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RESEARCH ARTICLE

Phytoremediatory efficiency of *Chrysopogon zizanioides* in the treatment of landfill leachate: a case study

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Abstract

A common approach for waste management is their disposal in landfills, which is usually associated with the production of dangerous gases and of liquid leachate. Due to its toxicity, polluted liquid negatively impacts on the environment with the possible contamination of large volumes of soil, groundwater, and surface water. Leachate remediation is therefore subject of intensive research, and phytoremediation has been achieving increasing interest in recent decades. We describe here the suitability of vetiver grass for the remediation of two leachates collected in urban landfills of northern Italy, characterized by different composition. Our objective was measuring the accumulation/tolerance potential of this species and the evapotranspiration ability in a pot experiment, to evaluate applicability of vetiver plants for the reduction and decontamination of landfill leachate. Plants were grown for 4 months in pots with a zeolite growth bed and watered with either tap water (control) or undiluted landfill leachate. Plant growth and fitness and elemental content in shoots and roots were evaluated at the end of the experiment. In these experimental conditions, the high bioaccumulation of metals highlights the suitability of this species for its employment in phytoremediation; however, vetiver growth under leachate treatment was strongly dependent on leachate composition, making a case-to-case evaluation of plant tolerance necessary before large-scale application.

Keywords *Chrysopogon zizanioides* · Vetiver · Landfill leachate · Rhizofiltration · Nitrogen · Metals

Introduction

In the past decades, widespread industrial and commercial growth has been accompanied by an increased production of municipal and industrial solid waste. Waste disposal in landfills remains to this day the most common method for waste management around the world, with in some instances up to 95% of generated refuse placed in landfills, because it is simple and relatively inexpensive (Kim and Owens 2010). However, landfills are associated with consistent pollution problems, primarily the production of potentially explosive gases and of liquid leachate (Sang et al. 2010). Due to the inherent water content of waste, the infiltration of rainwater, and to the chemical and biological reactions occurring in the refuse, a landfill site produces leachate during its life and after its decommission and may generate leachate for hundreds of years following its closure (Jones et al. 2006). This polluted liquid represents a very critical issue not only for its toxic impact on the environment, such as the possible contamination of large volumes of soil, groundwater, and surface water, but also for the potential negative effect to human health (Jones et al. 2006).'

The microbiological and chemical composition of landfill leachate is usually complex and variable and is dependent on a wide range of factors, including waste characteristics and age, climate and seasonal weather variations, and the operational practices in landfill management. Depending on environmental factors and the age of the landfill, leachate characteristics, such as chemical (COD) and biological oxygen demand (BOD), pH, nitrogen content and speciation, and heavy metal

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Elisa Fasani and Giovanni DalCorso contributed equally to this work.

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levels, can vary significantly (Kjeldsen et al. 2002; Renou et al. 2008). In any case, landfill leachates must be contained, collected, and treated adequately to prevent their dispersion in the environment (Dumble and Ruxton 2001); to this end, regulation in many countries requires the installation of leachate collection systems as well as plans for leachate management during and after landfill operating life. Landfill leachate treatments are conventionally carried out by the following methods, or a combination thereof: (i) in traditional sewage plants together with municipal wastewater; (ii) by biological processing performed by microorganisms able to degrade organic compounds to carbon dioxide in aerobic condition and to biogas in anaerobic conditions; or (iii) by chemical and physical means such as flotation, coagulation-flocculation, chemical precipitation, and air stripping (reviewed by Renou et al. 2008). Other non-conventional strategies have been designed for leachate treatment, including membrane-based technologies as reverse osmosis and nanofiltration (Van der Bruggen et al. 2003), oxidation processes (Li et al. 2010), and sequencing batch reactors (Shammas and Wang 2009).

Although a wide range of treatment processes had been developed and tested to remediate leachate with different degrees of success, phytoremediation has been achieving increasing interest in recent decades. Indeed, it is a promising system that exploits the innate plant accumulation capacity as well as the plant-rhizosphere system to detoxify, degrade, and inactivate pollutants in the leachate (Jones et al. 2006). This technology has gained social acceptance and can be considered an appealing alternative to the engineering-based remediation methods. Moreover, the implementation of phytoremediation in landfill areas may be easily acceptable by the inhabitants because of the non-environmentally disruptive nature of this system, as well as for its economic benefits and the possible re-use of its by-products (Gomes 2012; Tripathi et al. 2016).

There is a consistent literature on the use of plant species to treat leachates. Potential advantages include the provision of water and nutrient for plant growth (Nagendran et al. 2006), the reduction of leachate volume by evapotranspiration (Licht et al. 2004; Keizer-Vlek et al. 2014), and the exploitation of the rhizosphere processes for binding and degradation of pollutant in the leachate (Kaseva 2004; Akinbile et al. 2012; Calheiros et al. 2009). Various reports have ascertained good results with the use of aquatic plants. Tropical water lily (*Nymphaea spontanea*) was efficient in removing hexavalent chromium, although plants showed toxicity symptoms as reduction of chlorophyll, protein, and sugar contents (Choo et al. 2006). In a pilot-scale experiment, papyrus (*Cyperus papyrus*) showed effectiveness in the treatment of domestic wastewater (Perbangkhem and Polprasert 2010). Noticeably, the use of plants for removing contaminants is not devoid of restrictions and further research is needed to determine the suitability of different species for this purpose, considering their contaminant accumulation and tolerance, the depth and expansion of root system and biomass production, and the annual/perennial nature of the plant species. To add complexity, diverse types of pollutant may require different plant species (Zalesny and Bauer 2007; Rai 2008; Anning et al. 2013).

