



## Distribution and mass balance of hexavalent and trivalent chromium in a subsurface, horizontal flow (SF-h) constructed wetland operating as post-treatment of textile wastewater for water reuse

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### ABSTRACT

In this study, during a two-year period, we investigated the fate of hexavalent and trivalent chromium in a full-scale subsurface horizontal flow constructed wetland planted with *Phragmites australis*. The reed bed operated as post-treatment of the effluent wastewater from an activated sludge plant serving the textile industrial district and the city of Prato (Italy). Chromium speciation was performed in influent and effluent wastewater and in water-suspended solids, at different depths and distances from the inlet; plants were also analyzed for total chromium along the same longitudinal profile. Removals of hexavalent and trivalent chromium equal to 72% and 26%, respectively were achieved. The mean hexavalent chromium outlet concentration was  $1.6 \pm 0.9 \mu\text{g l}^{-1}$  and complied with the Italian legal limits for water reuse. Chromium in water-suspended solids was in the trivalent form, thus indicating that its removal from wastewater was obtained by the reduction of hexavalent chromium to the trivalent form, followed by accumulation of the latter inside the reed bed. Chromium in water-suspended solids was significantly affected by the distance from the inlet. Chromium concentrations in the different plant organs followed the same trend of suspended solids along the longitudinal profile and were much lower than those found in the solid material, evidencing a low metal accumulation in *P. australis*.

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### 1. Introduction

Textile wastewater contains great amounts of a number of toxic and recalcitrant chemicals and its treatment is therefore of great environmental importance. Moreover, textile districts consume large quantities of water thus producing high volumes of wastewater. Consequently, for textile manufacturing processes more than for other kind of industrial productions, a wastewater management system capable of guaranteeing water reuse is of paramount importance. Typical contaminants of textile effluents are organic compounds and solvents used for dyeing polyester fibres, reducing agents and sulphate salts employed as additives in dye baths, and heavy metal complexes with azo-dyes that are used for dye fixation in wool [1]. In this last process, one of the most representative heavy metals is chromium.

In aquatic environments chromium can be present as Cr(III) and/or Cr(VI), mainly depending on pH and redox conditions; the

two forms behave quite differently, since Cr(III) is much less soluble and therefore less mobile than Cr(VI).

The toxicity of chromium is highly dependent on its oxidation state; in fact, the hexavalent form is known to be toxic for many plants, animals and microorganisms, and clear evidence indicates that exposure to certain levels of Cr(VI) can result in significant risks for human health [2–4]. Conversely, the trivalent form is significantly less harmful and is an essential trace nutrient in the human diet [5,6]. According to these facts, limits for water reuse of  $100 \mu\text{g l}^{-1}$  and  $5 \mu\text{g l}^{-1}$  are enforced by the Italian regulation [7] for total and hexavalent chromium, respectively.

The heavy metal removal efficiency of SF-h constructed wetlands (CWs) has been monitored by different authors in full-scale systems and in pilot plants fed with real wastewater, both of domestic and industrial origin, evidencing good performances for several elements, including chromium [8–13]. Regarding plant chromium uptake in CW, few papers are currently present in literature and their results suggest that the actual phytoextraction efficiency is poor [10,14,15]; these papers also investigated the chromium vertical and horizontal distribution in SF-h systems. However, in all these studies, hexavalent chromium was not

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**Table 1**  
Major design parameters and management conditions of the SF-h CW investigated.

Start of operation	May 2003
Vegetated bed area (m <sup>2</sup> )	180
Length (m)	20
Width (m)	9
Depth (m)	0.8
Sealing	Clay
Average flow (m <sup>3</sup> d <sup>-1</sup> ) <sup>a</sup>	24 ± 2
Hydraulic loading rate (cm d <sup>-1</sup> ) <sup>a</sup>	13.3
Filtration material (mean size in mm)	Gravel (8)
Hydraulic residence time (d) <sup>b</sup>	2.1
Plant species	<i>Phragmites australis</i>

<sup>a</sup> Average value during the monitored period.

<sup>b</sup> Calculated according to Reed et al. [22].

investigated or, if speciation was performed, no Cr(VI) was found in wastewater [12]. The effectiveness of CW systems for Cr(VI) removal was demonstrated by Makos and Hrnčir [16], but this investigation was carried out at laboratory scale, using standard solution of Cr(VI) instead of real wastewater and, above-all, with a design approach very different from the current CW technology. Very recently, Fibbi et al. [17] reported a good Cr(VI) removal efficiency in a full-scale SF-h CW treating domestic and textile wastewater without providing any information about the fate of chromium inside the reed bed.

Therefore, to date, data are currently not available in literature regarding the fate of the different forms of chromium in a full scale CW fed with wastewater containing significant concentrations of Cr(VI).

In this work, the fate of chromium in the SF-h CW previously studied by Fibbi et al. [17] was investigated over a two-year period for the following purposes: (i) identification of the main chromium sink in the wetland and its speciation; (ii) evaluation of chromium distribution in the different aboveground and underground organs of macrophytes populating the wetland; (iii) calculation of the mass balance of chromium loaded into the system during the investigated period; (iv) evaluation of the importance of underground biomass present in the reed bed for chromium storage.

