

# Influence of different ornamental shrubs on the removal of heavy metals in a stormwater bioretention system

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**Abstract:** Several laboratory studies have shown the ability of bioretention systems to remove pollutants from stormwater. However, to our knowledge, no existing research has addressed the use of ornamental shrubs for improving water quality in bioretention systems in Italian cities. In this short note, we evaluated the potential of three ornamental shrub species (*Lonicera pileata* Oliver, *Cotoneaster horizontalis* Decne., *Hypericum hidcoteense* 'Hidcote') for the removal of heavy metals in a stormwater bioretention system. Pot experiments in "pot prototypes" using an alternative bioretention system filter media have been carried out under controlled conditions. The ornamental shrubs were irrigated with semisynthetic stormwater with known heavy-metal concentrations. Experimental results indicate that the removal of heavy metals by the system is very efficient. However, there was not a significant effect of the plant on the system's retention efficiency. The removal of lead and cadmium by the system was over 87%. In order to provide accurate information for bioretention design, future research should comparatively assess plant species in a laboratory-scale filter column and *in situ*.

## 1. Introduction

Urban stormwater runoff contains pollutants which can impact the quality of surface, seepage, and ground water (Eckley and Branfireun 2009; Göbel *et al.*, 2007). Stormwater carries different pollutants, both organic and inorganic (Barbosa *et al.*, 2012), including copper, zinc, lead, cadmium, sediments, polycyclic aromatic hydrocarbons, and de-icing salts (Muthanna *et al.*, 2007) so that its quality management is of crucial

importance to urban development and water resource planning (Zgheib *et al.*, 2012). In particular, cadmium has become an increasing problem because of its toxic effects on biological systems (Mishra and Tripathi, 2008). Additionally, contaminated soils and waters represent an environmental and human health problem, which may be partially solved by the phytoremediation technology (Mojiri 2012; Dadea *et al.*, 2017).

New approaches to improve water quality as well as water cycle in urban areas have been proposed, for example with Best Management Practices (BMP), Low Impact Design (LID), Sustainable Urban Drainage System (SUDS), Water Sensitive Urban Drainage Systems (WSUD) and sponge cities (Pompêo 1999; Raja Segaran *et al.*, 2014, Fletcher *et al.*, 2015; Griffiths 2017). These systems have been implemented around the world because they provide important environmental, economic and health benefits such as improving water quality, reducing flood risk, increasing amenity and increasing biodiversity in cities (Griffiths, 2017). Retention and degradation of stormwater pollutants using the above systems are becoming an important ecosystem service in urban environments (Kabir *et al.*, 2014). According to Kabir *et al.* (2014), more than 75% of metals, such as Pb, Zn, Cu, and Cd is retained by blue-green infrastructure.

In particular, bioretention systems, also known as biofilters or rain gardens, have been used to remove a wide range of pollutants, such as suspended solids, nutrients, metals, hydrocarbons, and microorganisms from stormwater runoff (Muthanna *et al.*, 2007; Sun and Davis 2007; Hatt *et al.*, 2009; Blecken *et al.*, 2010; Megharaj *et al.*, 2011; Trowsdale and Simcock 2011; Weerasundara *et al.*, 2016). Well-designed bioretention systems can remove several pollutants from the urban runoff via physical, chemical, and biological processes, including plant uptake, sedimentation, filtration, and sorption on mulch and soil layers, and biodegradation by soil microorganisms (Weerasundara *et al.*, 2016). A bioretention system consists of several layers of filter media, normally a soil/sand/organic media matrix (approximately 0.7 - 1 m deep), a mulch layer and both woody and herbaceous plants (Sun and Davis 2007; Davis *et al.*, 2009; Liu *et al.*, 2014).

Plants not only assimilate pollutants directly from wastewater and rooting media into their tissues, but also act as catalysts for purification reactions by increasing the environmental diversity in the rhizosphere and promoting a variety of chemical and bio-

logical reactions that enhance pollutant removal (Zhang *et al.*, 2011). The benefits of bioretention by vegetation have not been well quantified (Davis *et al.*, 2009) and the majority of studies have focused on herbaceous plants in bioretention systems (Sun and Davis 2007; Read *et al.*, 2008; Feng *et al.*, 2012; Barrett *et al.*, 2013; Payne *et al.*, 2014). Woody shrubs may also provide low maintenance and might be an attractive cover for stormwater systems (Environmental Services Division, 2009).