Vetiver grass (*Chrysopogon zizanioides* L. Roberty, formerly *Vetiveria zizanioides* L.) is one of the plant species that meet the required criteria for phytoremediation. It is a tall grass (1–2 m) with an abundant vegetative growth, characterized by a massive, finely structured and deep root apparatus, capable of reaching 3–4 m depth in the first year (Truong and Danh 2015). For this trait, vetiver grass is well known for its effectiveness in erosion and sediment control. Furthermore, it is of particular interest for its unique morphological, physiological, and ecological features, as well as for its tolerance to different extreme climatic and edaphic conditions such as drought and flooding, excess of nutrients, persistent organic, and heavy metal pollution (Chen et al. 2004; Angin et al. 2008; Truong and Danh 2015). This grass was recognized for having a “super absorbent” feature that could be reasonably exploited for the treatment of wastewater and leachate (Badejo et al. 2017). Given the above, the purpose of this work was to investigate the suitability of vetiver grass for the remediation of two leachates of diverse origin, both from urban landfills but with different composition, with the specific objective of measuring the accumulation/tolerance potential of this species and their evapotranspiration ability in a pot experiment, to evaluate its applicability for the containment of landfill leachate leakages and the treatment of collecting ponds.

Materials and methods

Plant material

Vetiver grass (*Chrysopogon zizanioides* L. Roberty) ecotype Monto, obtained from the Italian Vetiver consortium, was selected to be applied as mono-culture for the phytoremediation of landfill leachate. Plantlets were multiplied clonally by splitting mature plants into slips which were grown for 3 months in the greenhouse (16-h photoperiod at 23 °C) before the experiment (Truong et al. 2008). During acclimation, vetiver grass was irrigated with tap water. Ten days before the beginning of the experiment, the plants were watered with a mixture of plant growth-promoting rhizobacteria and mycorrhizal fungi (BioSoil Expert S.r.l., Rovereto, TN, Italy; the composition is reported in Table 1), previously selected for their beneficial effect on root and shoot growth and plant general fitness (data not shown). Chlorophyll concentration of random individual plants was assessed and no statistically significant differences were observed (data not shown), confirming the uniformity of plants subsequently initiated to the treatments. Shoots were pruned at about 20 cm before the start of the experiment.

Table 1 Microbial mixture fed to vetiver grass 3 days before the beginning of the experiment

Formulation commercial name	Concentration	Microbial composition
BSE_2	10 g/L	*Pochonia chlamydosporia*; *Frankia* spp.; *Glomus intraradices*
BSE_3	2 g/L	*Pisolithus tinctorius*; *Rhizopogon villosuli*; *Rhizopogon luteolus*; *Rhizopogon amylopogon*; *Rhizopogon fulvigleba*; *Scleroderma cepa*; *Scleroderma citrinum*; *Laccaria bicolour*; *Glomus intraradices*; *Glomus aggregatum*; *Glomus mosseae*; *Glomus brasilianum*; *Glomus monosporum*; *Glomus deserticola*; *Glomus clarum*; *Glomus etunicatum*; *Gigaspora margarita*
BSE_4	15 g/L	*Pseudomonas fluorescens*; *Rhodopseudomonas palustris*

Landfill leachates

Leachates were derived from two different municipal landfills in the Trento province (North Italy), Ischia-Podetti and Rovereto Loc. Lavini, both collecting urban solid wastes. Ischia-Podetti (IP) is the major municipal landfill in the Trento province and since January 2018 is the only one still active in the area. It now collects shredded unrecyclable urban solid wastes from the entire province and has a closure time predicted around year 2020. It is composed by four main plots: three are inactive and resting on a substrate of old municipal waste dating back to the 1970s, whereas the fourth has a volume of 150,000 m^3 and is still operating. The IP leachate collection system gathers leachates from plots 2, 3, and 4, whereas plot 1 is already mineralized; the average leachate flux is about 20,000 m^3/year (ADEP agency, personal communication). Rovereto landfill (R) used to collect unrecyclable urban solid wastes from the town of Rovereto and the neighboring municipalities and was closed and capped at the end of 2017, but was still active at the time of leachate sampling. At the end of its activity, it had reached a volume of disposed waste of about 170,000 m^3. The leachate and biogas collection systems are still operating: the annual leachate production is about 30,000 m^3/year (ADEP agency, personal communication). Leachates from both landfills are actually treated in the municipal sewage plant.

Leachate was sampled in October 2016 through the landfill leachate collection system, sampled and stored at − 20 °C. Leachate characteristics collected from the landfill, such as pH, conductivity at 20 °C (EC), suspended matter, COD, and BOD$_5$, were quantified by the landfill monitoring systems and are reported in Table 2. The elemental content prior to treatment was measured according to the procedures reported in a following paragraph.

Experimental design

Eighteen plastic pots were used for this experiment, divided as follows: three pots for control, IP, and R treatments planted with vetiver grass, and three pots of same treatments but not planted with vetiver, with three replicates for each set. The experimental setting, organized as a closed batch system, is schematized in Table 3. Pots (22 × 30 × 30 cm) were filled with 7.5 kg dry zeolite with 7–12 mm particle size (Europomice S.r.l., Pitigliano, GR, Italy). Half of the vessels were planted, whereas the other half remained non-planted as a control to estimate the time of leachate volume reduction and natural attenuation of pollution in the absence of plants. Six healthy vetiver plants, selected for uniform aspect and initial weight (2.4 ± 0.5 g/plant), were transferred in each of the 9 planted containers, achieving a density of 9 plants/m^2. Planted and non-planted containers were watered with 1 L of either IP or R undiluted leachate at the beginning of the experiment; irrigation with tap water was used as control. Further leachate or water in case of controls (1 L each time) was supplied to each system when free liquid was no longer present in the vessel. The experiment was conducted for 4 months in the greenhouse under controlled growth conditions (16-h photoperiod at 23 °C). Evapotranspiration rate was calculated by the application of the soil water balance equation (Rana and Katerji 2000). Since the experiment was conducted in pot in the greenhouse under controlled climatic conditions, the contribution given by precipitations, emergence from water table, surface runoff, and drainage was considered as null. The values for evapotranspiration rate are the mean of four measurements for the three replicates of each condition tested.