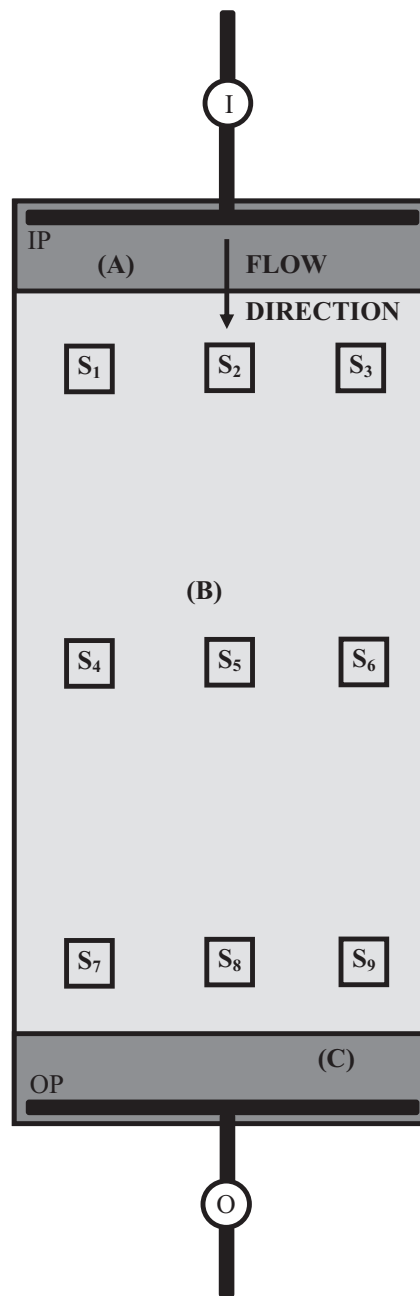
## 2. Materials and methods

### 2.1. Reagents

The reagents HNO<sub>3</sub> 65%, HCl 37% and H<sub>2</sub>O<sub>2</sub> 30% were purchased from Merck (Darmstadt, Germany) and were all Suprapur<sup>®</sup> grade. ICP chromium analytical standard (AA/ICP calibration/check standards for environmental analysis, 1 g l<sup>-1</sup>) and all the other reagents and salts were supplied by Sigma–Aldrich (Milwaukee, WI, USA) with a purity ≥ 99.0%. Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

### 2.2. Description of the study site and the constructed wetland

The SF-h CW treated the effluent of the Baciacavallo activated sludge plant that receives the industrial wastewater from the textile district of Prato (about 20,000,000 m<sup>3</sup> y<sup>-1</sup>), together with the domestic sewage from the urban area (about 18,000,000 m<sup>3</sup> y<sup>-1</sup>). Major design characteristics and management conditions, and a schematic depiction of CW are reported in Table 1 and Fig. 1, respectively. An inlet flow of about 1 m<sup>3</sup> h<sup>-1</sup>, corresponding to a hydraulic loading rate (HLR) of 13.3 cm d<sup>-1</sup>, was applied to the wetland. The flow was daily monitored with an electromagnetic flow-meter, model PROMAG 50P DN15 (Endress + Hauser, Reinach, Swiss) and adjusted when necessary.

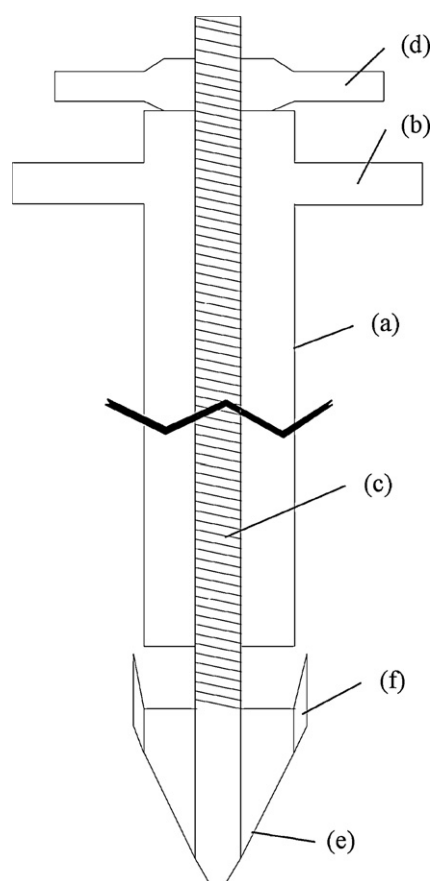


**Fig. 1.** Schematic depiction of the SF-h CW investigated (scale: 1 cm = 1.5 m). Inlet zone (A, dark gray) and drainage zone (C, dark gray) of the bed were originally filled with rubble (diameter: 5–7 cm), while the main medium (B, light gray) was gravel (mean diameter: 8 mm). I = inlet well provided with a flow-meter. IP = inlet distribution pipe. S<sub>1–3</sub> = sampling cells along the transect located at about 3 m from the IP. S<sub>4–6</sub> = sampling cells along the transect located at about 9 m from the IP. S<sub>7–9</sub> = sampling cells along the transect located at about 16 m from the IP. OP = outlet drainage pipe. O = outlet well.

### 2.3. Sampling

#### 2.3.1. Wastewater

Composite 24-h wastewater samples were collected at the inlet and the outlet of the CW, using two automatic samplers, model Liquiport 2000 RPT20 (Endress + Hauser, Reinach, Swiss) during the period September 2008–October 2010. Each week, 3–5 paired inlet and outlet samples were collected.



**Fig. 2.** Apparatus for the sampling of particulate material trapped in the reed bed. (a) Zincated steel tube; (b) handle; (c) threaded rod; (d) additional handle; (e) hollow tip ( $V=40$  ml); (f) rostrums.

