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RESEARCH ARTICLE

Solid vermicompost and liquid vermicompost leachate have contrasting impacts on cadmium, lead and zinc phytoextraction by the Syrian beancaper *Zygophyllum fabago* L.

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Abstract
Background and aims Vermicompost is a valuable amendment for phytomanagement of heavy metals contaminated areas but its impact on plant physiology remains poorly documented. It is available in liquid or solid forms, but these forms were never compared for their influence on pollutant accumulation by the plant in relation to soil heavy metals bioavailabilities.
Methods Solid vermicompost (SV) and liquid vermicompost leachate (LVL) were tested on spiked soils in the absence (control) or in the presence of either 10 mg.Kg^{-1} Cd, 250 mg.Kg^{-1} Zn or 500 mg.Kg^{-1} Pb and cultivated with *Zygophyllum fabago* L. in a column device allowing leachate recovery and analysis. Plant physiological parameters were determined during 10 weeks of culture.
Results SV reduced soil heavy metals bioavailability, improved plant growth and photosynthesis and reduced heavy metals concentrations in all organs. In contrast, LVL increased heavy metal bioavailability and root growth but inhibited shoot growth. LVL decreased root heavy metals concentrations but increased them in the shoots. Both types of amendments increased the total amount of heavy metal removed by the plant. Among the removed Cd and Zn less than 5% were removed by leaching and SV contributed to reduce percolation. SV contributed to stress avoidance by reducing pollutant uptake while LVL contributed to stress tolerance through reinforcement of endogenous protecting mechanisms.
Conclusions Both SV and LVL are beneficial amendments for phytostabilization and phytoextraction of heavy metals by *Z. fabago* but act through distinct mechanisms on the plant in relation to mineral nutrition, oxidative stress and osmotic adjustment.

Keywords Heavy metals · Phytoremediation · Soil pollution · Vermicompost · Vermicompost leachate

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Introduction

Soils have functions that are intrinsically linked to humans through multiple interactions. The degradation of soil quality through the accumulation of pollutants has harmful consequences for human health and ecosystems stability. According to Hou et al. (2020) 2.8 million sites in the European Union are potentially polluted and 38.4% of them are contaminated by heavy metals (HM). Beside mining areas and

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former industrial sites or waste disposal characterized by high concentrations of HM, agricultural soils may also be contaminated by HM originating from natural or anthropogenic sources (Yao et al. 2024). Inorganic phosphate fertilizers commonly obtained from pulverized phosphate rocks containing Cd, Pb and Zn also constitute a major risk of contamination (Hu et al. 2024).

Bioaccumulation of these toxic persistent pollutants through the food chain poses tremendous risks for human health (Huang et al. 2024). Therefore, reducing the amounts of HM in the soil or limiting their plant bioavailability appears as a priority (Huang et al. 2024; Liu et al. 2024). *Ex-situ* remediation technologies are available but imply excavation processes and transportation; they are expensive, especially for remediation of large areas and require high amounts of energy generating greenhouse gas (Amponsah et al. 2018). Other physical in situ technologies (electro-kinetics, vapor sparging,…) also require expensive facilities, frequently disrupt soil structure and lead to soil sterility (Pandey et al. 2023).

Phytoremediation is attracting a considerable attention as a cheap green alternative to physical and chemical treatments and consists in the use of plants and their associated microorganisms to manage polluted areas (Oladoye et al. 2022; Shen et al. 2022; Yang et al. 2022). The most classical strategies considered in phytoremediation are (i) phytoextraction which consists in pollutant absorption by the roots and translocation to the harvestable aerial parts in order to remove pollutant from the substrate and (ii) phytostabilization which helps to reduce pollutants mobility and to stabilize them in the substrate to avoid both shoot and groundwater contamination (Oladoye et al. 2022; Riyazuddin et al. 2022; Yang et al. 2022). Phytoextraction and phytostabilization are influenced in contrasting ways by soil HM bioavailability. HM are deposited onto sediment surfaces and could be immobilized through adsorption, coagulation, flocculation, precipitation and incorporation in the lattice structure of minerals (Zhang et al. 2014). While phytoextraction requires a high bioavailability to absorb pollutants and a high rate of HM translocation from the root to the shoot (Mnasri et al. 2015), the purpose of phytostabilization is to reduce their bioavailability and mobility. Bioavailability is directly influenced by numerous parameters such as pH, redox potential, soil texture, organic matter, mineralogical composition and rhizosphere activity (Lambrechts et al. 2011a, b; Zhang et al. 2014).

Phytoextraction and phytostabilization potentials are strongly influenced by plant growth capacities. Total amount of HM removed by the phyoextracting plant is a function of plant biomass and shoot concentration for the considered metal. Similarly, the phytostabilizing potential strongly depends on the root system depth and architecture which in turn determine the amounts of HM sequestered in the roots and the amounts of stabilizing exudates released into the rhizosphere (Kafle et al. 2022). It is however well established that HM have a deleterious impact on plant growth (Thakur et al. 2022; Feki et al. 2021). Numerous natural or synthetic agents are used to improve efficiency of the phytoremediation processes. These agents commonly act both on plant physiology and on metal bioavailability as demonstrated for chemical amendments (EDTA, EGTA, EDDS), natural amendments (composted sewage sludge, paper waste, biochar) or biological amendments (arbuscular mycorrhizal fungi, plant growth promoting bacteria) (Lambrechts et al. 2011a, b; Kafle et al. 2022; Wang et al. 2024; Palansooriya et al. 2023). Although an increase in bioavailability may improve plant uptake, it also increases the risk of leaching leading to groundwater contamination (Kafle et al. 2022). Alternatively, if a given amendment reduces HM bioavailability but if the resulting growth stimulation is higher than the recorded decrease in accumulated HM in the shoots, it may be assumed that such amendment increases phytoextraction efficiency in terms of total HM removed by surface area despite a decrease in pollutant bioavailability (Lambrechts et al. 2011b).

Vermicompost is the end-product from organic matter digestion by earthworms (*Eisenia foetida*) and microorganisms (Palansooriya et al. 2023). It contains high amounts of humic substances and nutrient in available forms and improves soil fertility through its positive impact on microbial activity and soil physical properties (Hoehne et al. 2016; Santana et al. 2018; Landorfa-Svalbe et al. 2022). The vermicomposting process produces solid vermicompost and a liquid by-product commonly referred to as vermicompost leachate (Kosem et al. 2022; Voko et al. 2022). Solid vermicompost (SV) has been tested as a useful amendment on HM-contaminated soils and usually improves plant growth through a decrease in HM bioavailability leading to a reduction of HM concentration within the plants (Hoehne et al. 2016; Zhang et al. 2019; Landorfa-Svalbe et al. 2022; Khosropour et al. 2022; Wang et al. 2021, 2022). However, the impact of vermicompost on HM uptake by plants may be quite different for Cd, Zn and Pb, the most frequently recorded HM in agricultural soils (Xia et al. 2024). In contrast to solid vermicompost, liquid vermicompost leachate (LVL) received far less attention regarding its remediation potential of HM-contaminated soils, although it has been shown to improve plant resistance to salinity (Benazzouk et al. 2018, 2019; García-Galindo et al. 2021) and phosphorous or potassium deficiencies (Arthur et al. 2012). Benazzouk et al. (2020) demonstrated that the beneficial impact of LVL may be related to its high concentration of phytohormones, especially salicylic acid and auxin allowing a strong root growth stimulation, as well as a high endogenous concentration of various antioxidants including flavonoids and phenolic compounds.