At the end of the experiment, plants were collected and weighted and roots washed in deionized water. Leaf samples for chlorophyll quantification (three for each replicate and condition) were cut between 2 and 4 cm above the crown or root collar: chlorophylls were extracted in NaCO$_3$-buffered 90% aqueous acetone and the total chlorophyll content was measured as described by Porra et al. (1989). The above-ground tissues (leaf samples) were collected, frozen in N$_2$ liquid, and reduced to powder. The same procedure was followed for the under-ground tissue (the root samples). Powdered leaf and root samples as well as zeolite samples for element quantification were dried at 60 °C.

Table 2 Characteristics of Ischia-Podetti and Rovereto leachates. *n.d.* not detectable

		Ischia-Podetti	Rovereto
pH		7.8	8
Color		Detectable in a 1:20 dilution	Detectable in a 1:20 dilution
Conductivity at 20 °C	µS/cm	7330.0	11,810.0
Suspended matter	mg/L	33.5	30.8
COD	mg/L	629.0	1070.0
BOD_5	mg/L	23.0	29.0
BOD_5/COD		0.037	0.027
Total C—CNS	mg/L	162.82	585.06
Total N—CNS	mg/L	614.84	1273.96
TKN	mg/L	386.91	1094.32
NO_2^-	mg/L	n.d.	n.d.
NO_3^-	mg/L	n.d.	n.d.
P	mg/L	3.30	11.22
Al	mg/L	0.41	0.47
As	mg/L	n.d.	n.d.
B	mg/L	2.02	4.83
Ba	mg/L	0.35	0.39
Ca	mg/L	148.86	80.44
Cd	mg/L	n.d.	n.d.
Cr	mg/L	0.06	0.19
Cu	mg/L	0.01	0.03
Fe	mg/L	1.65	4.35
Hg	mg/L	n.d.	n.d.
K	mg/L	307.94	714.92
Mg	mg/L	110.74	110.16
Mn	mg/L	0.55	0.12
Na	mg/L	523.88	1123.58
Ni	mg/L	0.09	0.16
Pb	mg/L	n.d.	n.d.
Zn	mg/L	0.09	0.13
Total bacterial count at 22 °C	UFC/mL	1.3×10^4	8.1×10^3
Total coliforms	UFC/100 mL	6000	Absent
Fecal streptococci	UFC/100 mL	Absent	96

N and C quantification

Total N and C contents were measured by CNS analysis, according to the AACCI Method 46-30.01 for plant tissues (AACC International 2012) and method VII of the Italian DM 13/09/1999 (Ministero per le politiche agricole e forestali 1999) for leachates and zeolite. Organic and ammonium N (total Kjeldahl nitrogen (TKN)) was measured by the Kjeldahl method, following the specifications of the AOAC Official Method 977.02 (AOAC International 2016) for plant material and method XIV of the Italian DM 13/09/1999 (Ministero per le politiche agricole e forestali 1999) for leachates and zeolite. Quantification of nitrate and nitrite was performed by ion chromatography, according to EPA Method 300.0 for plant tissues (Pfaff 1993) and method XIV.9 of the Italian DM 13/09/1999 (Ministero per le politiche agricole e forestali 1999) for leachates and zeolite.

Mineral content determination by inductively coupled plasma atomic emission spectrometry

Mineral quantification was performed following the specifications of the AOAC Official Method 2013.06 for the analysis of plant material (AOAC International 2016), and U.S. EPA method 3050B (U.S. EPA 1996) and method XI of the Italian DM 13/09/1999 (Ministero per le politiche agricole e forestali 1999) for the mineralization and analysis of leachates and zeolite, respectively. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was employed for the content determination of the

Table 3 Schematization of the experimental system, with indication of the follow-up analyses conducted for each condition

Watering	Planted/ non-planted	No. of replicates	Analyses
Water	Non-planted	3	Evapotranspiration rate, element quantification on zeolite
	Planted	3	Evapotranspiration rate, biomass and chlorophyll quantification, element quantification on zeolite, leaves and roots
Ischia-Podetti leachate	Non-planted	3	Evapotranspiration rate, element quantification on zeolite
	Planted	3	Evapotranspiration rate, biomass and chlorophyll quantification, element quantification on zeolite, leaves and roots
Rovereto leachate	Non-planted	3	Evapotranspiration rate, element quantification on zeolite
	Planted	3	Evapotranspiration rate, biomass and chlorophyll quantification, element quantification on zeolite, leaves and roots

following elements: Al, As, B, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Zn. One hundred fifty to two hundred milligrams of dried material was prepared by microwave closed vessel digestion by an Ethos 1600 Advanced Microwave Digestion Labstation (Milestone S.r.l., Sorisole, BG, Italy). Each sample was placed in a Teflon (TFM) vessel with 6 mL of Suprapur® quality concentrated (30%) hydrochloric acid and 3 mL of Suprapur® quality concentrated (65%) nitric acid (Merck Chemicals GmbH, Darmstadt, Germany). The so prepared samples were subjected to a microwave digestion as follows: step 1, 25–200 °C for 15 min at 1500 W with P max 45 bar; step 2, 200 °C for 18 min at 1500 W with P max 45 bar; step 3, 200–35 °C for 25 min. After cooling down to room temperature, the dissolved samples were diluted with ultrapure water (resistivity 18.2 M Ω cm at 25 °C) to a final volume of 50 mL, reaching a final concentration of 12% hydrochloric and 6% nitric acid.