### 2.3.2. Suspended solids

Suspended solids were collected along three transects at 3, 9 and 16 m from the inlet pipe; five samples were collected within each transect, at a distance of approximately 1 m from the edges and about 1.5 m one from the other (Fig. 1). The collection was made in November for three consecutive years (2008–2010), by using a home-made zincated-steel sampling apparatus (Fig. 2) at a depth of 60 cm. In 2009 the depth of 30 cm was also investigated along the same longitudinal profile. After the insertion until the desired depth, the sampler is raised about 2 cm and the tip is lowered about 1 cm by means of the handle (d) and the threaded rod (c), allowing the suspended solids and wastewater to enter the hollow tip and preventing any gravel from entering. The tip is raised, the sampler is extracted from the reed bed and the suspended solids are recovered from the tip and transferred into a jar (without head space) that is refrigerated at 0–4 °C until analysis.

### 2.3.3. Plants

To evaluate the plant biomass production, in October 2009 and 2010, three squared cells (length = 1 m) were chosen for each transect investigated for suspended solids and the reeds were harvested; in October 2010, the flowers were also collected. For each sampling point leaves, stems and flowers were separately collected, milled and dried at 60 °C until constant weight. Five representative samples were also collected for rhizomes and roots in different points of each cell and the material underwent the same treatment described above. All dried organs were weighed and five representative aliquots of each sample were analyzed for total chromium ( $Cr_{TOT}$ ), following the method described below.

**Table 2**

Digestion conditions of the different kinds of samples analyzed for  $Cr_{TOT}$ . A =  $HNO_3$  65%; B =  $H_2O_2$  30%; C =  $HCl$  37%.

Sample	Digested amount	Digestion mixture	Temperature program
Wastewater	12.5 ml	3.5 ml A and 0.5 ml B	From room temperature to 120 °C in 2 min, isotherm at 120 °C for 3 min, from 120 to 190 °C in 5 min and isotherm at 190 °C for 20 min
Sediment	0.04–0.20 g	3 ml A, 3 ml B and 9 ml C	From room temperature to 200 °C in 10 min and isotherm at 200 °C for 15 min
Plant material	0.5 g	7 ml A and 2 ml B	From room temperature to 180 °C in 10 min and isotherm at 180 °C for 10 min

At the end of the study (November 2010), the CW was drained and three squared sections (length = 1 m), located at 3, 9 and 16 m from the inlet (Fig. 1), were dug down to a depth of about 0.7 m. Rhizomes and roots were completely recovered, separated, milled and dried at 60 °C. For each distance from the inlet, five representative samples of each plant organ were analyzed for  $Cr_{TOT}$ .

## 2.4. Physical and chemical analysis

### 2.4.1. Hydraulic tracer test

Hydraulic residence time (HRT) was experimentally evaluated at the end of this study by adding in the inlet well 270 l of a potassium bromide solution ( $C=480$  g  $l^{-1}=4$  M) as a single input with duration of 5 min. Conductivity was continuously monitored in the outlet well using a WTW (Weilheim, Germany) conductivity meter model COND340i and data were stored for 96 h.

### 2.4.2. Macro-parameters

Oxidation–reduction potential (ORP), pH and dissolved oxygen (DO) of wastewater and wastewater suspended solids were measured as elsewhere reported [17].

Total suspended solids (TSS) were determined gravimetrically according to the IRSA-CNR Standard Method [18].

### 2.4.3. Chromium analysis and speciation

The total chromium concentration in wastewater, in suspended solids trapped inside the reed bed and in plant organs was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Perkin-Elmer (Waltham, MA, USA) Optimal DV 2000, after acidic-oxidant digestion with Suprapur® grade reagents, using a microwave system “ETHOS-1” (Milestone S.r.l., Bergamo, Italy) with pulsed-mode emission. Depending on the kind of matrix analyzed, the samples underwent the different digestion procedures described in Table 2 and the resulting solutions were made up to 25 ml with ultrapure water before ICP-OES analysis. Quantification was performed with external standard calibration curves.

Hexavalent and trivalent chromium in wastewater were determined according to the IRSA Italian guidelines [19,20] as reported by Fibbi et al. [17].

Chromium speciation was performed on a limited set of filtered and unfiltered inlet and outlet wastewater samples for the purpose of assessing the contribution of dissolved and particulate phases to the input and output of chromium in the CW.

Chromium speciation in water suspended solids was carried out according to the EPA method 3060A [21].

## 2.5. Quality control samples

### 2.5.1. Water samples

A quality control (QC) procedure for chromium analysis of water samples was performed by spiking 100  $\mu\text{l}$  of a freshly prepared aqueous solution of  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{CrCl}_3$ , both at a concentration of  $300\text{ mg l}^{-1}$ , in 500 ml of ultrapure water, thus obtaining a spiked solution of about  $20\text{ }\mu\text{g l}^{-1}$  in both Cr(VI) and Cr(III). The QC samples ( $n=3$ ) were analyzed for  $\text{Cr}_{\text{TOT}}$ , Cr(VI) and Cr(III) according to the methods described above and the achieved recoveries were included, respectively, in the ranges of 95–98%, 93–97% and 92–94%.

### 2.5.2. Plants

The QC protocol for plant tissues was carried out on a standard reference material (SRM 1573a-tomato leaves) with a certified chromium concentration equal to  $1.99 \pm 0.06\text{ mg kg}^{-1}$  dry weight (d.w.), supplied by the National Institute of Standard and Technology (NIST). Results of three replicated analyses were included between 1.84 and  $1.89\text{ mg kg}^{-1}$  d.w., corresponding to a recovery of 92–95% with respect to the certified value.