Because it is a long-term process, phytoremediation also requires the use of perennial plant species producing high amounts of biomass, a deep root system, and a high level of HM tolerance, especially for phytoextraction purposes. Although it is considered as a noxious weed in agricultural fields, the syrian beancaper (*Zygophyllum fabago* L.) can spontaneously colonize arid and HM-contaminated areas (Párraga-Aguado et al. 2016; Kabas et al. 2014). It displays high levels of cadmium and zinc resistance (Lefèvre et al. 2005, 2009, 2010) and accumulates these toxic elements in roots (Lefèvre et al. 2016) and leaves (Lefèvre et al. 2014). According to López-Orenes et al. (2018, 2021) the species is also able to cope with high Pb concentrations. López-Orenes et al. (2014) demonstrated that the Pb-induced responses in *Zygophyllum fabago* may be triggered by salicylic acid in an organ-dependent manner in relation to the stimulation of the osmoprotective proline synthesis. Similarly, salicylic acid may influence the management of oxidative stress induced by Pb (López-Orenes et al. 2020). According to Ferrer et al. (2018), oxidative pathways assume key functions in Pb tolerance in *Z. fabago* and a similar observation was reported by Yildiztugay and Ozdifan-Konakci (2015) for Cd toxicity.

The aim of the present study was to compare the impact of solid vermicompost (SV) and liquid vermicompost leachate (LVL) on Cd, Zn and Pb uptake and distribution in *Zygophyllum fabago* in relation to their impacts on HM bioavailability and leaching processes. The tested hypothesis are that (i) SV reduces HM bioavailability but strongly increases shoot growth allowing an increase in the amount of HM removed from the soil and (ii) LVL-induced root growth stimulation reduces the risk of HM leaching resulting from an increase in HM bioavailability.

Materials and methods

Soil analysis and vermicompost properties

Sandy-silt agricultural soil was collected in Beuzet (Belgium ; 50° 31’ 55’’ N; 4° 44’ 49’’ E), dried and sieved at 5 mm. Soil pH was measured after adding samples (5 g) to 25 mL of distilled water and mechanically shaking for 5 min. Soil electrical conductivity (EC; $mS.cm^{-1}$) was determined on the same supernatant. Organic carbon and total nitrogen were determined by combustion according to the NF ISO 13878 standards after ashing 50 mg of samples at 1,000 °C in the presence of O_2. $CaCO_3$ determination was estimated by NaOH 0.5 N titration (Page et al. 1982). Major cation concentrations were determined by loss ignition at 950 °C of soil samples, addition of lithium metaborate and lithium tetraborate. Heavy metal concentrations were obtained after acid attack of soil samples with HNO_{3conc} and HF_{conc} as previously detailed (Lambrecht et al. 2011a). Samples were prepared using ultrapure water with a resistivity of 18MΩ cm (Merck-Millipore, Germany). Measurements were performed with an inductively plasma emission spectrometry ICP-OES (Thermo Jarrel Ash Iris Advantage). The main properties of the considered soil are listed in Table 1.

Soil was disposed in a thin layer (3 cm) inside a greenhouse, and sprayed with solutions containing $CdCl_2$ or $ZnCl_2$ or $Pb(NO_3)_2$ with a Gardena 159179 hand sprayer (pressure of 3 bars) in order to reach a final soil concentration of 10 $mg.Kg^{-1}$ DW Cd or 250 $mg.Kg^{-1}$ DW Zn or 500 $mg.Kg^{-1}$ DW Pb. The polluted spiked soils were allowed to equilibrate during 4 months in darkness, under a mean temperature of 15 °C and a relative humidity of 60%, with gentle re-homogenization weekly. At the end of the incubation period, the four considered soils (control; Cd, Zn or Pb-polluted) were distributed in three lots. The first lot did not receive vermicompost (OVC), the second lot received 20% solid

Table 1 Physico-chemical properties of soil collected from Beuzet and used in this study. Soil was analyzed by standard procedures (Page et al. 1982) with slight modification according to Lambrecht et al. (2011a). Each value is the mean of three replicates ± S.E

Soil parameters	
pH H_2O	6.9 ± 0.3
Electrical conductivity (dS m^{-1})	1.08 ± 0.21
CEC (cmol$_c$ Kg^{-1} DW)	24.57 ± 1.11
Texture [%]	
Sand (2,000–50 µm)	15.8 ± 2.3
Coarse silt (50–20 µm)	42.3 ± 3.1
Fine silt (20–2 µm)	26.1 ± 2.4
Clay (< 2 µm)	15.8 ± 1.8
Major elements (g.kg^{-1}DW)	
Ca^{2+}	32.3 ± 3.1
K^+	14.6 ± 1.5
Mg^{2+}	4.2 ± 0.5
Na^+	1.9 ± 0.6
Fe^{2+}	16.3 ± 1.2
Exchangeable basis (meq.100 g^{-1}DW)	
Ca^{2+}	23.2 ± 3.6
K^+	1.6 ± 0.6
Mg^{2+}	2.8 ± 0.9
Na^+	0.4 ± 0.1
Minor elements and heavy metals (mg.kg^{-1}DW)	
As	6.3 ± 0.9
Cd	0.3 ± 0.1
Cr	11.7 ± 1.2
Cu	12.3 ± 2.6
Ni	17.4 ± 2.0
Pb	18.1 ± 1.6
Zn	69.6 ± 4.1
Organic matter (%)	1.9 ± 0.9
Total N (%)	0.17 ± 0.04

vermicompost (SV) thoroughly mixed with the substrate while the third lot received liquid vermicompost leachate (LVL) added to irrigation water during the time course of experiment as explained below.

The solid vermicompost was purchased from a private company (Vermicompost; Ferme du Moutta; Boueilh-Boueilho-Lasque, France) and its main properties are presented in Table 2. The liquid vermicompost leachate (LVL) was a gift from Dr. Z.E. Djazouli, University of Blida (Algeria) and was produced by earthworm (*Eisinia fetida*) digestion of domestic waste according to Ndegwa and Thompson (2001); LVL was analyzed as detailed by Chaichi (2018) and major properties are listed in Table 3. Heavy metal bioavailabilities in the spiked soils with or without solid vermicompost were estimated according to Lindsay and Norvell (1978).

Table 2 Major properties of solid vermicompost used in the experiment. Physicochemical and biological characters were determined according to Sengupta et al. (2022). Each value is the mean of three replicates ± S.E

Parameter	
pH :	7.15 ± 0.09
Dry matter percentage:	48.3 ± 2.37 %
WHC (%) :	75.34 ± 6.32
Bulk density (Kg.m^{-3}) :	0.72 ± 0.08
Particle size (%)	92.36 ± 9.21
Organic matter (%) :	27.6 ± 2.11 %
Nitrogen (%) :	2.64 ± 0.32 %
Phosphorus (P_2O_5) :	0.73 ± 0.07 %
Potassium (K2O) :	0.49 ± 0.08 %
Magnesium (MgO) :	0.49 ± 0.06 %
Calcium (CaO) :	6.14 ± 1.02 %
C/N :	12.8 ± 1.17
Electrical conductivity (dS/m) :	3.12 ± 0.21
CEC (mmol.Kg^{-1})	9.40 ± 1.11
Total Zn (mg.Kg^{-1})	54.2 ± 6.3
Total Cd (mg.Kg^{-1})	< 0.5
Total Pb (m.Kg^{-1})	17 ± 5
Total Na (mg.Kg^{-1})	432 ± 36
Fe (mg.Kg^{-1})	4211 ± 254
B (mg.Kg^{-1})	3 ± 1
S (mg.Kg^{-1})	925 ± 47
Dehydrogenase (µg TPF g^{-1} $24h^{-1}$)	75.21 ± 3.24
FDA enzyme assay (µg fluorescein g^{-1} h^{-1})	81.48 ± 6.25
Alkaline phosphatase (µg p-nitrophenol g^{-1} h^{-1})	176.9 ± 11.2

WHC; water holding capacity, TPF; triphenyl formazan

Soil was distributed in black PVC columns (15 cm diameter; 40 cm height), each column receiving 3 kg of soil. To prevent preferential water pathways during subsequent irrigation, hydrophobic cotton was introduced between the soil and the column walls. The upper part of the column received a layer of 1 cm gravels to reduce evaporation; the bottom part of the columns was fitted to a funnel containing three layers of cheesecloth and microperforated polyamide netting avoiding soil particle movement into the leaching tubes. The lower part of the funnel opens onto a Falcon tube designed to receive the percolates leaching from the soil.