The element quantification was conducted using an Arcos EOP ICP-AES analyzer (Spectro Analytical Instruments GmbH, Kleve, Germany). Operating parameters of the instrument were optimized as follows: plasma observation axial, nebulizer Crossflow, spray chamber Scott doublepass, torch injector quartz diameter 3.0 mm, plasma power 1350 W, coolant gas 12.0 L/min, auxiliary gas 0.8 L/min, nebulizer gas 0.90 L/min, additional gas 0.20 L/min, sample uptake rate 2.0 mL/min, replicate read time 28 s, replicates 3, pre-flush time 60 s. Calibration standards were prepared using multi-element and single-element standard solutions (Inorganic Ventures Inc. Christiansburg, VA, USA) in 12% hydrochloric and 6% nitric Suprapur® acid (Merck Chemicals) as the samples. Analytes were prepared at the concentration of 0, 0.02, 0.05, 0.2, 0.5, 2, 5, 20, and 50 mg/L. For Ca and P, standards concentrated 200, 500, and 2000 mg/L were also added to the calibration. The accuracy and precision of both methods were confirmed by analyzing blank solution, low-level control solution (recovery limits ± 30%), and medium-level control solution (recovery limits ± 10%) prepared as described above.

Bioconcentration and translocation factors

The bioconcentration factor (BCF, L/kg) was calculated as C_P/C_L where C_P is the metal concentration in the whole plant tissue (mg/kg dry weight (DW)) and C_L is the metal concentration in the leachate (mg/L) (Soda et al. 2012). A higher BCF indicates a better capability to accumulate and remove the metal from the leachate. The translocation factor (TF) was calculated as C_S/C_R wherein C_S is the metal concentration in the shoot (mg/kg DW) and C_R is the metal concentration in the roots (mg/kg DW) (Soda et al. 2012). Higher values of TF indicate a better capability to transfer metal to the aboveground tissues.

Statistical analysis

Statistical significance of the data was evaluated by one-way ANOVA, followed by a Tukey post hoc test, using the software GraphPad Prism v. 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). In the figures, data are represented as mean ± standard error (SE) and data with non-significant differences ($p > 0.05$) are indicated with the same letter.

Results and discussion

Characterization of leachates

Landfill effluents are usually combinations of inorganic, natural, and xenobiotic components, and their mixture determines the leachate pollution potential (Kjeldsen et al. 2002). Although the leachate composition is strongly influenced by the landfill type and age, there are features common to all leachates such as the high levels of dissolved organic carbon compounds, nitrogen, and metals, which make leachates an unbalanced nutrient medium for plant growth (Sang et al. 2010). In this case, physical and chemical properties of both leachate samples are summarized in Table 2 and are compatible with what observed for mature municipal landfills (Kjeldsen et al. 2002; Kulikowska and Klimiuk 2008; Renou et al. 2008). Indeed, both are characterized by an alkaline pH and low levels of organic matter, expressed as COD. Moreover, the very low BOD_5/COD ratio is indicative of the low residual biodegradable matter in the landfill. Both leachates have relatively high salinity (EC), particularly R, which is also high in P and B. However, the highly toxic elements As, Cd, Hg, and Pb were not detected in both leachates, whereas low levels for Cr (0.06 mg/L in IP and 0.19 mg/L in R) were measured; these values were indeed promising for the application of undiluted leachate to vetiver grass, except for EC in R. However, the levels of most measured elements, with the exclusion of Al, Ba, Ca, Mg, and Mn, are two to four times higher in R leachate than in IP one (Table 2). Therefore, the physiological response of vetiver grass in this study is strongly dependent on the features of the two different leachates and in particular on the consistent differences in elemental content.

Water removal during experimental period

During the 4-month experiment, vetiver plants were cultivated in zeolite-filled pots, supplemented before the experiment with commercial formulated microorganisms and periodically wetted with undiluted leachate. The choice of zeolite as growth bed was guided by the positive results achieved in previous works. Indeed, zeolite is able to act as ion exchanger and efficiently removes ammoniacal nitrogen, which constitutes the main part of TKN in mature landfill leachate, strongly inhibits microorganism-mediated biodegradation of pollutants, and is highly toxic for aquatic environments (Wen et al. 2006; Halim et al. 2010). For this reason, zeolite has been employed as bedding material in constructed wetlands for the treatment of wastewaters or leachates with positive results (Yalcuk and Ugurlu 2009; Bruch et al. 2011; Mojiri et al. 2016).

During the entire experiment, untreated and IP-treated vetiver plants were bigger and healthier than R-treated plants; the latter displayed a slower growth and earlier senescence (data not shown). Despite this, no difference was observed in the evapotranspiration rate in vetiver grass irrigated either with water or with IP or R leachates (Fig. 1). On the other hand, non-planted replicates showed lower evapotranspiration rate and twice as longer times for the complete removal of water/leachate (Fig. 1). Indeed, vetiver grass has demonstrated an excellent efficiency in volume reduction thanks to its high transpiration rate (Truong and Hart 2001; Truong and Danh 2015). Although evapotranspiration may have a negative effect in constructed wetlands by altering water and nutrient balance (Beebe et al. 2014; Białowiec et al. 2014), in this experiment, where leachate is fed to plants in a closed system, volume reduction is fundamental. It should be noted that our experiment was performed in the greenhouse, where evapotranspiration is limited if compared with open air. This means that the leachate volume reduction by evapotranspiration could be significantly increased by operating in open field.

Plant biomass and growth

The main effects of leachate irrigation on vetiver grass are shown in Fig. 2, reporting results after 4 months of treatment.