### 2.5.3. Suspended solids

The QC protocol for the analysis of chromium in suspended solids was performed by spiking 100  $\mu\text{l}$  of the above-mentioned  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{CrCl}_3$  solutions in 200 mg of dried suspended solids, thus obtaining a spiking of about 150 ppm in both Cr(VI) and Cr(III). The QC samples ( $n=3$ ) were analyzed for  $\text{Cr}_{\text{TOT}}$ , Cr(VI) and Cr(III) according to the methods described above; the achieved recoveries were included, respectively, in the ranges of 97–103%, 76–85% and 90–94%.

## 2.6. Blanks and limits of quantification

Blanks for  $\text{Cr}_{\text{TOT}}$ , Cr(VI) and Cr(III) analysis were performed following the same analytical procedures adopted for the sample analysis, without adding the matrix (wastewater, plant tissue or suspended solids).

For wastewater blanks were included in the ranges of  $0.8\text{--}1.1\text{ }\mu\text{g l}^{-1}$  for total and trivalent chromium, and  $0.15\text{--}0.24\text{ }\mu\text{g l}^{-1}$  for hexavalent chromium; therefore, the limits of quantification (LOQ) were considered equal to  $2\text{ }\mu\text{g l}^{-1}$  for  $\text{Cr}_{\text{TOT}}$  and Cr(III) and  $0.5\text{ }\mu\text{g l}^{-1}$  for Cr(VI). Since the method for the analysis of Cr(VI) is more sensitive than that of Cr(III), speciation in wastewater was carried out by analyzing Cr(VI).

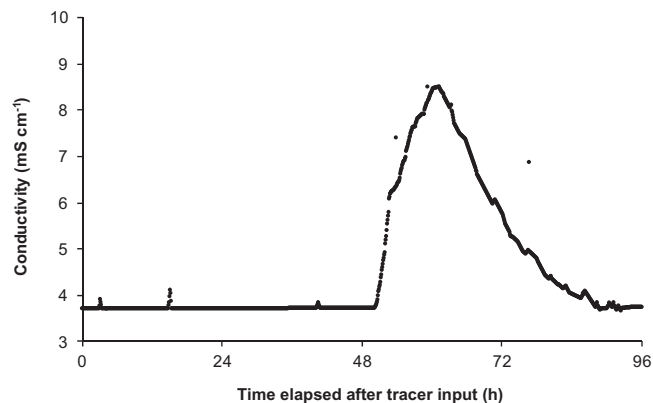
For the analysis of  $\text{Cr}_{\text{TOT}}$  in plants, the contribution of the blank ranged from 0.05 to  $0.15\text{ mg kg}^{-1}$  d.w. and therefore the LOQ was considered equal to  $0.3\text{ mg kg}^{-1}$  d.w.

For suspended solids, the blank of  $\text{Cr}_{\text{TOT}}$  was in the range of  $0.06\text{--}0.14\text{ mg kg}^{-1}$  d.w. and the corresponding LOQ was  $0.3\text{ mg kg}^{-1}$  d.w. The blank for Cr(VI) analysis in suspended solids was below quantification limits (bql) and therefore the corresponding LOQ was considered equal to the instrumental limit of detection, i.e.  $12\text{ mg kg}^{-1}$  d.w.

In order to verify whether a chromium release occurred from the sampler, 40 ml of ultrapure water was added to the tip of the sampler, maintained overnight and then analyzed for  $\text{Cr}_{\text{TOT}}$ . The results did not show any contamination significantly higher than the blank contribution reported above, since  $\text{Cr}_{\text{TOT}}$  concentration was  $0.9\text{ }\mu\text{g l}^{-1}$ .

## 2.7. Data analyses

Data plots were performed by Microsoft® Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA). In order to evaluate the significance of the differences between mean values, the *T* test



**Fig. 3.** Residence time distribution curve obtained measuring conductivity in the reed bed outlet after a single input of a KBr solution ( $V=270\text{ l}$ ;  $C=480\text{ g l}^{-1}=4\text{ M}$ ).

**Table 3**

Mean values  $\pm$  standard deviation (S.D.) and ranges of physico-chemical and chemical parameters, found in the inlet ( $n=398\text{--}452$ ) and outlet ( $n=382\text{--}447$ ) of the investigated SF-h CW, between September 2008 and September 2010. Within each row, mean values with the same letters are not statistically different, according to the Dunnett T3 nonparametric test for multi comparisons of mean values ( $P<0.01$ ). ORP=oxidation–reduction potential; DO=dissolved oxygen; bql=below quantification limit.

	Inlet		Outlet	
	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.	Range
pH	$7.34 \pm 0.21$ (a)	6.32–8.03	$7.15 \pm 0.18$ (a)	7.05–7.22
ORP (mV)	$214 \pm 12$ (a)	205–233	$147 \pm 29$ (b)	50–250
DO (% saturation)	$92.6 \pm 3.5$ (a)	90.1–95.2	$35.8 \pm 8.7$ (b)	30.6–41.3
TSS ( $\text{mg l}^{-1}$ )	$14 \pm 3$ (a)	2–22	$2 \pm 1$ (b)	bql–5
Cr(VI) ( $\mu\text{g l}^{-1}$ )	$5.5 \pm 2.9$ (a)	0.8–15.8	$1.6 \pm 0.9$ (b)	bql–4.6
Cr(III) ( $\mu\text{g l}^{-1}$ )	$12.7 \pm 5.7$ (a)	bql–27.9	$9.4 \pm 3.6$ (b)	bql–26.0
$\text{Cr}_{\text{TOT}}$ ( $\mu\text{g l}^{-1}$ )	$18.2 \pm 7.2$ (a)	5.4–43.2	$11.0 \pm 3.5$ (b)	2.7–26.9

and the nonparametric Dunnett T3 contrast test were carried out using the statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results and discussion