Feng *et al.* (2012), conducted a large-scale stormwater biofilter column study and found that vegetation and the type of filter are significant factors for the treatment of metals. While most studies evaluated individual plant performance for metal uptake, some plant species have been shown to improve the performance of stormwater biofiltration systems (Read *et al.*, 2008; Houdeshel *et al.*, 2012). Therefore, the assemblage of different species may be suitable for increasing biofilter efficiency and maximizing the spectrum of removed pollutants, but this topic remains largely unexplored.

Species mixes might also be preferred for aesthetic and ecological reasons (Read *et al.*, 2008). However, higher concentration of heavy metals can cause damage to plants by reducing growth and the rates of photosynthesis and respiration, so that further understanding on species' tolerance to pollution is needed (Hossain *et al.*, 2012; Ovečka and Takáč 2014). Plant species suitable for the use in bioretention systems are provided by North American and Australian bioretention design guidelines (Environmental Services Division, 2009; Houdeshel *et al.*, 2012). However, this information is not based on data from replicated experiments (Dylewski *et al.*, 2011) and little is known about the most suitable type of plant for bioretention systems in terms of survival and performance for Italian cities. Therefore, the objectives of our study were: i) to evaluate an alternative bioretention filter media; and ii) to test the hypothesis that species association may increase heavy-metal retention by the system constituted by different plant combinations and substrates; and iii) to understand the heavy-metal effect on chlorophyll and root/shoot ratios.

## 2. Materials and Methods

### *Experimental setup and planting material*

Three species potentially suitable for planting in bioretention systems were chosen across a range of

evergreen ornamental shrubs commonly grown in urban areas in Central-Northern Italy. 70 plastic pot prototypes (Fig. 1) with a truncated pyramid shape (418 x 310 mm, 347 x 245 mm base, and 575 mm height) with lateral taps at the bottom, were put in a greenhouse facility at the University of Florence in Sesto Fiorentino, Italy, in October 2013 (Fig. 2). The pots consisted of four layers: (1) The drainage layer at the bottom of the pot was filled with 150 mm of perlite (AGRILIT 2, Perlite Italiana) and (2) a filter sheet (DRENALIT F130, Perlite Italiana) was placed to separate the 300 mm substrate layer (3) (AgriTERRAM TV, Perlite Italiana) from the drainage layer, followed by a 50 mm mulch layer (4) (GEOBARK Pine Bark) to cover the soil and improve pollutant retention (Muthanna *et al.*, 2007). The substrate basic properties were pH 6-7, EC <40 mS/m, cation-exchange capacity (CEC) 55-60 meq/100 g, total organic content <20-25%, bulk density  $400 \text{ kg/m}^3 \pm 5\%$ , and vertical permeability >13 mm/min. The system consisting of AGRILIT 2 and AgriTERRAM TV (Perlite Italiana), known as PERLIROUND™, is used for the greening of roundabouts and traffic islands (Perlite Italiana, 2011). Three-year-

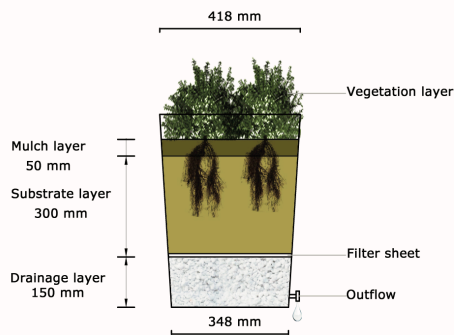


Fig. 1 - Schematic drawing of the bioretention pot prototype. Not to scale.



Fig. 2 - Photo of the greenhouse experiment at the University of Florence, Italy: (a) bioretention pot prototypes, (b) 200-L plastic water storage tanks.

old plants of *Lonicera pileata* Oliver, *Cotoneaster horizontalis* Decne., and *Hypericum hidcoteense* 'Hidcote' were potted in the containers. Each pot contained 2 plants of the same species, namely *Lonicera pileata* (Lp), *Cotoneaster horizontalis* (Ch), and *Hypericum hidcoteense* 'Hidcote' (Hh), or plants of two species, in all possible combinations (Lp + Ch, Lp + Hh, and Ch + Hh). 5 additional pots were prepared as previously described but left unplanted. The experiment was carried out from October 2013 until June 2014. Plants were grown at 28/18°C day/night temperatures and exposed to natural daylight, and the light transmission was of 90%. Relative humidity was always above 60%.

