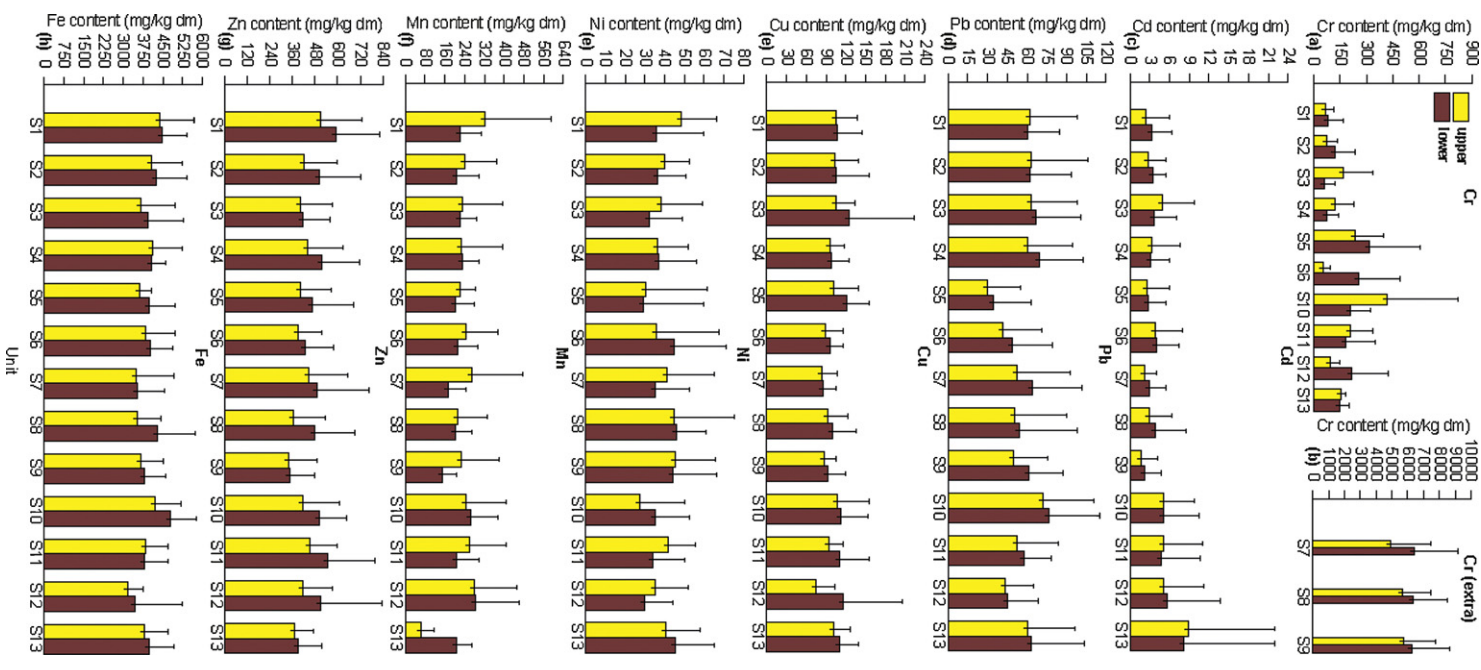
Fig. 3. Mean heavy metal content in the upper and lower half of the residual sludge layer during the entire period of operation (October 2007–October 2010) for all units.

biomass than in the aerial plant parts, as also reported by others [27–29].