Fig. 3 shows the HRT distribution curve of the SF-h CW herein investigated, as measured by the KBr tracer test. The tracer trend as a function of time elapsed after the salt input highlighted the absence of short-circuiting phenomena; the curve did not exhibit ideal plug flow response, but it was closer to plug flow than to the complete-mix model. Based on this curve, a peak HRT of about 61 h was found. A similar HRT (about 63 h) was also calculated as the time corresponding to the half of the area subtended by the HRT distribution curve. These values were quite in agreement with the theoretical HRT (50.4 h) calculated on the basis of actual dimensions of the CW (Table 1), using 35% as the value of porosity percentage [22].

Table 3 shows mean values, standard deviations and ranges of the parameters monitored in the inlet and outlet of the SF-h CW during the two-years investigation period. Inlet standard deviation values were fairly low, evidencing that the reed bed was fed with a wastewater having quite homogeneous characteristics; on the other hand, the outlet standard deviations were low as well, indicating the homogeneous behaviour of the CW during the entire period of investigation.

Inlet TSS were included in the range of  $2\text{--}22\text{ mg l}^{-1}$ , with a mean value of  $14\text{ mg l}^{-1}$ ; these values were lower than or similar to others observed at the inlet of SF-h CW tertiary treatments in various countries [23–25]. Outlet TSS, which are also an important parameter for water reuse, above all for industrial purposes, were



frequently below the quantification limit ( $1 \text{ mg l}^{-1}$ ) and constantly lower than the legal limit for water reuse ( $10 \text{ mg l}^{-1}$ ).

### 3.1. Chromium speciation and removal in wastewater

Inlet concentrations of Cr(VI), Cr(III) and Cr<sub>TOT</sub> were in the ranges of 0.8–15.8, bql–27.9 and 5.4–43.2  $\mu\text{g l}^{-1}$ , respectively (Table 3). Therefore, inlet Cr<sub>TOT</sub> concentrations were always much lower than the Italian legal limit for water reuse ( $100 \mu\text{g l}^{-1}$ ). Conversely, hexavalent chromium concentrations in the influent were often higher than the maximum value permitted by the Italian regulation ( $5 \mu\text{g l}^{-1}$ ), evidencing that wastewater must be treated before being reused. As previously reported by Fibbi et al. [17], the high Cr(VI) inlet concentrations were the result of the ozonation treatment carried out on wastewater before entering the wetland to destroy the complexes between azo-dyes and Cr(III). Chromium speciation in filtered and unfiltered inlet samples evidenced that Cr(VI) was only present in the dissolved phase, while Cr(III) was found in both phases, albeit with a much higher abundance (about 80%) in the dissolved phase. This behaviour was not compliant with pH conditions and the presence of ppm levels of particulate matter in the inlet that should promote precipitation and/or sorption of trivalent chromium. The high Cr(III) levels in the dissolved phase could be attributed to the presence of azo-dye soluble complexes, only partially destroyed during the ozonation process before entering the wetland owing to their recalcitrance. It should however be noted that the concentration of chromium adsorbed onto particulate matter was anything but negligible, averaging  $271 \text{ mg kg}^{-1} \text{ d.w.}$  (range:  $197\text{--}356 \text{ mg kg}^{-1} \text{ d.w.}$ ).

Chromium concentrations in the CW outlet were significantly lower than those found in the inlet for both Cr(III) and Cr(VI) ( $P < 0.001$ ) and mean removals of 26% and 72% were obtained for the two forms. Therefore, mean Cr(III) removal percentage was very similar to the fraction of this form that is present in the inlet particulate matter, in agreement with the above-mentioned hypothesis regarding the presence of recalcitrant soluble azo-dye complexes with Cr(III). A different fate can be attributed to the trivalent chromium originating from the reduction of the hexavalent one. Cr(III), in fact, can be trapped in the reed bed through precipitation and/or sorption processes, even mediated by biological activity [26,27]. By assuming that all Cr(VI) is removed by reduction to Cr(III), the actual removal percentage of Cr(III) would be approximately 43%.

Based on these results, the wetland gave rise to an effluent that was much less toxic than the inlet with regard to the presence of Cr(VI) and appears to be still effective in the removal of either hexavalent or trivalent chromium after seven years' operation, when a hydraulic loading rate (HLR) as high as  $13.3 \text{ cm d}^{-1}$  was adopted (Table 1). In particular, effluent Cr(VI) concentrations were included in the range of bql– $4.6 \mu\text{g l}^{-1}$  (Table 3) and therefore it can be stated that, under the management conditions reported in Table 1, the wetland successfully treated hexavalent chromium for the water recycling.

### 3.2. Chromium accumulation in suspended solids inside the reed bed

Total chromium concentrations found in suspended solids collected inside the reed bed at various distances from the inlet as a function of sampling depth and sampling year, are reported in Fig. 4A and B, respectively.