#### Measurement of pollutants and plant growth

Synthetic stormwater runoff was prepared using tap water that was left to stand at room temperature in 200-L plastic water storage tank for 24 h to dechlorinate and thermally equilibrate (Fig. 2) (Sun and Davis, 2007). The first irrigation with synthetic stormwater started on April, 3<sup>rd</sup> 2014 after approximately 6 months of plant growth in the pots. Plants were irrigated with synthetic stormwater with heavy metal concentrations (Pb and Cd) once per week for 3 weeks. The total volume of runoff applied to each pot was 5 L, this amount was based on rainfall precipitation in Florence (Vijaya Kumar *et al.*, 2013). The concentrations ( $\text{mg L}^{-1}$ ) of pollutants in our synthetic stormwater were 2.02 ( $\text{mg L}^{-1}$ ) in the first irrigation and 1.97 in the successive irrigations for Pb and 0.37 ( $\text{mg L}^{-1}$ ) in the first irrigation and 0.39  $\text{mg L}^{-1}$  in the successive irrigations for Cd. These values are the highest concentrations of highway runoff reported in the literature (Kayhanian *et al.*, 2012). To determine the effect of plants on pollutant removal from stormwater, the water that drained from the tap (outflow) was collected during the first and second irrigations. We collected 60 samples from the "stormwater plants" and 10 from the unplanted containers "stormwater soil". We also collected stormwater (inflow) in order to assess its quality, before each irrigation. Furthermore, pH was measured immediately after each sampling using a pH Electrode LE407. Samples were filtered through 0.45  $\mu\text{m}$  membrane filter (Swinnex Filter Holder) and acidified with 1% of Nitric Acid. The samples were sent to an accredited analytical chemistry laboratory (Research Centre for Agriculture and Forestry, Laimburg, Italy) and analyzed according to standard methods for Pb and Cd using ICP. The removal efficiency was calculated as percentage of inflow concentrations.

A Minolta SPAD-502 leaf chlorophyll meter was

used for non-destructive data collection. The instrument is able to provide a rapid and reasonably accurate estimate of leaf Chl. Measurements were made before the first irrigation and after the second irrigation. SPAD readings were recorded for 3 positions on each leaf and for 3 different leaves on a single shrub (Table 1). At the end of the experiment, dry weight (DW) of roots, stems and leaves was determined in 36 treated plants and in 36 control plants. The total plant DW and shoot/root ratio were calculated.

**Experimental design and statistics**

The experiment was a randomized complete block with five blocks (Rao, 2007). The outflow data were checked for normality using Kolmogorov-Smirnov and Ryan-Joiner tests using Minitab 17. The data did not fit a normal distribution and we used a non-parametric Kruskal-Wallis test to analyse statistical differences among treatments. In order to determine whether there was a statistically significant effect between treatments on the plant-growth parameters, including stem, roots and leaves, a post-hoc comparison on means was conducted by Duncan’s test (SPSS Statistics) with p<0.05.

**3. Results and Discussion**

Mean outflow concentrations and reduction are shown in Table 2. Outflow Pb concentrations ranged in the first irrigation from 4.13 µg/L in *Lonicera + Cotoneaster* to 9.37 µg/L in *Lonicera pileata + Hypericum hidcoteense* ‘Hidcote’. Cd concentrations ranged in the first irrigation from 1.57 µg/L in

*Lonicera* and *Cotoneaster* to 3.23 µg/L in *Cotoneaster + Hypericum*. However, Pb concentrations ranged in the second irrigation from 5.88 µg/L in soil to 237.80 µg/L in *Lonicera + Lonicera*. Cd concentrations ranged in the second irrigation from 1.44 µg/L in soil to 8.34 µg/L in *Cotoneaster* as single species.