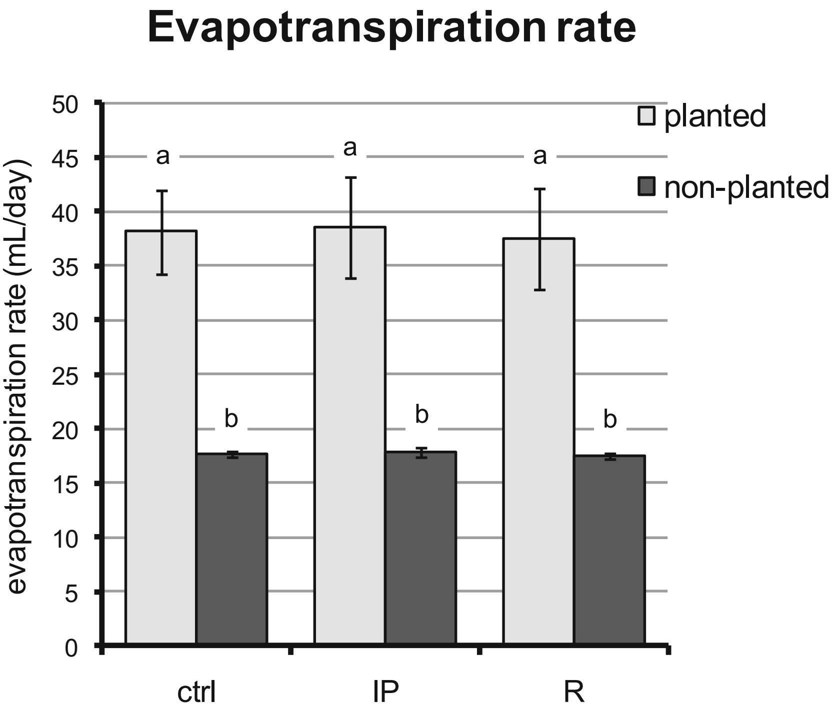


Fig. 1 Analysis of volume reduction for control solution (water), Ischia-Podetti (IP), and Rovereto (R) leachates in planted and non-planted experimental systems; volume reduction is evaluated in terms of rate of water/leachate removal by evapotranspiration. Data are represented as mean ± SE; lowercase letters indicate statistical significance, evaluated by one-way ANOVA followed by a Tukey post hoc test ($p < 0.05$)

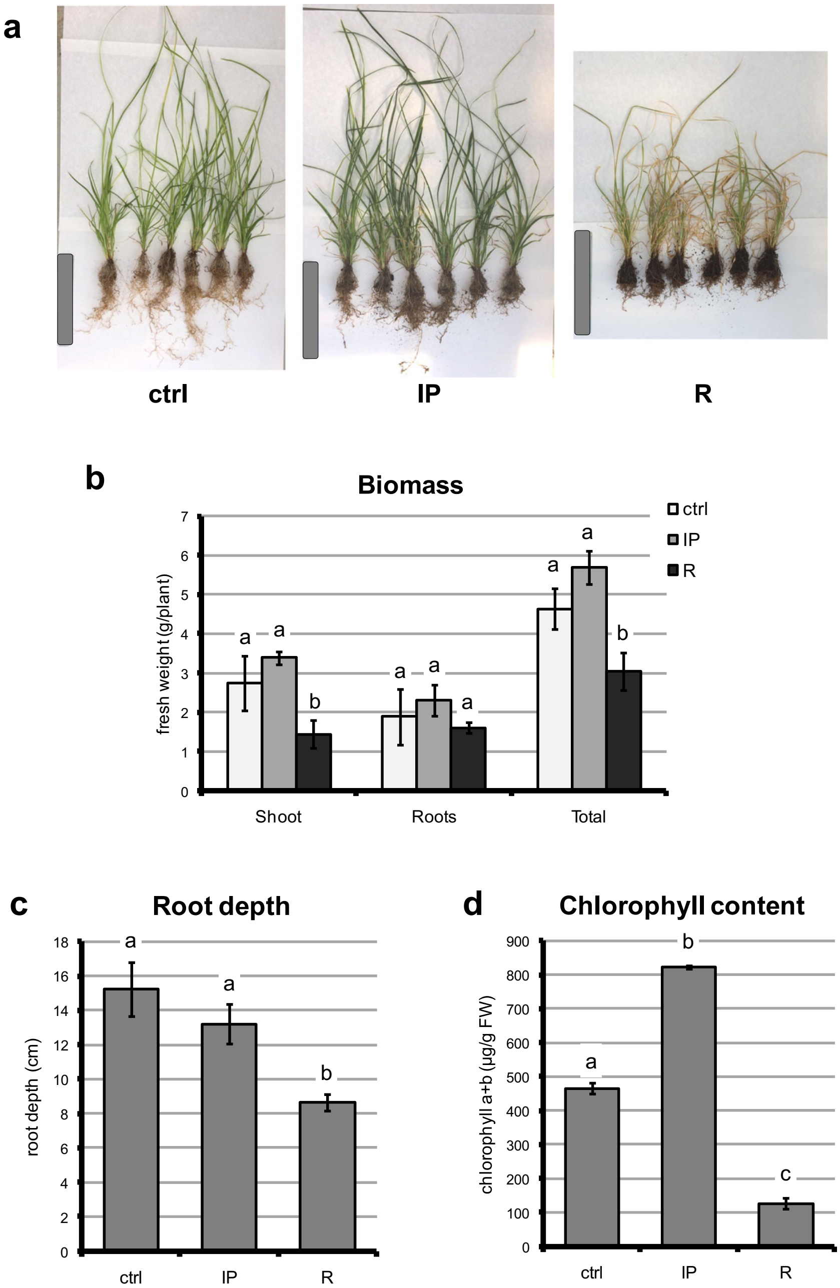
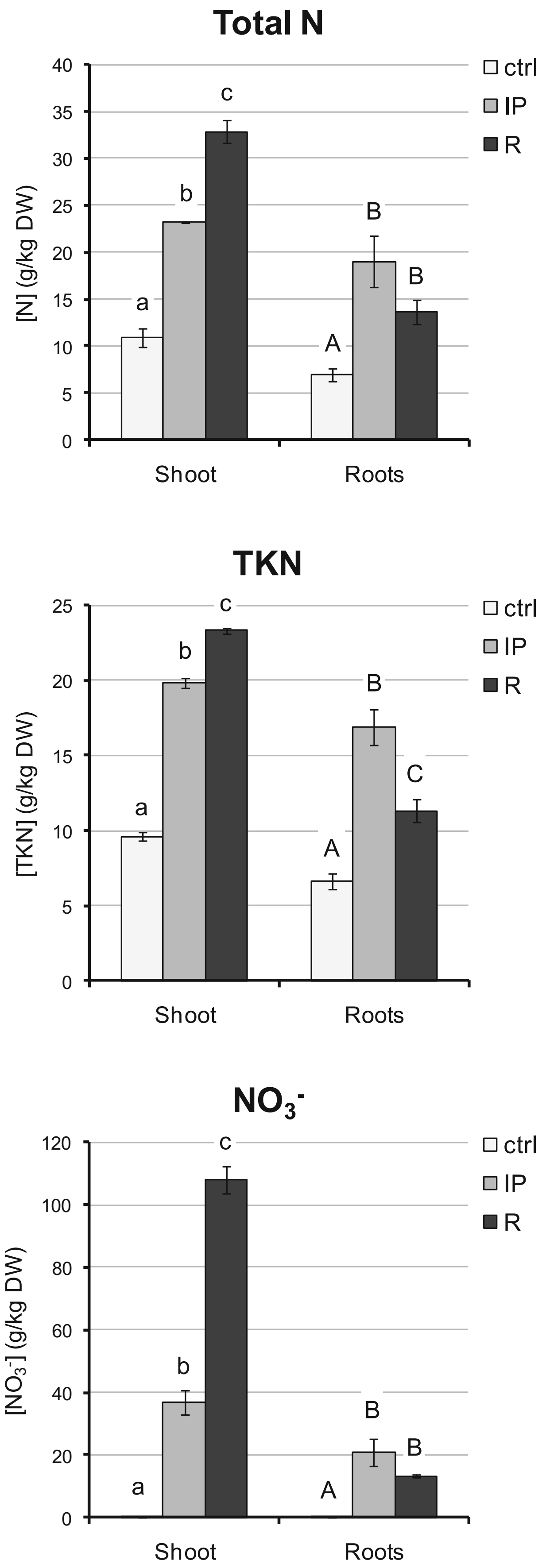