In suspended solids mean Cr<sub>TOT</sub> concentrations ranged from 189 to  $653 \text{ mg kg}^{-1} \text{ d.w.}$  and were essentially in the trivalent form, since Cr(VI) was found at concentrations lower than the LOQ ( $12 \text{ mg kg}^{-1} \text{ d.w.}$ ). This finding, that has been highlighted here for

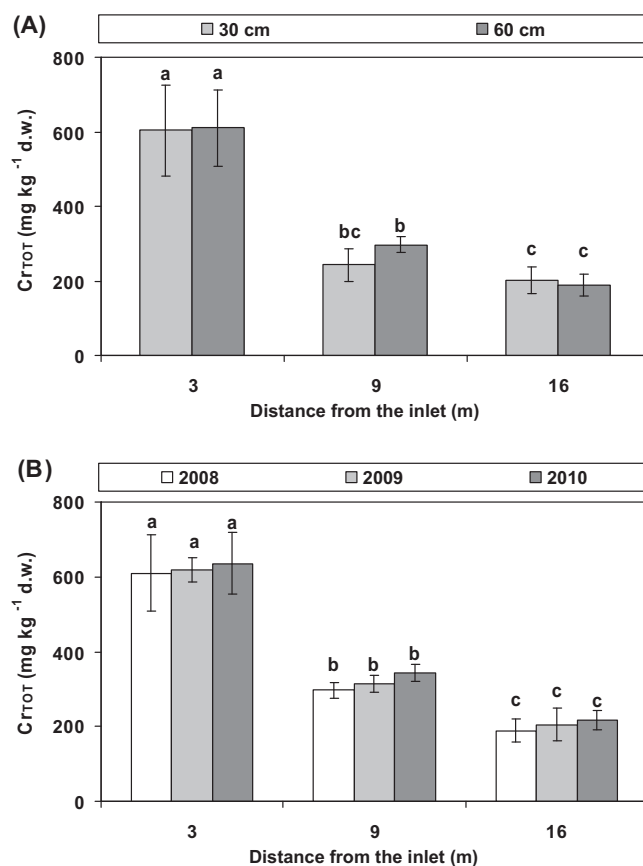


Fig. 4. Total chromium (Cr<sub>TOT</sub>) concentrations found in the SF-h CW suspended solids collected at various distances from the inlet as a function of depth in 2009 (A) and the sampling year (B). Values with the same letter are not significantly different according to the Dunnett T3 nonparametric test for multi comparisons of mean values ( $P < 0.1$ ).

the first time, was in agreement with ORP and pH data found in suspended solids freshly collected at different depths and distances from the inlet, that were included in the ranges of  $-25$  to  $60 \text{ mV}$  and  $7.62\text{--}7.84 \text{ pH}$  units; in these pH and redox conditions in fact, chromium is known to be present in the trivalent form [28]. Thus, the removal mechanism previously proposed, based on the reduction of Cr(VI) to Cr(III) followed by its storage in the reed bed, is confirmed.

Regardless of the investigated year and depth, a statistically significant ( $P < 0.1$ ) decrease in chromium concentrations was observed as the distance from the inlet increased (Fig. 4). More specifically, concentrations found at 3 m were more than twofold higher than the ones found at 9 m, whereas the concentration decrease between 9 and 16 m was much less pronounced. This pattern indicated that both the reduction of hexavalent to trivalent form and the precipitation and/or sorption and/or sedimentation of the latter occurred to a greater extent in the first 3 m of CW during the seven operational years.

In a study regarding metal accumulation in a SF-h CW, Lesage et al. [14] postulated strong reducing conditions occurring at the beginning of the bed to explain the behaviour of Mn and reported a Cr<sub>TOT</sub> longitudinal profile very similar to the one observed herein. Even though Lesage et al. [14] did not carry out chromium speciation, it can be hypothesized that in their study chromium was in the trivalent form, since the fraction present in the particulate phase was predominant and the wastewater was of domestic origin. According to these considerations, the trend found in their study should be due to precipitation and/or sorption

phenomena only. Anyhow, since our results and those of Lesage et al. [14] referred to similarly designed CW, it can be deduced that the observed behaviour is typical for these kinds of wetlands.

Concentrations of water-suspended solids followed the same chromium trend along the longitudinal profile of the bed, and their mean concentrations at 3, 9 and 16 m from the inlet were 22.3, 12.0 and 4.5  $\text{g l}^{-1}$ , respectively. Based on these data, the contribution of the reed bed area closest to the inlet appeared to be even more important for the Cr(III) storage process.

The samples collected at the same distance from the inlet but at different depths did not show any statistically significant variations (Fig. 4A); an absence of variations within a vertical profile of a SF-h CW was also evidenced by Vymazal and Krása [29] for Mn, Al, Cu and Zn in a similarly designed system.

The pattern of chromium concentrations as a function of the longitudinal and vertical profiles is in agreement with the hydraulic behaviour of the reed bed shown in Fig. 1.

When chromium concentrations found within each transect in different sampling years were compared, a slightly increasing trend was observed (Fig. 4B), confirming that the chromium storage process still continues in the wetland. Even though Cr(III) removal is based on its storage in the reed bed and is therefore more sensitive to the CW age, the longitudinal and temporal trends suggest that the system is still far from being saturated, even after seven years of operation at an HLR of 13.3  $\text{cm d}^{-1}$ , which is two or three-fold higher than the ones commonly applied to SF-h CWs [13]. These findings are of utmost importance for the future applications of SF-h CW in the polishing of wastewater containing significant concentrations of chromium, especially in the hexavalent oxidation state.