We found that the different shrub species did not affect the reduction and there was no significant difference in metal concentration between the effluent from soil-only controls and shrubs or mix of species. Based on the results above, heavy metals are mainly retained by physical processes (i.e., sedimentation and chelation) within the PERLIROUND substrate and we were unable to determine removal by vegetation uptake. However, previous studies have highlighted the limited role of plant uptake in the removal of metals from storm water in bioretention systems (Read *et al.*, 2008; LeFevre *et al.*, 2015). Several factors could interact with the Cd uptake, for example the interaction of soil composition, pH, organic matter, and available mineral elements may decrease or increase the plant availability of Cd (Chizzola and Lukas, 2006). Furthermore, effective vegetation metal removal performance in bioretention has been attributed to species (i.e. hyperaccumulating plants), root architecture, plant age, and leaf area and the species chosen may not be metal accumulators or alter the soil chemistry/ecology to enhance metal retention (Muerdter *et al.*, 2018). Based on the average effluent concentrations, reduction efficiency for Pb and Cd was more than 87%. Removal was very high in non-vegetated bioretention containers >99.4%, this is due to the absence of roots and soil compaction (Rycewicz-Borecki *et al.*, 2016). Similarly,

Table 1 - Effects of Cd and Pb on the SPAD chlorophyll in three ornamental shrubs

Treatments		A		B		C		D		E		F	
		Lp	Lp	Hh	Hh	Ch	Ch	Lp	Hh	Lp	Ch	Ch	Hh
Control - without heavy metals	Mean	69.03	68.10	38.90	38.23	49.70	53.80	42.67	36.93	58.60	67.17	41.53	47.43
		(6.55)	(3.73)	(1.54)	(0.29)	(1.39)	(1.41)	(7.61)	(4.83)	(0.10)	(3.10)	(0.58)	(3.47)
	Mean	44.37	56.17	43.50	44.60	56.13	60.70	49.40	38.67	54.50	61.43	63.43	47.03
Treatment with heavy metals		(4.08)	(2.76)	(8.44)	(3.75)	(4.22)	(5.47)	(4.76)	(4.36)	(5.60)	(5.75)	(8.13)	(5.55)
	Mean	51.27	52.80	42.83	42.17	62.93	61.67	54.43	38.83	53.70	66.30	66.20	45.50
		(1.58)	(1.57)	(1.81)	(1.29)	(6.37)	(3.21)	(1.66)	(3.97)	(4.47)	(2.41)	(4.22)	(3.74)
	Mean	75.40	66.53	38.57	41.70	60.57	69.70	66.30	43.57	77.03	65.53	62.70	44.50
		(8.59)	(9.37)	(4.40)	(2.17)	(4.30)	(4.25)	(8.83)	(2.61)	(4.74)	(6.33)	(2.98)	(2.85)
	Mean	70.90	63.73	43.27	42.33	61.57	61.53	52.97	43.80	69.20	60.23	60.93	40.33
	(10.62)	(14.17)	(5.43)	(2.81)	(7.09)	(5.89)	(6.75)	(5.16)	(12.33)	(2.97)	(4.34)	(4.44)	
	Mean	60.87	70.57	41.23	46.30	44.77	44.40	55.20	41.43	65.77	65.00	65.63	40.37
		(13.55)	(9.64)	(3.09)	(3.64)	(3.49)	(1.75)	(2.67)	(2.61)	(7.16)	(2.31)	(1.91)	(1.75)

Lp= *Lonicera pileata*, Hh= *Hypericum* ‘Hidcote’, Ch= *Cotoneaster horizontalis*.

Standard deviation in brackets. SPAD readings were recorded for 3 positions on each leaf and for 3 different leaves on a single shrub. Treatments were at 2 plants per pot, each pot contained 2 plants of the same species (column A, B and C) and plant mix (2 species, column D, E and F).



Rycewicz-Borecki et al. (2016), found that compacted soil conditions of unplanted controls retained significantly more Cu, Pb, and Zn than *Carex praegracilis*, and *Carex microptera* treatments.

The outflow concentrations changed over time and the removal efficiency was lower in the second irrigation for the majority of planted pots and not for the unplanted ones. This may be due to soil compaction. The lower removal rate could be attributed to leaching of Pb and Cd from the bioretention media as the concentration of heavy metals in the bottom layer increases (Muthanna et al., 2007).

Reduction rates in this study agree with the rates observed in previous experiments carried out on biore-

tention systems in laboratory (Davis et al., 2003; Kabir et al., 2014; Wang et al., 2017; Muerdter et al., 2018).

The results suggested that plant growth was not influenced by heavy-metal treatments for the majority of species. It is likely that the heavy metal concentrations were below the tolerance limits of these species or the length of exposure time was not long enough.