Fig. 2 Phenotype of vetiver grass grown for 4 months in a zeolite bed and irrigated with water (ctrl) or with Ischia-Podetti (IP) or Rovereto (R) landfill leachate. **a** Plant appearance after 4 months of treatment; the gray bars correspond to 20 cm. **b** Shoot, root, and total plant biomass, measured as fresh weight. **c** Root deepening. **d** Total chlorophyll content in the aerial part of vetiver grass. Data in **b**, **c**, and **d** are represented as mean ± SE; lowercase letters indicate statistical significance, evaluated by one-way ANOVA followed by a Tukey post hoc test ($p < 0.05$)

Overall, untreated and IP-treated plants had a normal growth and were bigger and healthier than R-treated plants (Fig. 2a). Untreated control plants had a slightly deeper root system than IP-treated plants, although the difference was not statistically significant (Fig. 2c). Moreover, both global biomass and root and shoot weight showed no statistically significant difference between these two conditions, although IP-treated plants were marginally bigger than untreated ones (Fig. 2b). On the other hand, R-treated plants were significantly smaller, with a reduced root and shoot development, and showed evident chlorosis symptoms (Fig. 2a–c). This result was confirmed by the evaluation of chlorophyll content, where IP-treated plants had by far the highest total chlorophyll levels and R-treated plants the lowest (about one-eighth of that in IP-treated plants; Fig. 2d).

The markedly different effect on plant growth between the two leachate treatments reflects their different composition: indeed, although the most toxic elements were not detected in either landfill effluent, the levels of macronutrients and metals were generally higher, particularly EC in the R leachate (Table 1). Accordingly, the IP leachate had a global positive effect on plant growth whereas the R treatment definitely inhibited it. Truong et al. (2002) reported that the saline threshold of vetiver is at $EC_{se} = 8$ dS/m and soil with EC_{se} values between 10 and 20 dS/m would reduce yield by 10% and 50% respectively. These results indicate that the depressed


Total N
[N] (g/kg DW)
0
5
10
15
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40
a
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TKN
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0
20
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B
Shoot
Roots
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◀ **Fig. 3** Nitrogen content in leaves and roots of vetiver plants irrigated with water (ctrl) or with Ischia-Podetti (IP) or Rovereto (R) landfill leachate; graphs report the main N forms measured, respectively total N (analyzed by CNS), total Kjeldahl N (TKN, analyzed by the Kjeldahl method), and nitrate (analyzed by ion chromatography). Data are represented as mean ± SE; lowercase and uppercase letters indicate statistical significance for shoot and root measurements respectively, evaluated by one-way ANOVA followed by a Tukey post hoc test ($p < 0.05$)

growth of vetiver grass in R treatment is most likely also due to high salinity levels (EC = 11.81 dS/m); thus, dilution of leachate may be needed. This hypothesis is consistent also with evidences from other previous studies (Deifel et al. 2006; Edelstein et al. 2009). The fertilizing effect of landfill leachate has already been reported for both woody species and grasses (Zalesny et al. 2007a; Dimitriou and Aronsson 2010; Justin et al. 2010; Cheng and Chu 2011): in these cases, irrigation with leachate resulted in higher biomass yields in comparison with water-irrigated conditions, although the increase was generally lower than that observed with traditional fertilizing. Conversely, evidences have also emerged indicating that landfill leachates may be detrimental to plant growth, depending on plant genotype and leachate composition (Zalesny et al. 2007b; Justin et al. 2010); in such cases, as for R leachate in this work, the application of diluted leachate may be a viable strategy. Since abundant biomass production is of major importance for successful phytoremediation, different leachate loads and dilutions should be tested prior to establishing a large-scale phytoremediation system, to effectively balance plant fitness and efficient nutrient and metal removal (Aronsson et al. 2010).