Table 3 Chemical composition of liquid vermicompost leachate (LVL) used in the experiment. Standard procedures were used as detailed in Chaichi (2018) and phytohormones concentrations were determined according to Benazzouk et al. (2020)

Parameter	
pH	7.69 ± 0.13
Electrical conductivity (dS/m)	15.7 ± 0.8
Density	1.023 ± 0.102
Osmotic potential (Ψs ; MPa)	−0.64 ± 0.09
Soluble sugars (µg.mL^{-1})	267 ± 36
Polyphenols (µg.mL^{-1})	32.11 ± 5.65
Anthocyanes (µg.mL^{-1})	10.3 ± 1.2
Humic + fulvic acid (mg.L^{-1})	832 ± 36
Total N (g.L^{-1})	0.415 ± 0.042
NO3-N (mg.L^{-1})	314.2 ± 29.6
NH4-N (mg.L^{-1})	61.2 ± 5.5
Organic C	18.9 ± 1.14
C/N ratio	45.54 ± 3.98
Total P (g.L^{-1})	0.24 ± 0.02
Total Ca (g.L^{-1})	0.157 ± 0.007
Total Mg (g.L^{-1})	0.178 ± 0.026
Fe (mg.L^{-1})	83 ± 5
Mn (mg.L^{-1})	1.12 ± 0.13
Cu (mg.L^{-1})	1.94 ± 0.21
Zn (mg.L^{-1})	3.21 ± 0.15
Cd (mg.L^{-1})	nd
Pb (mg.L^{-1})	< 0.01
Cytokinin (nmol.L^{-1})	12.13 ± 1.02
Auxin (nmol.L^{-1})	20.1 ± 2.14
ABA (nmol.L^{-1})	9.87 ± 0.57
Jasmonates (nmol.L^{-1})	2.13 ± 0.02
Salicylic acid (nmol.L^{-1})	169.7 ± 12.7
Benzoic acid (nmol.L^{-1})	412.3 ± 33.3
ACC (nmol.L^{-1})	173.2 ± 14.6
Gibberellins (nmol.L^{-1})	12.6 ± 1.37
Putrescine (µmol.L^{-1})	10.11± 2.13
Spermidine (µmol.L^{-1})	9.87 ± 0.69
Spermine (µmol.L^{-1})	14.17 ± 1.39
Proline (mmol. L^{-1})	nd
Glutathione(µmol.L^{-1})	nd

nd; not detected

Plant material and growing conditions

Seeds of *Zygophyllum fabago* L. were harvested on mature plants spontaneously colonizing a marginal area in southeast Spain (Mazarrón 37°35’ N; 1°18’ W). Seeds collected from 32 plants were pooled and the pooled sample was used for subsequent experiments. Seeds were sown in trays filled with a mixture of sand and compost (50/50) in a greenhouse under natural light supplemented with LED LumiGrow lights (650 W; red-blue) to provide a minimal PAR (photosynthetic active radiation) of 300 µmol m^{-2} s^{-1} at the top of the canopy for 12 h day^{-1}. The day/night temperature and relative humidity were set at 25 °C/20°C and 55%/67%, respectively and substrate was regularly moistened with deionized water.

After four weeks, uniform-sized plants (all plants at the 4 node stage with a mean length of 7.25 ± 0.15 cm) were transferred to the filled column with one plant per column. The substrate was moistened to 70% field capacity (volumetric soil water content of 28.3%). The experiment lasted 10 weeks. During the first five weeks of the experiment, plants were irrigated every three days with 100 mL of either deionized water (for treatments OVC or SV) or with the same volume of water containing 5 mL LVL for the LVL treatment. Volume was increased up to 150 mL containing 7.5 mL LVL during the subsequent five weeks to meet the transpiratory needs of the growing plants and thus avoid drought stress. The soil water content was measured twice per week using a ThetaProbe soil moisture sensor type ML1 (Delta-T Devices Ltd., UK) based on changes in apparent dielectric constant, and converted to gravimetric water content based on calibration for the considered substrate. The amount of water was sufficient to maintain a soil water content comprised between 60 and 75% field capacity. The leachates were collected at the bottom of the columns twice per week and were transferred to a brown glass bottle and stored at 4 °C in darkness until final analysis. For each treatment, three columns without plants (CWP) were maintained as control to assess the soil behavior in the absence of *Zygophyllum fabago*.

The total setup thus involves 12 crossed treatments: four types of contamination (control; Cd, Zn, Pb) and three types of vermicompost treatments (OVC, SV or LVL). Each treatment comprised 15 columns (12 columns with *Zygophyllum fabago* and three columns without plants); the 180 columns were randomly distributed in the greenhouse and were randomly rearranged each week. After 10 weeks of experiment, soils were carefully removed from the columns; roots were separated from surrounding soils and rinsed for 30 s in demineralized water to remove adhering soil particles. Roots, stems and leaves were then separated and for each plant, organs were individually weighed (fresh weight determination). Three plants were then placed in an oven for incubation at 70 °C during 48 h until reaching constant dry weight and used for water content (WC) calculation. Six remaining plants (roots, stems and leaves) were flash frozen in liquid nitrogen, ground to a fine powder and stored at −80 °C until final analysis. Cadmium, zinc and lead total content remaining in the soil and element bioavailabilities of all spiked soils (including LVL-treated one) were again determined according to Lambrecht et al. (2011a) and to Lindsay and Norvell (1978), respectively to compare the obtained values with the initial content and bioavailability values at the beginning of the experiment.

Plant photosynthesis-related parameters and plant water status

Stomatal conductance (g_s), net photosynthesis (A) and instantaneous transpiration (E) were estimated after eight weeks of treatment on leaflets located on the 6th and 7th nodes and which developed during the time course of experiment but already reached final elongation at the time of measurement. Gaz exchange measurements were performed using an Infrared Gaz Analyzer (LCA4 8.7., ADC Biocientific) with a Parkinson leaf cuvette, under an air flow of 300 mL min^{-1} during 1 min (20 records) at 23 ± 2°C and on a leaf segment of 6.25 cm^2 at a light intensity of 725 μmol m^{-2} s^{-1}. The net CO_2 assimilation rate (A) and instantaneous transpiration (E) were measured. The instantaneous water use efficiency ($WUE = A/E$) was then calculated. The stomatal conductance (g_s) was measured with an AP4 porometer system (Delta-T Devices). All measurements were performed between 11.00 a.m. and 2.00 p.m. during a sunny day with no clouds. The leaves used for photosynthetic measurements were then collected and cut in small segments (3×2 cms). Photosynthetic pigments ($Chla + Chlb$) and total carotenoids (xanthophylls and β carotene) were quantified after extraction with cold acetone 80% and spectrophotometric analysis according to Lichtenthaler (1987).

The water potential (Ψ_w) of the main stem was measured on six plants per treatment at the time of harvest with a pressure chamber (PMS Instrument Co; Corvallis, Oregon USA). Osmotic potential (Ψ_s) was determined for leaves and roots on frozen tissues just after harvest. Tissues were cut in small pieces and tissue sap was extracted according to Bajji et al. (2000). The osmolarity of the extracted sap was estimated using a vapor pressure osmometer (model 5500; Wescor, Logan, Utah, USA) and converted to osmotic potential (Ψ_s) according to the Van T'Hoff equation.

Ion concentration in plant samples

After incubation of fresh samples (leaves, stems and roots) for 48 h in an oven at 70 °C, dry matter (ca.50 mg) was digested in the presence of 37% HCl and 68% HNO_3 at 80 °C. After evaporation of the mixture, elements were resuspended in HCl 0.1 N and filtered (Whatman n° 1). Elements were quantified by ICP-OES inductively plasma emission spectrometry (Thermo Jarrel Ash Iris Advantage). Samples were injected through a Mainhard nebulizer and carried through an argon gas plasma condition (reflected power of 7 W and forward power of 1300 W). Gas flow rates of plasma, auxiliary, and nebulizer were set at 16.0, 1.0 and 1.0 L min^{-1}, respectively. The internal standard multi-element stock solution obtained from Agilent (USA) was used to control stability of the instrument while certified reference material ERM-CA713 was obtained from Sigma-Aldrich (Germany).