3.3. Heavy metal mass balance

Fig. 4 presents the allocation of the influent SAS among the various components of the SDRB units, which include the residual sludge layer, the plants, the drained water and the substrate
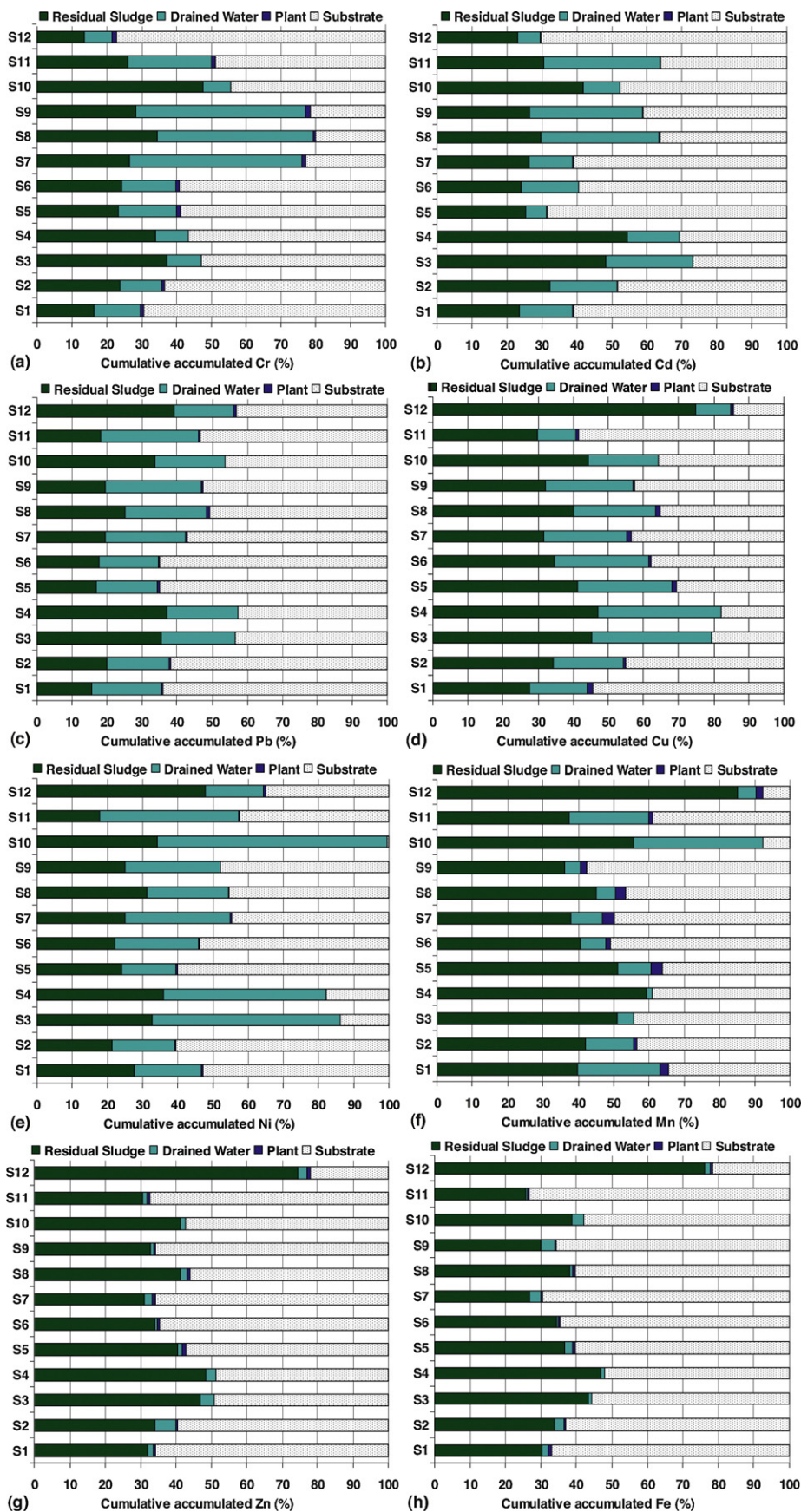
Fig. 4. Heavy metal mass balance in the SDRB units for: (a) Cr, (b) Cd, (c) Pb, (d) Cu, (e) Ni, (f) Mn, (g) Zn, and (h) Fe.