Elemental content in plants is indicative of their ability to remove specific pollutants from contaminated matrices. In this experiment, the application of both IP and R leachates induced a significant increase in N content in shoots and roots of vetiver plant in comparison to control conditions, considering the total N, TKN, and nitrate parameters (Fig. 3; Supplementary Table 1 in Online Resources). Vetiver grass was reported to well tolerate extreme N supplies and to efficiently remove N from wastewaters and leachates; in previous works, N shoot content increased up to 2.5% with increasing N supply (Wagner et al. 2003) and from 0.8 to 1.9% upon leachate treatment (Cheng and Chu 2011). In this case, shoot total nitrogen grew from 1% in control conditions to 2.3% and 3.3% upon IP and R treatments, comparably with the value previously reported. However, it must be considered that the fitness in R-treated vetiver plants was significantly compromised. This negatively impacts on the global nutrient removal; therefore, an adequate balance between biomass increase and elemental accumulation should be achieved.

Regarding P content, no statistically significant difference was observed between treated and untreated plants (Supplementary Table 1 in Online Resources). However, vetiver requirements of P are moderate and its excess has an inhibitory effect on growth (Wagner et al. 2003); therefore, it is likely that P excess supplied with the leachates is not absorbed by the plant.

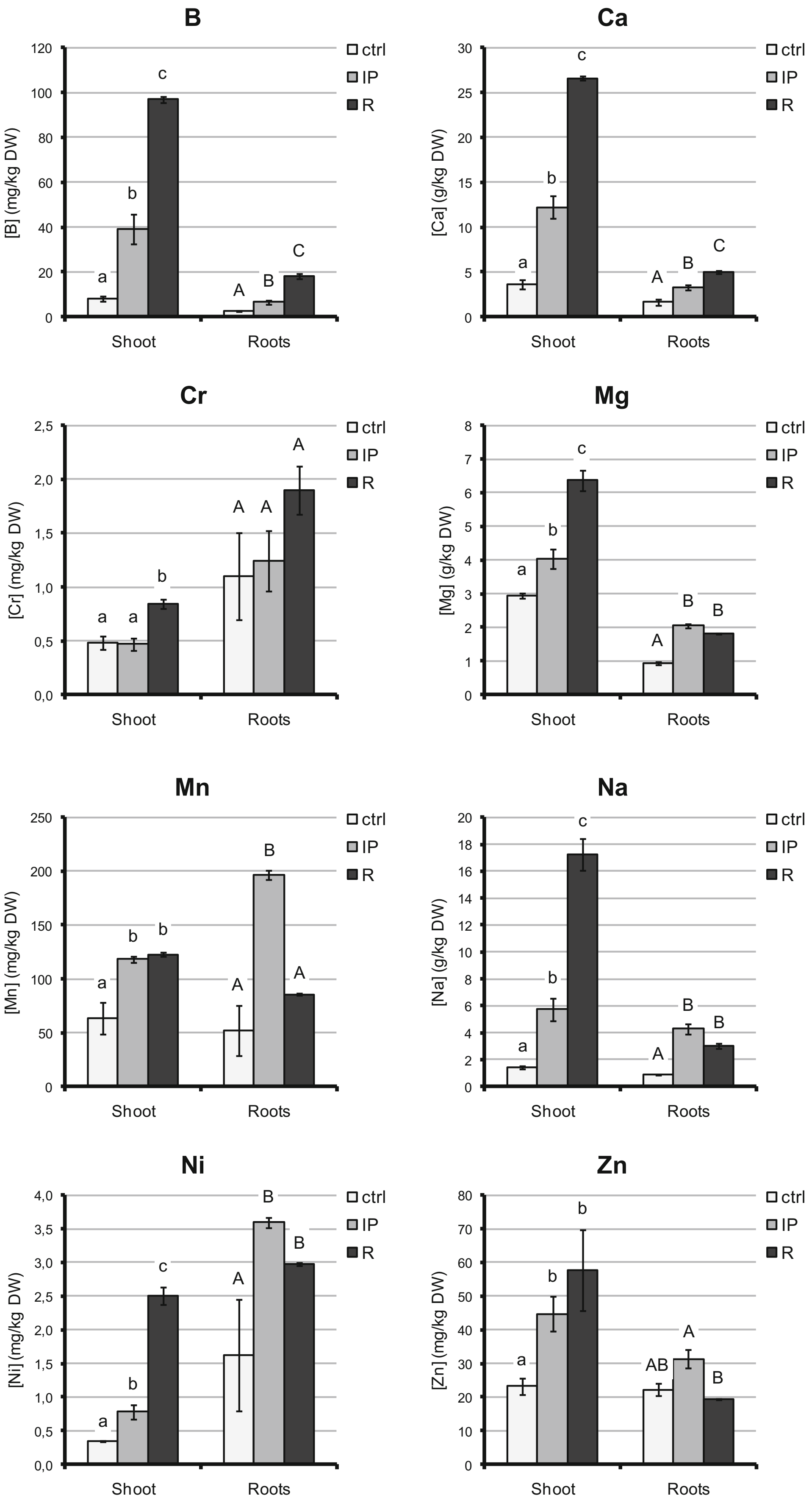
Efficiency of metal removal

Vetiver grass was able to accumulate different elements with varying degrees of efficiency. The levels of some elements (B, Ca, Mg, Mn, Na, Ni, and Zn) in root and shoot were significantly higher than in control (Fig. 4; Supplementary Table 2 in Online Resources); concentrations in the plants were mostly correlated with the amount of element present in the leachate, with R-treated plants reaching in most cases the highest concentrations (Table 1). The BCF for these elements ranged between 10 for Na in the IP treatment and 923 for Mn in the R treatment. Interestingly, TF for B, Ca, Mg, Na, and Zn exceeded one in all conditions, with Zn < Na < Mg < Ca < B in most conditions (Supplementary Table 2 in Online Resources). This evidence indicates that vetiver grass has the ability to efficiently transport and accumulate these elements in shoots. In particular, high values of both BCF and TF were observed for B, a micronutrient whose excess can produce significant toxicity symptoms (Nable et al. 1997). The high B tolerance of vetiver grass, associated with an efficient accumulation and translocation to shoots, has been previously reported and makes this species suitable for the phytoremediation of B-polluted matrices (Xin and Huang 2017, 2018). In addition, Na, Zn, and Mn tolerance and accumulation have also been reported (reviewed in Danh et al. 2009).