### 3.3. Chromium accumulation in plants

$\text{Cr}_{\text{TOT}}$  concentrations found in different organs of *Phragmites australis* as a function of the distance from the inlet in 2009 and 2010, are reported in Fig. 5A and B, respectively; they ranged from 0.66 to 41.6  $\text{mg kg}^{-1}$  d.w., depending on the organ, the sampling point and the year of plant harvest. Chromium accumulated in underground and aboveground tissues was low when compared to the one found in suspended solids. In all cases roots showed chromium concentrations significantly higher than the ones found in the other plant tissues ( $P < 0.05$ ), indicating a preferential metal allocation in this plant part. Besides a limited shoot translocation, this higher metal amount in roots may also be due to the propensity of Cr(III) to bind to the root cell walls [30]. Concentration differences among the other organs were much lower and did not follow a univocal trend; however, in most cases chromium accumulation was in the order of rhizome > leaves > stem (Fig. 5). This chromium allocation pattern was in agreement with the ones found elsewhere for *P. australis* and other aquatic plants [14,31–33]. According to different authors, wetland plants, including *P. australis*, are able to reduce hexavalent chromium into the much less toxic trivalent chromium, either inside their tissues after assimilation of Cr(VI) or outside through release of root exudates. In any case, only Cr(III) is accumulated in the plant organs [34,35]. This kind of metal allocation is of fundamental importance for limiting the possibility of CW being a source of contaminants. In this study chromium concentrations in shoots were in the ranges of 0.66–4.62 and 1.12–2.83  $\text{mg kg}^{-1}$  d.w., for leaves and stems, respectively. No limits for domestic animal toxicity are assessed for chromium. However, for elements much more toxic than trivalent chromium, the United States National Research Council (US-NRC) [36] reported limits higher than the above-mentioned shoot chromium concentrations (lead: 100  $\text{mg kg}^{-1}$ ; cadmium: 10  $\text{mg kg}^{-1}$ ; arsenic 30  $\text{mg kg}^{-1}$ ). Therefore, an absence of a significant risk due to chromium intake for

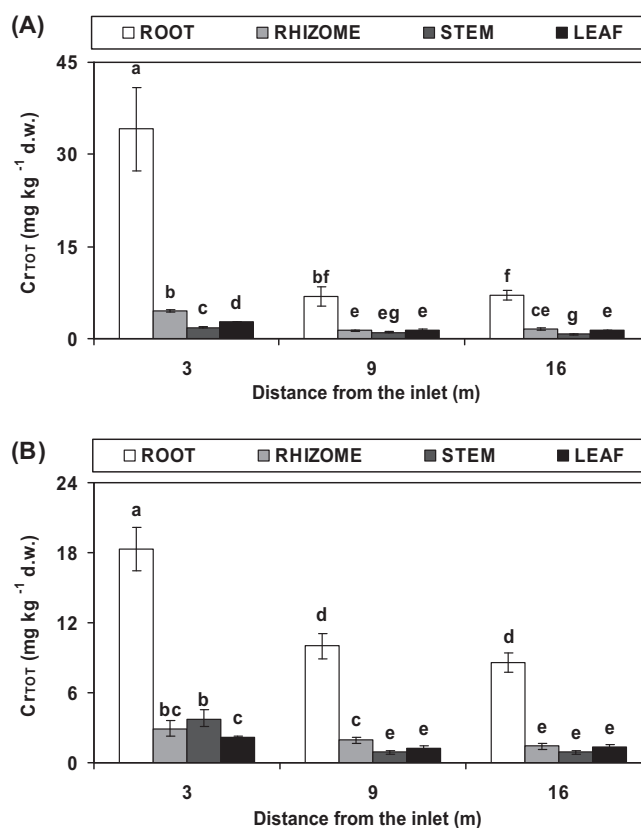


Fig. 5. Total chromium ( $\text{Cr}_{\text{TOT}}$ ) concentrations found in roots, rhizomes, stems and leaves of *Phragmites australis* collected from the SF-h CW at various distances from the inlet, in 2009 (A) and 2010 (B). Values with the same letter are not significantly different according to the Dunnett T3 nonparametric test for multi comparisons of mean values ( $P < 0.1$ ). Note the different scale on the y-axis.

foragers, such as cattle or other wildlife that may consume these plants, can be hypothesized.

Another possible contamination source, that has not been taken into account before, is related to the wind-dispersion of pollen from flowers of *P. australis*. Pollen collected in 2010 along the longitudinal profile evidenced mean  $\text{Cr}_{\text{TOT}}$  concentrations equal to  $1.59 \pm 0.17$ ,  $1.32 \pm 0.16$  and  $1.22 \pm 0.08$   $\text{mg kg}^{-1}$  d.w. at 3, 9 and 16 m from the inlet, respectively. Since these values were quite similar, they were combined to give a general mean of  $1.38 \pm 0.20$   $\text{mg kg}^{-1}$  d.w.; based on the mass values of pollen collected within each squared cell, it was possible to estimate that pollen produced by *P. australis* averaged 232  $\text{g d.w./m}^2$ ; thus a total annual pollen production of about 42  $\text{kg d.w.}$  was estimated. As a consequence, chromium that can be dispersed around the CW during the entire period of pollen production is at maximum about 60 mg, corresponding to a chromium concentration of approximately 0.3  $\text{mg m}^{-3}$  in an air volume above the reed bed with a height of 2 m. This value, which is strongly overestimated since it does not take into account any dilution effect or the fact that the release of pollen into atmosphere is not instantaneous, is in any case lower than the recommended threshold limit value for inhalation of Cr(III) for the entire human lifecycle (0.5  $\text{mg m}^{-3}$ ) [37]. It can therefore be drawn for the first time the important conclusion that the chromium dispersion by pollen should not represent an inhalatory risk for humans.