material. Values refer to total Cr, i.e., the sum of Cr(III) and Cr(VI). The figure shows that, on the average, more than 35% of the influent HM content is accumulated within the residual sludge layer. Mn showed the higher accumulation rate in the residual sludge (mean 48.5% of all units), while Pb and Ni accumulation was the lowest (mean 24.8%). Mn appears to be less available for plants because it is precipitated as hydrous oxides. The increasing trend of metal accumulation in the residual sludge was Pb < Ni < Cr < Cd < Fe < Cu < Zn < Mn. HM losses in drained water reached 16.8% (mean value of all units). The highest concentration in drained water was observed for Ni (mean 31.4%) and the lower for Fe (mean 1.9%), while the increasing trend was Fe < Zn < Mn < Cd < Pb < Cu < Cr < Ni. The lower Fe, Zn and Mn concentration in the drained water could be explained by the precipitation of respective oxides within the well-aerated bed. Direct plant uptake was low (<3%) for all units. Cd showed the lowest plant uptake (mean 0.18%), since Fe, Mn and Zn are antagonistic to Cd, and Mn showed the highest (mean 2.13%) [24]. The increasing trend was Cd < Ni < Fe < Pb < Cr < Zn < Cu < Mn. Low uptake of Ni could also be linked to the presence of antagonistic metals (Cu, Fe, Zn) during its absorption by plants [5]. Generally, the high uptake of Cu, Mn and Zn seems to limit the uptake of other metals [24], which present a limited bioavailability in sediments. Especially, Zn within the plant is linked to the metabolism of proteins, sugars and phosphate [4]. Finally, the accumulation in the gravel layers appears to be the main HM sink. Subtracting the percent values of the other components (residual sludge, plant, drained water), it appears more than 47% of the influent metal content is retained there. Binding to sediments and organic matter and precipitation and adsorption onto substrate material is the major HM removal process in wetland systems [25]. The highest gravel accumulation was observed for Fe (mean 59.2%) and the lower for Cu (mean 36.4%). The increasing trend was Cu < Mn < Ni < Cr < Cd < Pb < Zn < Fe. Yadav et al. [27] presented the mass balance for metal removal from wastewater using a VFCW and reported even higher gravel accumulation rates reaching 68–80% .

HM accumulation in the residual sludge is not intensive. The mass balance study implies that the gravel layers play the major role in removal of HMs. The main removal mechanisms include sedimentation, adsorption to suspended matter (organic substances), and precipitation as oxides, carbonates and sulfides [22,25]. Particularly, sorption of HM is favoured, since the residual sludge pH values in all units varied between 6 and 7. The measured slight decrease of sulfate in the drained water (data not presented here), combined with the feeding regime of these systems, implies that aerobic conditions generally dominate within the bed, thus, sulfide precipitation is limited. Gravel layers function as a sink for metals, since adsorption and sedimentation of polluted with HM particles results in intense metal accumulation [29]. Furthermore, annual plant harvesting accounted for only 1–3% on the average HM mass removal, while the high accumulation rate in roots indicate respective availability in the substrate materials.

3.4. Comparison between units

3.4.1. Effect of porous media origin

Interesting remarks can be drawn by comparing the various SDRB units. Units S1 and S2 were similar, except of the porous media origin (igneous and carbonate rock respectively; Table 1). Small differentiations appeared between these two units. The differences between the metal content in the residual sludge (Table 2) were found to be statistically significant only for Ni (paired t-tests: $p < 0.05$; Table SM-2). Ni content was higher in unit S1, possibly due to the higher Fe content of the igneous rock of this unit which is antagonistic to Ni adsorption. Unit S1 showed higher accumulation rates for most metals in the substrate, while both units had

comparable HM concentrations in drained water (Table 3). The reed growth in unit S1 seems to be favored (mean plant biomass density 853.8 and 595.6 g/m^2 in unit S1 and S2, respectively) and plant uptake was double in unit S1 compared to unit S2 (Table 5). The duration of the operation appears to significantly affect the content of most HM in the residual sludge (two-way ANOVA: $p < 0.05$; Table 6), while no interactions were observed between the material origin and the time of operation. This indicates the relation between HM adsorption on the material and the material gradual saturation with time, irrespectively of the origin of the two materials tested. It is also interesting that, as post hoc tests revealed, the differences occurred between the first and the other two years of operations, which implies a possible start-up period until the plants are fully developed before the system reaches its higher performance.