In general, the BCF values observed in this work are significantly higher than those reported in literature for vetiver grass (Ghosh et al. 2015; Banerjee et al. 2016; Gautam and Agrawal 2017), in particular for Al, Cu, Fe, Mn, and Zn, whose values are in the order of 10^2. However, such results are comparable or lower than those obtained for other species (Wang et al. 2002; Liao and Chang 2004; Soda et al. 2012; Jerez and Romero 2016). These high BCF values were achieved when plants were grown in hydroponics or constructed wetlands, since elements are significantly more bioavailable when dissolved in aqueous solution than in soil; for example, BCFs in water hyacinth (*Eichhornia crassipes* Mart. Solms.) are about two orders of magnitude higher when plants are grown in water than in sediments (Liao and Chang 2004). In this view, conditions carried out in this work closely resemble those of constructed wetlands, with leachate added to a relatively inert growth bed, thus allowing for high bioaccumulation of metals.

Element persistency in the zeolite bed

An elemental analysis was performed on the zeolite, to evaluate a possible persistency of pollutants in the growth bed. The potential contamination of the substrate is important, as


B
[B] (mg/kg DW)
0
20
40
60
80
100
120
a
b
c
A
B
C
Shoot
Roots
Ca
[Ca] (g/kg DW)
0
5
10
15
20
25
30
a
b
c
A
B
C
Shoot
Roots
Cr
[Cr] (mg/kg DW)
0,0
0,5
1,0
1,5
2,0
2,5
a
a
b
A
A
A
Shoot
Roots
Mg
[Mg] (g/kg DW)
0
1
2
3
4
5
6
7
8
a
b
c
A
B
B
Shoot
Roots
Mn
[Mn] (mg/kg DW)
0
50
100
150
200
250
a
b
b
A
B
A
Shoot
Roots
Na
[Na] (g/kg DW)
0
2
4
6
8
10
12
14
16
18
20
a
b
c
A
B
B
Shoot
Roots
Ni
[Ni] (mg/kg DW)
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
a
b
c
A
B
B
Shoot
Roots
Zn
[Zn] (mg/kg DW)
0
10
20
30
40
50
60
70
80
a
b
b
AB
A
B
Shoot
Roots
☐ ctrl
▨ IP
■ R

◀ **Fig. 4** Content of the most significant elements in leaves and roots of vetiver plants irrigated with water (ctrl) or with Ischia-Podetti (IP) or Rovereto (R) landfill leachate; the analysis of B, Ca, Cr, Mg, Mn, Na, Ni, and Zn was performed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Data are represented as mean ± SE; lowercase and uppercase letters indicate statistical significance for shoot and root measurements respectively, evaluated by one-way ANOVA followed by a Tukey post hoc test ($p < 0.05$)

it dictates both the long-term operability of the system and the disposal of the solid material. Indeed, national legislations generally prescribe limits to metal contents to determine the specific disposal procedure of wastes from remediation practices (in Italy, these processes are regulated by the D. Lgs. 152/2006). In this instance, total N, TKN, and nitrate levels are significantly higher upon leachate treatment than in control conditions in both non-planted and planted experiments (Supplementary Table 3 in Online Resources). This is consistent with the nature of zeolite that, as previously indicated, has excellent properties in ammonia and nitrate retention (Sepaskhah and Yousefi 2007). Interestingly, nitrite concentration in the zeolite bed was abated by the presence of vetiver plants, indicating the ability of this grass and associated rhizosphere to promote nitrite oxidation and removal.

On the other hand, the levels of most other elements in zeolite did not vary among the different treatments, except Na. The latter is retained in the zeolite bed in the non-planted R-treated experiment (Supplementary Table 3 in Online Resources); vetiver grass effectively removes this element from the zeolite bed and stores it at extremely high levels in the shoot (Fig. 4). Overall, the persistence of metals in zeolite is negligible; therefore, the growth bed would not require special disposal, according to the Italian law, since no limitation is posed to N levels (D. Lgs. 152/2006). However, element retention in the bed should be evaluated on a case-by-case basis.

Conclusion

Vetiver grass is an extremely promising plant for the treatment of municipal landfill leachate. In this work, efficiency of vetiver in water removal by evapotranspiration was confirmed, as well as its tolerance to metals and the ability to accumulate them. In particular, vetiver grass proved particularly efficient in removing B from solution and translocating it to shoots; moreover, it has a good accumulation of different N forms, as well as Na, Zn, and Mn, making this species suitable for its employment in phytoremediation strategies. Although constructed wetlands with a continuous flow are more commonly considered for the treatment of polluted waters, it is also important to consider methods allowing the complete removal of polluted liquids: applications of these include for example the containment of landfill leachate leakages and the treatment of collecting ponds, in particular where the role of plants is to prevent the dispersion of polluted liquids and toxic elements by rapidly evaporating water and removing pollutants.

However, highly polluted leachates may be detrimental to plant growth, due to intrinsic characteristics such as high salinity; therefore, case-by-case considerations and pilot experiments should be performed ahead of large-scale planting, to assess the applicability of vetiver grass to the specific conditions.

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Compliance with ethical standards

Conflict of interest Zerminiani A., Ferrarese A., and Campostrini P. are founders and managers of the company Bio Soil Expert srl.

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