However, we found statistically significant differences (Duncan multiple range test;  $p < 0.05$ ) in root/shoot weight ratios for *Hypericum* sp. The addition of heavy metals appeared to increase the root/shoot ratio (Table 3). This observation may be due to the fact that low and moderate doses of Cd could stimulate multiplication, rooting, and biomass

Table 2 - Outflow concentrations and reduction efficiencies for Pb and Cd

	Soil (Unplanted pots)	<i>Lonicera</i> sp. & <i>Lonicera</i> sp.	<i>Hypericum</i> sp. & <i>Hypericum</i> sp.	<i>Cotoneaster</i> sp. & <i>Cotoneaster</i> sp.	<i>Lonicera</i> sp. & <i>Hypericum</i> sp.	<i>Lonicera</i> sp. & <i>Cotoneaster</i> sp.	<i>Cotoneaster</i> sp. & <i>Hypericum</i> sp.
Outflow concentration (Pb) (µg/L) 1 <sup>st</sup> irrigation	7.36 (8.97)	8.88 (8.54)	4.17 (1.84)	7.03 (11.02)	9.37 (5.67)	4.13 (1.58)	7.07 (12.17)
<i>p</i> value (Kruskal-Wallis test)	NS	NS	NS	NS	NS	NS	NS
Reduction % (Pb)	99.6	99.6	99.8	99.7	99.5	99.8	99.7
Outflow concentration (Pb) (µg/L) 2 <sup>nd</sup> irrigation	5.88 (1.87)	237.80 (313.60)	53.04 (79.04)	49.42 (31.49)	20.52 (4.69)	13.94 (6.52)	80.32 (107.64)
<i>p</i> value (Kruskal-Wallis test)	NS	NS	NS	NS	NS	NS	NS
Reduction % (Pb)	99.7	87.9	97.3	97.5	99.0	99.3	95.9
Outflow concentration (Cd) (µg/L) 1 <sup>st</sup> irrigation	2.08 (2.32)	1.45 (0.88)	1.77 (1.05)	2.38 (4.28)	2.68 (3.41)	1.57 (1.82)	3.23 (3.78)
<i>p</i> value (Kruskal-Wallis test)	NS	NS	NS	NS	NS	NS	NS
Reduction % (Cd)	99.4	99.6	99.5	99.4	99.3	99.6	99.1
Outflow concentration (Cd) (µg/L) 2 <sup>nd</sup> irrigation	1.44 (0.93)	3.78 (2.28)	2.54 (1.52)	8.34 (6.97)	2.22 (1.69)	2.04 (1.72)	7.34 (10.42)
<i>p</i> value (Kruskal-Wallis test)	NS	NS	NS	NS	NS	NS	NS
Reduction % (Cd)	99.6	99.0	99.3	97.9	99.4	99.5	98.1

Standard deviation in brackets. Kruskal-Wallis test; significant at  $p < 0.05$ . NS= not significant.

Table 3 - Effect of heavy metals on stem dry weight (SDW), root dry weight (RDW) leaf dry weight (LDW), total dry weight (TDW) and root/shoot