3.4.2. Effect of porous media size

The effect of material size appears to be more important than material origin. Units S1 and S11 were similar, but the material in unit S11 was coarser, which resulted in higher drained water volume. Accumulation in the substrate was lower in S11 (Fig. 4), possibly due to the faster drainage and shorter contact time, and smaller available surface of the coarse-grained material. Plant accumulation was more than double in unit S1 compared to unit S11 (Table 4). Accumulation in sludge was higher in unit S1 for Cr, Cd, Pb and Cu. Statistically significant differences were found for Cr, Cd, Ni and Fe content in the residual sludge (paired t-tests: $p < 0.05$; Table SM-2). As before, the time parameter significantly affected the content of most HM (except of Cr and Pb) in the residual sludge (two-way ANOVA: $p < 0.05$; Table 6), while no interactions were observed between the material size and the operation time. Post hoc tests also revealed that the differences existed again between the first and the other two years of operation, implying again the presence of a start-up period.

3.4.3. Effect of vegetation

The contribution of plants in HM removal is revealed through the comparison of unit S1 with unplanted units S3 and S4. Unplanted units showed almost double accumulation rate in the residual sludge, increased metal concentrations in the drained water (Table 3) and about 40% decrease in accumulation in the substrate (Fig. 4). The major role of plants is not in direct uptake, but in substrate stabilization, oxidation of the bed, and provision of attachment area for microbes, which affect metal mobility, bioavailability and toxicity [22]. The role of plants was statistically confirmed, since significant differences (paired t-tests: $p < 0.05$; Table SM-2) were found for most metals (Cr, Cd, Pb, Cu and Ni) in the residual sludge, comparing unit pairs S1–S3 and S1–S4. The similar unplanted units S4 and S3 did not show statistically significant differences (paired t-tests: $p < 0.05$; Table SM-2), and metal allocation among the various bed components was comparable. This indicates that the experimental conditions remained similar within the beds and system performance did not alter from bed to bed. There were also significant differences of the HM content between the planted and unplanted units with time of operation (two-way ANOVA: $p < 0.05$; Table 6), while the plant species did not affect the system performance. Significant interactions between the plant species and time were observed only for Pb (two-way ANOVA; Table 6). Post hoc tests also revealed that the differences existed between the first and the other two years of operations, while for Cr and Pb significant differences were observed for all the three years of operation.

3.4.4. Effect of aeration tubes

Unit S10 contained no aeration tubes and was unplanted. Compared to unplanted units S3 and S4 with aeration tubes, significant differences were detected only for Cr (paired t-tests: $p < 0.05$;

Table 6
F-values and significance of a two-way ANOVA of heavy metals in residual sludge from the various comparisons between the pilot-scale SDRB units with different characteristics during the entire operational time.