Oxidative stress : malondialdehyde and carbonyl quantification, total antioxidant, glutathione, non-protein thiol and phytochelatin estimation

Oxidative stress was quantified by estimating the malondialdehyde (MDA) resulting from lipid peroxidation and the carbonyl residue concentrations issued from protein damages in roots and leaves. Malondialdehyde (MDA) concentrations were estimated on roots and leaves of five plants per treatment, following the thiobarbituric acid reactive substances method of Heath and Packer (1968). Protein carbonylation was quantified according to Levine et al. (1994) using 2,4-dinitophenylhydrazine and expressed per mg of protein. Protein concentrations were determined according to Bradford (1976).

The estimation of the antioxidant capacity of the samples was based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Chen et al. (1999). The absorbance at 517 nm was measured against a methanol blank. The scavenging percentage of DPPH radical was calculated according to Maisuthisakul et al. (2007): DPPH radical scavenging capacity (%) = $[A_0-(A_1-A_S)]/A_0\times100$ where A_0 is the absorbance of the control solution (containing only DPPH), A_1 is the absorbance for the plant extract in the presence the DPPH solution, and A_S is the absorbance of the sample extract solution without DPPH.

For reduced (GSH) and total (GSHt) glutathione quantification, 200 mg of frozen samples were extracted and derivatized by orthophthalaldehyde according to Cereser et al. (2001). OPA derivatives were separated on a reversed-phase HPLC column using an acetonitrile-sodium acetate gradient system and detected fluorimetrically as previously detailed (Lefèvre et al. 2016). GSH was quantified using nine-point calibration curves with custom-made external standard solutions ranging from 0.0625 to 50 µM. The recovery was determined using GSH as an internal standard. The total non-protein thiol (NPT) concentration was determined according to De Vos et al. (1992). Phytochelatin content was evaluated as the difference between NPT and GSH levels (Schäfer et al. 1997).

Total phenolic compounds, flavonoids, salicylic acid, and proline concentration

Polyphenols were extracted from frozen leaf samples (0.3 g) with 2 mL of 80% methanol. The mixture was centrifuged at 10,000 *g* for 10 min at 4 °C. Total phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton's method (Singleton and Rossi 1965) Total phenolic contents were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg of GAE g^{-1} FW) through the calibration curve with gallic acid. Total flavonoid content was measured using a colorimetric assay as developed by Dewanto et al. (2002).

Salicylic acid (SA) was extracted and quantified according to Gharbi et al. (2016). Salicylic acid was quantified by high performance liquid chromatography (HPLC) using an Agilent 1260 series equipped with an automatic injector and a column (Inertsil ODS-3 (250 × 3.0 mm, 3 µm) oven at 30 °C. Salicylic acid was detected by a fluorescence detector with the emission and the excitation wavelengths respectively fixed at 315 nm and 408 nm.

Proline was quantified using the acid ninhydrin method according to Bates et al. (1973) on 200 mg of frozen fresh ground plant material. Absorbance was read at 520 nm in a quartz cuvette after strong vortex of the samples. Proline concentration was calculated on the basis of a standard curve established with L-proline (Sigma Aldrich chemicals).

Statistical treatment of the data

Normality of the data was checked by the Shapiro-Wilk tests and homosedasticity was verified using the Levene test. Data were transformed ($log10(x+1)$ or *asin(x/Σx)* when required to ensure normal distribution. Variance analysis (ANOVA II) was then performed using the R software considering the nature of contamination and the vermicompost treatments as independent factors. Post hoc analyses were performed using the Tukey test.

Results

Impact of amendments and plant culture on heavy metal bioavailability

Before plant culture in control soil, bioavailability (Table 4) expressed as a percentage of the total HM content (%T) was the lowest for Pb and the highest for Zn. Cadmium, Zn and Pb bioavailabilities strongly increased in their respective spiked soils compared to control soil; the addition of SV decreased bioavailabilities of HM in all cases ($P<0.01$).

After the plant culture, HM bioavailabilities were estimated on the bulk soil from the columns and expressed as percentages of the total HM remaining after the plant removal and leaching processes. Bioavailabilities were also estimated in column without plant maintained as control but irrigated in the same way (Table 4). The amounts of bioavailable HM were lower in columns which did not receive vermicompost (OVC) after the plant culture compared to the same columns before plant culture ($P<0.05$). Bioavailabilities of all HM significantly decreased

Table 4 Bioavailabilities of cadmium, zinc and lead estimated on control or spiked soils in the absence of vermicompost (OVC) or in soil amended with solid vermicompost (SV) or liquid vermicompost leachate (LVL) before and after 10 weeks of culture with *Zygophyllum fabago*

Element soil	Amendment	Before culture: Bioavailability (in µg g^{-1} DW)	Before culture: %T	After culture: Bioavailability (in µg g^{-1} DW)	After culture: %T
Cadmium					
Control	OVC	12 ± 1 a	3.94 ± 0.65 *c*	15 ± 2 a	3.25 ± 0.43 *c*
	SV	ND	–	10 ± 1 a	1.96 ± 0.01 *a*
	LVL	–	–	19 ± 1 b	3.99 ± 0.21 *c*
Spiked soil	OVC	537 ± 34 e	5.22 ± 0.33 *d*	412 ± 25 d	4.56 ± 0.27 *d*
	SV	240 ± 19 c	2.33 ± 0.18 *b*	216 ± 17 c	2.47 ± 0.19 *b*
	LVL	–	–	519 ± 43 e	6.02 ± 0.08 *e*
CWP	OVC	–	–	497 ± 51 e	5.07 ± 0.52 *d*
	SV	–	–	242 ± 16 c	2.45 ± 0.16 b
	LVL	–	–	615 ± 44 f	6.35 ± 0.45 e
Zinc					
Control	OVC	5658 ± 358 b	8.13 ± 0.51 *d*	5532 ± 451 b	7.95 ± 0.64 c
	SV	5185 ± 569 ab	7.45 ± 0.24 *d*	4998 ± 102 a	6.11 ± 0.12 *b*
	LVL	–	–	4986 ± 201 a	8.26 ± 0.33 *cd*
Spiked soil	OVC	38575 ± 1254 fg	12.07 ± 0.40 *f*	32223 ± 2514 e	10.32 ± 0.80 *e*
	SV	20199 ± 2133 d	6.32 ± 0.67 *b*	16143 ± 888 c	5.18 ± 0.28 *b*
	LVL	–	–	40561 ± 2631 g	13.07 ± 0.85 *f*
CWP	OVC	–	–	31097 ± 3002 e	9.88 ± 0.95 *e*
	SV	–	–	14364 ± 1287 c	4.56 ± 0.40 *a*
	LVL	–	–	36124 ± 2052 f	11.48 ± 0.65 *ef*
Lead					
Control	OVC	170 ± 23 a	0.94 ± 0.18 *b*	154 ± 25 a	0.75 ± 0.12 *a*
	SV	ND		ND	–
	LVL	–	–	253 ± 35 b	1.32 ± 0.18 *c*
Spiked soil	OVC	7046 ± 987 f	1.36 ± 0.19 *c*	5399 ± 124 e	1.08 ± 0.02 *b*
	SV	4507 ± 1164 d	0.87 ± 0.22 *ab*	3700 ± 143 d	0.74 ± 0.03 *a*
	LVL	–	–	9748 ± 651 g	1.95 ± 0.12 *d*
CWP	OVC	–	–	6299 ± 227 f	1.26 ± 0.04 *c*
	SV	–	–	3149 ± 365 c	0.63 ± 0.07 *a*
	LVL	–	–	10047 ± 841 g	2.01 ± 0.16 *d*

Columns without plants (CWP) containing spiked soil, amended and irrigated in the same way were used as controls. Bioavailabilities for columns amended with LVL during the culture, and for columns without plants were analyzed at the end of the experiment only. Data are provided in µg.g^{-1} soil DW and as percentage of total heavy metal (%T) present in the soil. Data are means of 5 replicates ± S.E. For a given element, means followed by the same letters for bioavailability on the one hand and %T (italics) on the other hand, were not different according to the Tukey test at $P = 0.05$. ND: not detected

in SV columns: this was valid for columns with and without plants and was even detected in columns containing non-polluted control soil, at least for Cd and Zn. In contrast, LVL application increased HM bioavailabilities in all cases ($P < 0.001$). Whatever the vermicompost treatment, there was no obvious difference in bioavailability of HM between columns containing spiked soil with or without plants.