Irrespective of the harvest year, a decreasing trend of chromium concentrations was observed in each type of plant organ as the distance from the inlet increased; the greatest variations were observed when data at 3 m were compared with those at 9 m, which were all statistically significant ( $P < 0.1$ ), the only exception being

the rhizome collected in 2010 (Fig. 5B). Variations in chromium accumulation between 9 and 16 m were much lower and statistically not appreciable, except for the rhizome sampled in 2010. The longitudinal profile observed was in accordance with data in suspended solids and indicated a direct dependence of chromium concentrations in plant organs on the chromium concentrations inside the bed.

#### 3.4. Chromium mass balance and relative importance of chromium accumulation in aboveground and underground biomass

In order to estimate the yearly mass loading of chromium during the period September 2008–October 2010, the mean influent and effluent flow rates should be multiplied by the corresponding mean inlet and outlet  $Cr_{TOT}$  concentrations.

The inlet flow rate averaged  $24 \pm 2 \text{ m}^3 \text{ d}^{-1}$  (Table 1) and the yearly hydraulic loading was approximately  $8760 \text{ m}^3 \text{ y}^{-1}$ . The outlet flow (OF), which was not measured, is the result of three different contributions. (i) Inlet flow (IF); (ii) precipitations (P); (iii) evapotranspiration (ET), according to the following equation:

$$OF (\text{m}^3 \text{ y}^{-1}) = IF (\text{m}^3 \text{ y}^{-1}) + P (\text{m}^3 \text{ y}^{-1}) - ET (\text{m}^3 \text{ y}^{-1}) \quad (1)$$

Cumulative yearly precipitations in 2009 and 2010 were 1091 and  $1214 \text{ mm y}^{-1}$  (Fondazione Prato Ricerche, personal communication), corresponding to a mean yearly value of  $207 \text{ m}^3 \text{ y}^{-1}$  for the total reed bed.

Evapotranspiration was not measured in this study; however, literature data regarding Italian SF-h systems planted with *P. australis*, indicated that the cumulated yearly evapotranspiration is about  $4000 \text{ mm y}^{-1}$  [38], corresponding to  $720 \text{ m}^3 \text{ y}^{-1}$  for the total reed bed. According to Eq. (1), the outlet flow was therefore  $8247 \text{ m}^3 \text{ y}^{-1}$ .

Based on the mean yearly inlet and outlet flow and  $Cr_{TOT}$  concentrations (Table 3), the masses inflowing and outflowing from the wetland were 159 and  $91 \text{ g y}^{-1}$ .

Since metals cannot be degraded, the only way to remove chromium from the system is shoot harvest. In order to estimate the chromium that can be removed annually by plant harvesting, mean  $Cr_{TOT}$  concentrations found in aboveground organs should be multiplied by the corresponding yearly biomass productions. To evaluate the yearly overall biomass production of the reed bed, the system was ideally divided into three zones, each with a surface area of  $60 \text{ m}^2$  and represented by the nine squared cells located at 3, 9 and 16 m from the inlet. A linear interpolation of biomass data obtained in the nine squared cells allowed us to calculate an aboveground biomass production of 1.79, 2.65 and  $3.04 \text{ kg m}^{-2} \text{ d.w.}$  in 2009 and 1.61, 2.32 and  $2.68 \text{ kg m}^{-2} \text{ d.w.}$  in 2010, at 3, 9 and 16 m from the inlet, respectively. Based on these aboveground biomass data the overall chromium removal by plant harvest was estimated to be 0.58 g in 2009 and 0.63 g in 2010, which is less than 1% of the amount of chromium removed from wastewater.

In order to assess the role of underground plant tissues as a sink for chromium in the reed bed, the roots and rhizomes present in three sections located along the above-mentioned longitudinal profile were collected and the overall biomass of the wetland was estimated by means of linear interpolation. In accordance with data obtained on aboveground tissues, underground organs showed an increasing trend of biomass production along the longitudinal profile of the system with mean values equal to 0.042, 0.079 and  $0.087 \text{ kg m}^{-2} \text{ d.w.}$  for roots and 4.05, 10.03 and  $10.84 \text{ kg m}^{-2} \text{ d.w.}$  for rhizomes, at 3, 9 and 16 m, respectively. Based on these biomass data the overall chromium accumulation in underground tissues was estimated to be 2.9 g, referring to the total life of the reed bed. As a result, it is evident that the only significant chromium sink

in the wetland is represented by the solids present inside the reed bed.

#### 4. Conclusions

Our results showed how SF-h CWs represent a technology suitable for the removal of hexavalent and non-complexed trivalent chromium from wastewater.

For the first time it was demonstrated that, in a full-scale SF-h CW, Cr(VI) is strongly reduced to Cr(III), the latter being accumulated inside the wetland, almost completely through precipitation and/or sorption onto water-suspended and settled solids, thereby evidencing the double role of SF-h CWs in acting as reducing and trapping sites of hexavalent and trivalent chromium.

According to the concentration pattern of chromium and its temporal trend along the longitudinal profile of CW, the zone closest to the inlet was the one most intensely involved in the storage process, indicating that the bed is far from being saturated and that the removal can still be sustained long-term, even if the system will unavoidably arrive at saturation with the increase in operating time.

Shoot chromium concentrations were as low as to exclude any significant risk of chromium intake for foragers and an inhalatory risk for humans due to wind diffusion of pollen.

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