Comparison		Cr	Cd	Pb	Cu	Ni	Mn	Zn	Fe
Material origin (S1 vs S2)	Time	5.438*	12.652*	0.133	26.437*	13.455*	11.489*	10.666*	12.587*
	Material × time	0.294	0.713	0.400	0.711	3.029	0.529	0.367	0.254
Porous media size (S1 vs S11)	Time	2.981	10.083*	1.943	24.531*	9.983*	11.934*	8.939*	17.999*
	Size × time	0.710	0.105	1.047	0.405	0.995	0.320	1.370	0.117
Presence of plants (S1 vs S3–S4)	Time	16.386*	17.984*	5.399*	27.883*	29.012*	15.182*	10.590*	7.049*
	Plants × time	0.990	1.703	4.322*	0.420	0.249	0.899	0.891	0.490
Presence of aeration tubes (S10 vs S3–S4)	Time	3.090*	13.187*	14.176*	15.242*	36.135*	8.600*	4.293*	7.894*
	Aer. tubes × time	3.683*	1.725	1.123	0.481	0.848	0.059	0.546	0.796
Replication of experimental setup (S5 vs S12)	Time	2.584	5.234*	1.853	1.220	0.656	2.507*	1.493	4.676*
	Replication × time	8.065*	0.061	4.101*	5.853*	1.414	1.863	4.941*	0.123
Loaded vs unloaded (S1 vs S13)	Time	5.550*	9.633*	0.267	16.015*	7.960*	9.440*	4.803*	8.682*
	Load × time	0.030	0.510	1.065	7.074*	0.401	1.044	1.036	2.530
Effect of Cr addition	(A): time	21.717*	13.417*	1.848	14.824*	14.532*	10.404*	9.070*	10.856*
A: S1 vs S7	Cr × time	24.178*	0.454	0.797	3.173*	1.815	0.283	0.550	0.551
B: S5 vs S8	(B): time	22.101*	16.122*	1.558	17.794*	2.927	7.872*	2.401*	5.829*
C: S6 vs S9	Cr × time	20.962*	0.137	1.030	0.015	0.334	1.207	0.235	0.435
	(C): time	33.953*	14.462*	0.336	14.365*	3.283*	7.997*	0.702	7.100*
	Cr × time	39.323*	1.261	1.288	0.431	0.266	1.167	0.387	0.226

* Significance at the 0.05 level.

Table SM-2) in the residual sludge. This means that the lower oxygen amount within the bed, possibly limited the transformation of Cr(VI) to Cr(III) and the subsequent precipitation. Mass balance of unit S10 was comparable to those of units S3 and S4. Significant differences of the HM content were also found with time of operation ($p < 0.05$; Table 6). Two-way ANOVA revealed that the Cr content was dependent on both the presence of aeration tubes and time of operation, which implies that the continuous better aeration of the substrate enhances the Cr transformations. This also counts for all the comparisons between the three years of operations, as post hoc tests showed ($p < 0.05$).

3.4.5. Effect of loading and resting phases

The comparison of the two similar units receiving low SLR (S5 and S12), which operated for 2.5 and 3.5 years, respectively, did not reveal any statistical difference except for Cu (paired *t*-tests: $p < 0.05$; Table SM-2). Mean metal accumulation in the residual sludge of unit S12 was about 20% higher compared to unit S5 (54.3% and 32.4%, respectively; Fig. 4). Moreover, metal content differences in units S5 and S12 were around 10%, as Table 2 shows, implying that the bed possesses, in the long term, high accumulation capacity. Thus, the gradual increase of accumulated HM with time is not anticipated to exceed respective limit values. Based on two-way ANOVA, for most metals, there was no significant effect of time, while the interactions between the total time of operation and the additional year of unit S12 showed significant differences for Cr, Pb, Cu and Zn (Table 6). These differences for these metals, however, should be attributed to the differences of the first two years of operation (post hoc tests). Therefore, the one additional year operation of unit S12 does not seem to significantly alter the system operation.

S13 unit, with a longer resting phase (more than two years), showed increased content of most metals (Cr, Cd, Pb, Ni, Zn and Fe; Table 2) in the residual sludge, compared to unit S1, since the faster decrease of the residual sludge volume in unit S13 resulted in higher HM accumulation. Lower content for Cu and Mn implies that oxidation and gradual leaching and drainage of these HMs also took place with precipitation water, which was the only inflow, while the absence of drained water in unit S13 indicates that part of the accumulated in sludge HMs was transferred to the gravel layers. Significant differences were found with time of operation (two-way

ANOVA; Table 6), while post hoc tests showed that these differences existed between the resting years of unit S13 and the loading years of unit S1, indicating the effect of the prolonged resting phase in unit S13.