Plant growth, photosynthesis and water status

Figure 1 shows the dry weight of roots (Fig. 1A), stems (Fig. 1B) and leaves (Fig. 1C) for plants exposed to the treatments for 10 weeks. In the absence of vermicompost (OVC) HM significantly decreased the dry weight of all organs comparatively to control plants maintained in non-spiked soil. As far as roots are concerned (Fig. 1A), SV significantly increased the root DW in all treatments including controls ($P < 0.01$). The recorded growth stimulation was even higher in response to LVL application ($P < 0.001$). In response to HM exposure, both stems (Fig. 1B) and leaves DW (Fig. 1C) were more affected by Pb than by Cd or Zn. Vermicompost mitigated the deleterious impact of HM. In shoot organs contrary to roots, SV appeared more efficient than LVL for growth

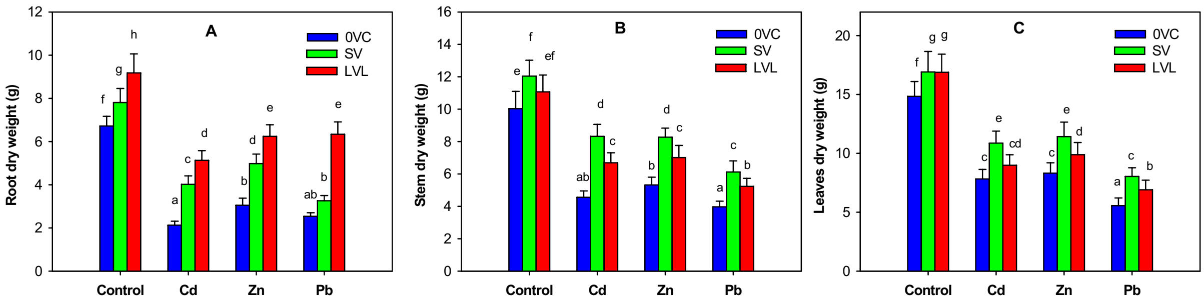

Fig. 1 Root (**A**), stem (**B**) and leaves (**C**) dry weights in plants of *Zygopyllum fabago* growing for 10 weeks in non-polluted control soil, or in soil contaminated with cadmium (10 mg.Kg^{-1} DW), zinc (250 mg.Kg^{-1} DW) or lead (500 mg.Kg^{-1} DW) in the absence of vermicompost (0VC), in the presence of solid vermicompost 20% (SV) or irrigated with liquid vermicompost leachates 5% (LVL). Each value is the mean of three replicates and vertical bars are standard errors. Values sharing a similar letter are not significantly different at P = 0.05 according to the Tukey test

stimulation. Plants exposed to LVL consequently exhibited the lowest shoot/root ratio in response to all HM ($P<0.05$). For plants maintained in the absence of vermicompost, shoot/root ratio was the highest in response to Cd and the lowest in response to Pb while Zn-treated plants exhibited an intermediate value (data not shown).

In the absence of vermicompost (OVC), heavy metals drastically decreased net photosynthesis (A) ($P<0.001$; Fig. 2A). The recorded decrease was higher in Pb-treated plants than for Cd-stressed ones. Addition of SV increased A values in all stressed plants, but not in control ones. Similarly, addition of LVL improved net photosynthesis, although to a lower extent than SV. Stomatal conductance (g_s; Fig. 2B) also decreased in plants cultivated on polluted spiked soils in the absence of vermicompost ($P<0.001$). The addition of SV, however, mitigated stomatal closure in plants exposed to Cd or Zn ($P<0.05$), but not in plants cultivated in the presence of Pb. In contrast to SV treatments, LVL application had no beneficial impact on g_s values. Instantaneous transpiration (E; Fig. 2C) exhibited a similar trend than g_s with the noticeable difference that both SV- and LVL-treated control plants exhibited lower values than control plants cultivated in the absence of vermicompost. Instantaneous water use efficiency (WUE; Fig. 2D) for plants cultivated in the absence of vermicompost increased in response to Zn ($P<0.05$) but remained unaffected in the other treatments. Instantaneous WUE significantly increased in all plants cultivated in the presence of SV or in the presence of LVL, including in controls. The recorded increase in WUE was similar for SV- and LVL-treated plants, except for the Zn-treated plants which exhibited a higher WUE in response to LVL than in response to SV.

Vermicompost treatments had no impact on plant water status-related parameters for plants growing on non-polluted control soil (Table 5; leaf WC, shoot Ψ_w, root and leaf Ψ_s). Plants maintained on spiked soils in the absence of vermicompost exhibited lower leaf WC than controls (Table 5), the lowest value being recorded for plants exposed to Pb. All vermicompost treatments increased the leaf WC ($P<0.001$). The shoot Ψ_w also decreased in response to HM. SV and LVL treatments accentuated the recorded decrease in shoot Ψ_w: in both cases the lowest Ψ_w value was observed in Cd-treated plants. A similar trend was observed for root Ψ_s. Heavy metals in the absence of vermicompost decreased the leaf Ψ_s. SV mitigated the recorded decrease in leaf Ψ_s in Zn- and in Pb-treated plants. In contrast, LVL treatment strongly decreased leaf Ψ_s which reached similar values in response to all considered HM.

Chlorophyll a (Table 5) in plants cultivated in the absence of vermicompost decreased in Zn- and Pb-treated plants. SV treatments increased *Chl*a concentration in all stressed plants which reached similar values than in controls, while a lower increase was observed in plants receiving LVL treatment. Chlorophyll b also decreased in plants exposed to HM and the lowest value was observed in plants exposed to Pb stress in the absence of vermicompost. SV, and to a lower extent LVL increased *Chl*b concentrations in