Treatments	SDW (g)	RDW (g)	LDW (g)	TDW (g)	Root/Shoot (g)
<i>Lonicera</i> sp. & <i>Lonicera</i> sp. without heavy metals	22.93 (4.07)	10.37 (3.73)	19.53 (3.71)	52.83 (7.93)	0.63 (0.26)
<i>Lonicera</i> sp. & <i>Lonicera</i> sp. with heavy metals	27.97 (4.04)	11.85 (1.64)	24.13 (5.11)	63.95 (10.28)	0.60 (0.06)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	NS
<i>Hypericum</i> sp. & <i>Hypericum</i> sp. without heavy metals	19.92 (8.43)	9.32 (4.51)	13.38 (7.41)	42.62 (19.70)	0.46 (0.21)
<i>Hypericum</i> sp. & <i>Hypericum</i> sp. with heavy metals	22.40 (5.70)	6.75 (4.14)	20.48 (7.61)	49.63 (16.22)	0.71 (0.14)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	<0.01
<i>Cotoneaster</i> sp. & <i>Cotoneaster</i> sp. without heavy metals	55.03 (11.37)	11.45 (7.62)	16.73 (7.01)	83.22 (24.79)	0.24 (0.06)
<i>Cotoneaster</i> sp. & <i>Cotoneaster</i> sp. with heavy metals	65.87 (6.79)	16.35 (3.19)	18.00 (2.92)	100.22 (10.72)	0.22 (0.03)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	NS
<i>Lonicera</i> sp. & <i>Hypericum</i> sp. without heavy metals	28.28 (7.68)	10.73 (4.87)	20.50 (6.45)	59.52 (15.85)	0.54 (0.14)
<i>Lonicera</i> sp. & <i>Hypericum</i> sp. with heavy metals	26.65 (4.89)	8.92 (3.01)	16.50 (2.75)	52.07 (9.38)	0.47 (0.07)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	NS
<i>Cotoneaster</i> sp. & <i>Hypericum</i> sp. without heavy metals	43.07 (18.61)	11.62 (6.98)	17.28 (4.23)	71.97 (23.38)	0.37 (0.21)
<i>Cotoneaster</i> sp. & <i>Hypericum</i> sp. with heavy metals	45.90 (24.22)	10.23 (5.04)	18.75 (6.51)	74.88 (33.52)	0.38 (0.18)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	NS
<i>Lonicera</i> sp. & <i>Cotoneaster</i> sp. without heavy metals	45.38 (21.95)	13.35 (4.47)	19.07 (6.50)	77.80 (28.10)	0.35 (0.11)
<i>Lonicera</i> sp. & <i>Cotoneaster</i> sp. with heavy metals	49.18 (17.28)	16.23 (5.78)	18.95 (7.81)	84.37 (16.94)	0.31 (0.15)
<i>p</i> value (Duncan multiple range test)	NS	NS	NS	NS	NS

Standard deviation in brackets. Duncan multiple range test; significant at  $p < 0.05$ . NS=not significant.

production in heavy metal-tolerant shrubs (Wiszniewska *et al.*, 2017). Furthermore, the genus *Hypericum* L. has been described as a cadmium hyperaccumulator (Gardea-Torresdey *et al.*, 2005).

SPAD readings ranged from 36.93 *Hypericum* sp. to 77.03 in *Lonicera*. Differences in chlorophyll content (Table 1) were statistically significant (One-Way ANOVA Test;  $p < 0.05$ ) in mono-specific pots between *Hypericum*, *Lonicera* and *Cotoneaster* (Table 1, columns A,B,C) as well as in mixed pots containing, respectively, *Hypericum* and *Lonicera*, and *Lonicera* and *Cotoneaster* plants (Table 1, columns D and F). This result agrees with previous studies that found that mixed heavy metals decrease the chlorophyll content in various plants (Chandra and Kang, 2016). The concentration of non-essential metals like Pb and Cd may be the cause of low chlorophyll content and could also have several negative impacts via oxidative stress (Nadgórska-Socha *et al.*, 2013).

Recent studies have suggested that laboratory-scale filter columns do not satisfactorily replicate field-scale conditions leading to calls for in situ evaluation of bioretention systems (Trowsdale and Simcock, 2011; Liu *et al.*, 2014). Furthermore, previous studies conducted in greenhouses in which plants were grown in pots have shown that pot size can have a limiting effect on plant growth, nutrient efficiency and photosynthesis rates (Ray and Sinclair, 1998). Future research should comparatively assess plant species in a laboratory-scale filter column and in situ.

#### 4. Conclusions

This study tested an alternative bioretention system filter media and species design. The reduction of Cd and Pb concentrations was over 87% similar to other studies, however there were no differences between replicates with plants and the soil-only control. Therefore, the presence of vegetation did not significantly affect heavy metal removal. Some species appeared Cd and Pb tolerant suggesting they would be appropriate in selections for bioretention systems in Mediterranean cities. The long-term effects of these, and other, metal contaminants is however advisable for future studies. Plant selection for bioretention systems has received considerably more research attention in recent years than previously, but important research gaps still remain, e.g. the impact of bioretention vegetation on emerging contaminants (Muerdter *et al.*, 2018). Our alternative bioretention system filter media can be used to

assess other plant species and different pollutants (e.g. nutrients, metals and emerging contaminants). More in depth study is recommended to help landscape architects and horticulturalists in the selection of suitable species or species mixes for bioretention systems.

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