3.4.6. Effect of additional Cr loading

The comparison of the three units which received additional Cr (S7, S8 and S9) with the respective units S1, S5 and S6 (same SLRs) showed significant differences (paired *t*-tests: $p < 0.05$; Table SM-2) for Cr content in the residual sludge. The main Cr removal process is the reduction of Cr(VI) to Cr(III) by bacteria under both anoxic and aerobic conditions, and the concomitant precipitation as trivalent oxides [22]. The higher influent Cr load resulted in more intensive Cr accumulation in the residual sludge and higher losses in the drained water (Fig. 4). Accumulation in gravel was lower, since adsorption is a finite process. Generally, the units managed to receive the excessive Cr load with no obvious toxicity signs, but the legal limit values were quickly outmarched. The two-way ANOVA showed the Cr content in the units was very dependent on both the additional Cr loaded and the time of operation, while significant interactions were observed between these two factors (Table 6). These imply that as the loadings with extra Cr content proceed with time, so does the Cr accumulation within the residual sludge, reaching higher concentrations in the three units (S7–S9).

4. Summary and conclusions

Thirteen pilot-scale SDRBs were constructed and operated for three years. The systems were effective in HM mitigation from SAS. HM removal in the residual sludge was in the following order: Pb > Cr > Ni > Cd > Cu > Zn > Fe > Mn. The apportionment of the influent metal load showed that the major portion is retained within the gravel layers, through sedimentation, filtration, microbial interactions, chemical precipitation and adsorption. Accumulation of HMs in the residual sludge was limited, and tended to be greater in the surface sludge layer, while plant accumulation accounted for less than 3% on the average of the annual metal removal. Below-ground biomass showed a greater accumulation capacity. Metal losses through drained water reached about 16% of the overall removal.

Concerning the various design and operational parameters, the systems managed to handle the HM content even at the higher SLR (75 kg dm/m^2/yr), which implies that higher loads (e.g., 85–90 kg dm/m^2/yr) can be applied. A fine-grained material, rich in Fe and Al oxides proved to be more appropriate to be used as substrate. Planted beds with aeration tubes appear as the optimum combination, since the presence of plants and aeration tubes contributes to increased metal removal and reed growth, respectively. Moreover, high Cr concentration can be applied to the beds without subverting the reed function, but with simultaneous increased metal accumulation in the residual sludge.

On the whole, the use of SDRB systems for activated sludge treatment produces a stabilized residual sludge product, which favours the immobilization of heavy metals. The accumulation rate is not intense and the final product fulfils the legal prerequisites for land application for environmental or agricultural purposes.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.02.016.

References

[1] European Commission, DG Environment, Environmental, economic and social impacts of the use of sewage sludge on land, Final Report, Part III: Project Interim Reports, Milieu Ltd., Belgium, Brussels, 2010.

[2] European Union, Council Directive on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture, 86/278/EEC, Official Journal of the EC No L181/6-12, 4/7/1986.

[3] European Union, Council Directive concerning urban wastewater treatment, 91/271/EEC, Official Journal of the EC No L135/40, 30/5/1991.

[4] European Commission, DG Environment, Working Document on Sludge, 3rd Draft, Brussels, 2000, Available from: http://ec.europa.eu/environment/waste/sludge/pdf/sludge _en.pdf.

[5] European Commission, DG Environment, Disposal and Recycling Routes for Sewage Sludge (Parts I, II and III), Brussels, 2001, Available from: http://ec.europa.eu/environment/waste/sludge/pdf/sludge_disposal1.pdf.

[6] European Commission, DG Environment, Working Document – Sludge and Biowaste, Brussels, 2010, Available for download from: http://ec.europa.eu.

[7] E. Uggetti, I. Ferrer, E. Llorens, J. García, Sludge treatment wetlands: a review on the state of the art, Bioresour. Technol. 101 (2010) 2905–2912.

[8] J.L. De Maeseneer, Constructed wetlands for sludge dewatering, Water Sci. Technol. 35 (5) (1997) 279–285.

[9] S. Nielsen, Sludge drying reed beds, Water Sci. Technol. 48 (5) (2003) 101–109.

[10] P.S. Burgoon, K.F. Kirkbride, M. Henderson, E. Landon, Reed beds for biosolids drying in the arid northwestern United States, Water Sci. Technol. 35 (5) (1997) 287–292.