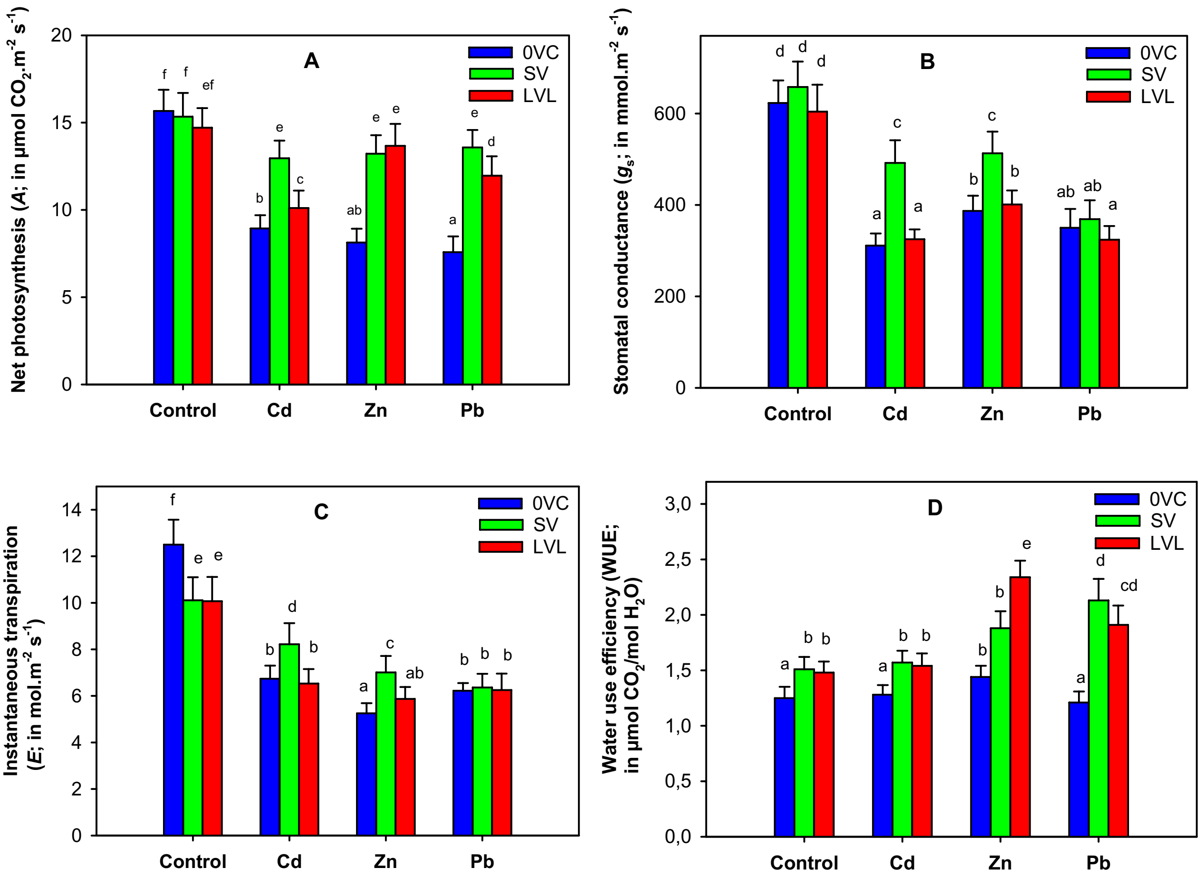

Fig. 2 Net photosynthesis (*A* in µmol.m^{-2} s^{-1}) (**A**), stomatal conductance (gs, in mmol.m^{-2} s^{-1}) (**B**), instantaneous transpiration (E, in mol.m^{-2} s^{-1}) (**C**) and instantaneous water use efficiency (WUE ; in µmol CO_2 mol^{-1} H_2O (**D**) in plants of *Zygopyllum fabago* growing for 10 weeks in non-polluted control soil, or in soil contaminated with cadmium (10 mg.Kg^{-1} DW), zinc (250 mg.Kg^{-1} DW) or lead (500 mg.Kg^{-1} DW) in the absence of vermicompost (0VC), in the presence of solid vermicompost 20% (SV) or irrigated with liquid vermicompost leachates 5% (LVL). Each value is the mean of six replicates and vertical bars are standard errors. Values sharing a similar letter are not significantly different at P = 0.05 according to the Tukey test

plants exposed to HM. In contrast, HM had no effect on carotenoid concentration and SV application did not modify carotenoid content. It is noteworthy that LVL strongly increased the carotenoid concentration in the absence as well as in the presence of HM ($P<0.01$).

Plant ion concentration and leaching process

Concentration of cadmium, zinc and lead in roots and in shoots (stems and leaves) are provided in Fig. 3. for plants growing on spiked soil. In all plants, HM concentrations were higher in the roots than in the shoots.

Cadmium concentrations remained at very low values in plants cultivated on non-polluted substrate (15.8 ± 1.2, 7.3 ± 0.6 and 4.7 ± 0.5 µg.g^{-1} DW for roots, stems and leaves, respectively) and vermicompost had no impact on Cd concentration in plants growing in the control soil (detailed data not shown). Cadmium concentrations strongly increased in plants cultivated on Cd-spiked soil but were lower in response to SV treatment compared to OVC ($P<0.05$; Fig. 3A and B). LVL treatment decreased Cd concentrations in the roots but increased them in both stems and leaves (Fig. 3B).

Plants maintained in non-polluted control soil contained 313 ± 23, 69 ± 5 and 58 ± 13 µg.g^{-1} DW

Table 5 Leaf water content (in %), water potential (*Ψ*w ; in MPa), root and leaves osmotic potentials (*Ψ*s ; in MPa) and photosynthetic pigments chlorophyll a (*Chl* a), chlorophyll b (*Chl* b) and carotenoids (Car) (in mg.g-1 FW) in plants of *Zygophyllum fabago* cultivated during 10 weeks in control soil or in spiked soils contaminated either with cadmium (10 $mg.Kg^{-1}$ DW), zinc (250 $mg.Kg^{-1}$ DW), or lead (500 $mg.Kg^{-1}$ DW))

Soil	Leaf WC (%)	*Ψ*w (MPa)	Root *Ψ*s (MPa)	Leaf *Ψ*s (MPa)	*Chla* ($\mu g.g^{-1}$ FW)	*Chl*b ($\mu g.g^{-1}$ FW)	Car ($\mu g.g^{-1}$ FW)
Control							
OVC	88.3 ± 0.4 f	−1.25 ± 0.15 a	0.87 ± 0.04 a	−1.52 ± 0.10 b	912 ± 45 c	253 ± 15 d	196 ± 16 a
SV	89.6 ± 0.7 f	−1.27 ± 0.07 a	−0.84 ± 0.09 a	−1.64 ± 0.16 b	942 ± 54 d	226 ± 19 c	201 ± 11 a
LVL	89.5 ± 0.5 f	−1.17 ± 0.10 a	−0.88 ± 0.04 a	−1.52 ± 0.16 b	937 ± 12 d	238 ± 20 cd	269 ± 13 c
Cd-spiked							
OVC	81.2 ± 0.8 b	−1.62 ± 0.21 c	−1.12 ± 0.10 b	−2.32 ± 0.07 cd	895 ± 61 c	188 ± 11 b	217 ± 19 a
SV	87.2 ± 0.3 e	−1.82 ± 0.11 d	−1.88 ± 0.07 e	−2.17 ± 0.15 cd	922 ± 55 cd	241 ± 20 d	193 ± 14 a
LVL	85.3 ± 0.2 d	−2.99 ± 0.08 e	−2.14 ± 0.06 f	−3.12 ± 0.14 e	894 ± 34 c	238 ± 21 cd	274 ± 16 c
Zn-spiked							
OVC	79.5 ± 0.6 ab	−1.53 ± 0.12 bc	−1.48 ± 0.11 cd	−2.49 ± 0.17 d	822 ± 74 b	223 ± 10 c	202 ± 12 a
SV	86.3 ± 1.0 de	−1.42 ± 0.07 b	−1.54 ± 0.09 d	−2.07 ± 0.01 c	938 ± 45 d	263 ± 24 d	211 ± 17 a
LVL	84.2 ± 0.9 cd	−2.05 ± 0.13 d	−1.88 ± 0.07 e	−3.24 ± 0.23 e	915 ± 15 cd	241 ± 5 cd	253 ± 14 bc
Pb-spiked							
OVC	78.4 ± 1.6 a	−1.45 ± 0.12 b	−1.45 ± 0.12 b	−2.35 ± 0.17 d	710 ± 19 a	165 ± 9 a	195 ± 17 a
SV	83.4 ± 0.2 c	−1.40 ± 0.05 b	−1.01 ± 0.05 ab	−1.02 ± 0.09 a	925 ± 26 cd	214 ± 18 c	189 ± 13 a
LVL	83.6 ± 0.5 c	−1.99 ± 0.08 d	−2.01 ± 0.09 ef	−3.32 ± 0.19 e	851 ± 30 bc	187 ± 10 b	241 ± 8 b

Soil was maintained in the absence of vermicompost (OVC), mixed with 20% of solid vermicompost (SV) or irrigated with water containing 5% (v/v) of liquid vermicompost leachate. Each value is the mean of 6 plants per treatment ± S.E. For a given parameter, values followed by the same letter did not differ at $P = 0.05$ according to the Tukey test

Zn in roots, stems, and leaves, respectively. SV had no impact on control plant Zn nutrition while LVL treatment increased Zn concentration in stems and roots by 17% and 23%, respectively (detailed data not shown). In Zn-polluted conditions, SV decreased Zn concentrations in all organs (Fig. 3C and D). LVL also decreased the root Zn concentration although root Zn concentration was lower for SV- than for LVL-treated plants. LVL slightly increased stem and leaves Zn concentrations. A similar pattern was recorded for Pb concentrations which were clearly lower in all organs of *Z. fabago* than Cd and Zn concentrations (Fig. 3E and F).

The total amount of HM in the plant are provided in Table 6. In all cases, vermicompost increased the total amount of HM removed by the plant ($P < 0.01$) and LVL was more efficient than SV for Cd, Zn and Pb removal. This is also valid when only amounts of HM in the shoots are considered (Table 6). The comparison between shoot and root behavior may be considered on the basis of total amount present in the organs ($[shoot/root]_{amount}$) or on the basis of the mean concentration of a specific element present in the two plant parts ($[shoot/root]_{conc}$.). For all elements and amendments $[shoot/root]_{amount}$ was higher than $[shoot/root]_{conc}$. (Table 6). For plants cultivated on spiked soil in the absence of vermicompost, the highest ($[shoot/root]_{amount}$ ratio was recorded for Cd-treated plants and the lowest for Pb-exposed ones. SV and LVL treatments reduced $[shoot/root]_{amount}$ in plants exposed to Cd or Zn, but the recorded decrease was not significant for Pb-treated plants. As far as $[shoot/root]_{conc}$. ratios were concerned, it is noteworthy that a strong increase was observed for all metals in response to LVL application ($P < 0.01$).

As expected, the total volume of leachates (V_l, Table 6) was the highest for columns without plants. For column containing plants, the total volume of leakage decreased in response to the SV treatment ($P < 0.05$). The concentration of heavy metals in the leachates (C_l; Table 6) increased for columns receiving the LVL treatment ($P < 0.01$) and this was valid for the three considered HM and was observed in columns with or without plants. The total amounts of HM removed by leaching (TR_l; Table 6) were higher in columns without plants than in column with plants and were in all cases lower for Pb than for Cd or

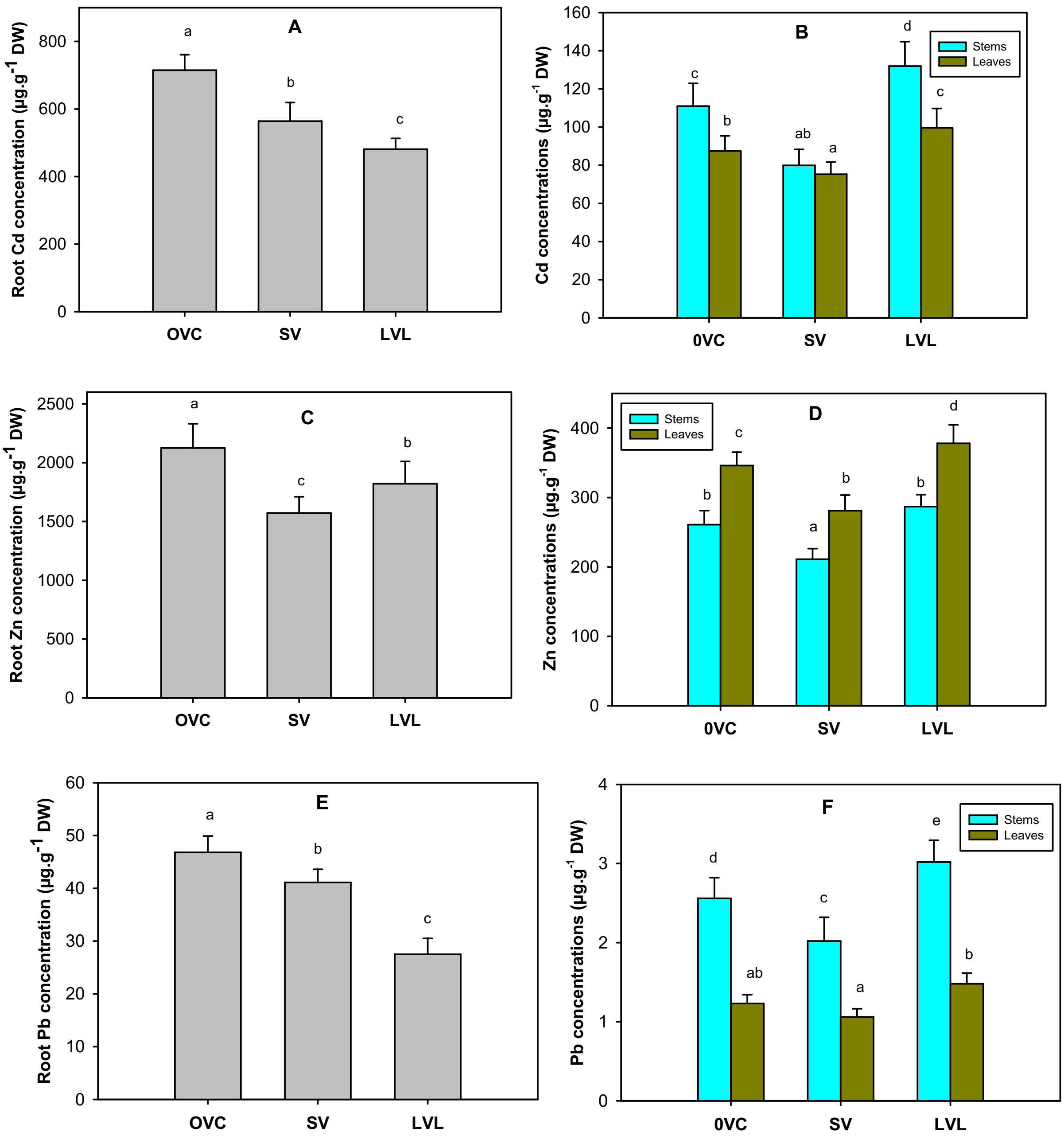

Fig. 3 Roots (**A**, **C** and **E**) and stems and leaves (**B**, **D** and **F**) heavy metals concentrations in plants of *Zygopyllum fabago* growing for 10 weeks in non-polluted control soil, or in soil contaminated with cadmium (10 mg.Kg^{-1} DW) (**A** and **B**), zinc (250 mg.Kg^{-1} DW) (**C** and **D**) or lead (500 mg.Kg^{-1} DW) (**E** and **F**) in the absence of vermicompost (0VC), in the presence of solid vermicompost 20% (SV) or irrigated with liquid vermicompost leachates 5% (LVL). Each value is the mean of three replicates and vertical bars are standard errors. Values sharing a similar letter are not significantly different at P = 0.05 according to the Tukey test

Zn. The TR_1 values were always higher in response to LVL than in columns receiving no vermicompost (OVC) while an opposite trend was recorded for SV which significantly decreased TR_1. As far as columns with plants are concerned, the proportion of HM removed by the plant (%P; Table 6) was by far higher

Table 6 Total amount of HM removed by the plant (TR_{pl}), total amount of HM removed by the shoot (TR_{sh}), ratio shoot/root calculated on the basis of HM amount ([shoot/root]$_{amount}$) or on the basis of HM concentration ([shoot/root]$_{conc.}$), total volume of leachate (V_l ; in mL), heavy metal concentration in leachate (C_l ; in mg.L^{-1}), total amount of HM removed by leaching (TR_l ; in mg), percentage of HM removed by plants (%P) or by the leachate (%L)