[11] J.S. Begg, R.L. Lavigne, P.L.M. Veneman, Reed beds: constructed wetlands for municipal wastewater treatment plant sludge dewatering, Water Sci. Technol. 44 (11–12) (2001) 393–398.

[12] A.I. Stefanakis, C.S. Akratos, P. Melidis, V.A. Tsihrintzis, Surplus activated sludge dewatering in pilot-scale sludge drying reed beds, J. Hazard. Mater. 172 (2009) 1122–1130.

[13] A.I. Stefanakis, V.A. Tsihrintzis, Dewatering mechanisms in pilot-scale sludge drying reed beds: effect of design and operational parameters, Chem. Eng. J. 172 (1) (2011) 430–443.

[14] S. Nielsen, N. Willoughby, Sludge treatment and drying reed bed systems in Denmark, J. Water Environ. Manage. – WEJ 19 (4) (2005) 296–305.

[15] J. Vymazal, Removal of nutrients in various types of constructed wetlands, Sci. Total Environ. 380 (2007) 48–65.

[16] B.J. Kim, E.D. Smith, Evaluation of sludge dewatering reed beds: a niche for small systems, Water Sci. Technol. 35 (6) (1997) 21–28.

[17] E. Uggetti, E. Llorens, A. Pedescoll, I. Ferrer, R. Castellnou, J. García, Sludge dewatering and stabilization in drying reed beds: characterization of three full-scale systems in Catalonia, Spain, Bioresour. Technol. 100 (17) (2009) 3882–3890.

[18] E. Peruzzi, G. Msciandaro, C. Macci, S. Doni, S.G. Mora Ravelo, P. Peruzzi, B. Ceccanti, Heavy metal fractionation and organic matter stabilization in sewage sludge treatment wetlands, Ecol. Eng. 37 (2011) 771–778.

[19] A.I. Stefanakis, V.A. Tsihrintzis, Effect of various design and operation parameters on performance of pilot-scale sludge drying reed beds, Ecol. Eng. 38 (1) (2012) 65–78.

[20] A.I. Stefanakis, D.P. Komilis, V.A. Tsihrintzis, Stability and maturity of thickened wastewater sludge treated in pilot-scale sludge treatment wetlands, Water Res. 45 (2011) 6441–6452.

[21] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, USA, 1998.

[22] D.B. Kosolapov, P. Kuschk, M.B. Vainshtein, A.V. Vatsourina, A. Wiessner, M. Kästner, R.A. Müller, Microbial processes of heavy metal removal from carbon-deficient effluents in constructed wetlands, Eng. Life Sci. 4 (5) (2004) 403–411.

[23] E. Uggetti, I. Ferrer, J. Molist, J. García, Technical economic and environmental assessment of sludge treatment wetlands, Water Res. 45 (2) (2010) 573–582.

[24] N. Staelens, C. Polprasert, T. Koottatep, P. Parpian, Fate of metals and heavy metals in constructed wetlands for septage dewatering, in: Proceedings of International Seminar on CWs – A Promising Technology for Septage Management and Treatment, AIT, Bangkok, Thailand, 1999.

[25] T.Y. Yeh, Removal of metals in constructed wetlands: a review, J. Hazard. Toxic Radioact. Waste 12 (2) (2008) 96–101.

[26] S. Cheng, W. Grosse, F. Karrenbrock, M. Thoennessen, Efficiency of constructed wetlands in decontamination of water polluted by heavy metals, Ecol. Eng. 18 (2002) 317–325.

[27] A.K. Yadav, N. Kumar, T.R. Sreekrishnan, S. Satya, N.R. Bishnoi, Removal of chromium and nickel from aqueous solution in constructed wetland: mass balance, adsorption–desorption and FTIR study, Chem. Eng. 160 (2010) 122–128.

[28] S. Khan, I. Ahmad, M.T. Shah, S. Rehman, A. Khaliq, Use of constructed wetlands for the removal of heavy metals from industrial wastewater, J. Environ. Manage. 90 (2009) 3451–3457.

[29] G. Bonanno, R. Lo Giudice, Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators, Ecol. Indic. 10 (2010) 639–645.