Element	Vermi.	TR_{pl} %L (mg)	TR_{sh} (mg)	[shoot/root]$_{amount}$	[shoot/root]$_{conc.}$	V_l (mg.L-1)	C_l (mg)	Tr_l	%P	%L
Cadmium										
Spiked soil	OVC	2.77 d	1.25 c	0.82 f	0.14 c	356 c	0.263 e	0.094 d	96.73 de	3.27 c
	SV	3.68 e	1.42 d	0.62 d	0.16 c	209 a	0.188 cd	0.039 c	98.94 f	1.06 a
	LVL	4.03 f	1.56 e	0.63 d	0.23 d	313 b	0.356 g	0.111 d	97.31 e	2.69 bc
CWP	OVC	–	–	–	–	429 d	1.265 ij	0.543 h	95.19 d	4.81 e
	SV	–	–	–	–	311 b	0.999 i	0.311 f	97.63 ef	2.37 b
	LVL	–	–	–	–	478 e	1.657 j	0.792 i	95.34 d	4.66 e
Zinc										
Spiked soil	OVC	10.75 g	4.27 f	0.66 e	0.15 c	429 d	1.265 ij	0.543 h	95.19 d	4.81 e
	SV	12.78 gh	4.95 g	0.63 d	0.16 c	311 b	0.999 i	0.311 f	97.63 ef	2.37 b
	LVL	16.20 h	5.46 h	0.51 c	0.22 d	478 e	1.657 j	0.792 i	95.34 d	4.66 e
CWP	OVC	–	–	–	–	2001 g	1.879 k	3.760 k	0	100
	SV	–	–	–	–	1907 g	1.587 j	3.026 j	0	100
	LVL	–	–	–	–	1987 g	2.013 k	4.032 k	0	100
Lead										
Spiked soild	OVC	0.13 a	0.017 a	0.15 ab	0.039 a	546 f	0.062 a	0.034 b	79.81 a	20.19 h
	SV	0.16 b	0.022 b	0.16 b	0.037 a	405 d	0.054 a	0.022 a	87.66 c	12.34 f
	LVL	0.21 c	0.024 b	0.14 a	0.075 b	398 d	0.107 b	0.043 c	82.10 b	17.90 g
CWP	OVC	–	–	–	–	1879 g	0.165 c	0.310 f	0	100
	SV	–	–	–	–	1803 g	0.136 bc	0.245 e	0	100
	LVL	–	–	–	–	1916 g	0.201 d	0.385 g	0	100

Plants of *Zygophyllum fabago* were cultivated on spiked soils in the absence of vermicompost (OVCL) or in soil amended with solid vermicompost (SV) or liquid vermicompost leachate (LVL) during 10 weeks. Columns without plants (CWP) containing spiked soil, amended and irrigated in the same way as columns with plants were considered for leaching estimation in the absence of plants. Data are means of 5 replicates (standard errors are not provided for the sake of clarity). For a given parameter, means followed by the same letters were not different according to the Tukey test at $P = 0.05$. ND : not detected than the proportion of HM removed by leaching (%L; Table 6). The maximal %P value was recorded for plants exposed to SV treatment. The highest proportion of HM removed by leakage (%L) was recorded for Pb. The %L was lower for Cd and Pb in columns containing plants receiving LVL than in those from the OVC treatment.

Oxidative stress, endogenous glutathione and phytochelatin concentration

Malondialdehyde concentrations remained low in roots of control plants (Fig. 4A) and were not affected by vermicompost. Cadmium treatment induced a strong increase in the root MDA ($P < 0.001$) which was partly mitigated by LVL application and a similar trend was recorded for the roots of Zn- and Pb-treated plants. As far as leaves are concerned, LVL reduced MDA concentrations in the leaves of control plants. The highest MDA concentration was recorded in leaves of plants exposed to Cd. SV treatment had no significant impact on the leaf MDA concentrations of HM-treated plants while LVL significantly decreased it except for plants exposed to Zn excess.

Protein carbonyl residues also increased in both roots and leaves of Cd-treated plants ($P < 0.01$; Fig. 4B) and LVL treatment mitigated Cd-induced increase. Protein carbonyl residues did not increase in the roots of Zn-treated plants but it increased at the leaf level ($P < 0.05$). It also increased in Pb-treated plants. LVL treatment slightly reduced carbonyl residues in the leaves of HM-treated plants.

Total antioxidant power estimated on the basis of the DPPH radical scavenging test is provided in Fig. 4C. In the absence of vermicompost, antioxidant power decreased in the roots of plants exposed to Cd but increased in Zn- and Pb-treated roots. HM had no significant impact on antioxidant power in leaves of OVC plants, except a small decrease recorded in response to Pb. SV had no impact on DPPH radical scavenging, except a slight increase noticed for Zn-treated leaves. In contrast, leaves of LVL-treated plants exhibited a strong increase in antioxidant power in the absence as well as in the presence of HM.

Vermicompost treatment hardly modified the root and leaf GSH concentration (Table 7) while HM significantly increased them ($P < 0.05$). The highest GSH content was recorded for Zn-treated plants and the lowest values among stressed plants were recorded for Cd-exposed ones, Pb-treated plants exhibiting intermediate values. The oxidized form GSSG (Table 7) in both roots and leaves were in the same range for control and Cd-treated plants but it increased in Pb-treated plants, whatever the applied vermicompost treatment. The total glutathione (GSH_t ; Table 7) was significantly higher in roots and leaves of Pb-treated plants while

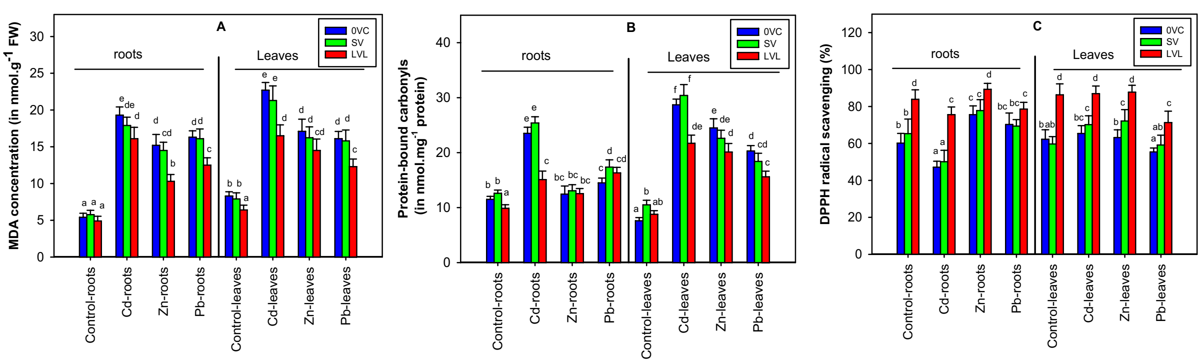

Fig. 4 Malondialdehyde concentration (MDA, nmol.g^{-1} FW) (A), carbonyl residues (nmol.mg^{-1} proteins) (B) and total antioxidant activity (radical scavenging capacity in %) in roots and leaves of *Zygopyllum fabago* growing for 10 weeks in non-polluted control soil, or in soil contaminated with cadmium (10 mg.Kg^{-1} DW), zinc (250 mg.Kg^{-1} DW) or lead (500 mg.Kg^{-1} DW) in the absence of vermicompost (0VC), in the presence of solid vermicompost 20% (SV) or irrigated with liquid vermicompost leachates 5% (LVL). Each value is the mean of six replicates and vertical bars are standard errors. Values sharing a similar letter are not significantly different at $P = 0.05$ according to the Tukey test

Table 7 Reduced glutathione (GSH ; in nmol.g^{-1} FW), oxidized glutathione (GSG ; in nmol.g^{-1} FW), total glutathione (GSHt ; in nmol.g^{-1} FW), GSSG/GSH ratio and total concentrations of phytochlatin (PC, in nmol GSHequi. g^{-1} FW) in roots and leaves of *Zygophyllum fabago* maintained during 10 weeks on non-polluted control soil, or on spiked polluted soil containing either Cd (10 mg.Kg^{-1} DW), Zn (250 mg.Kg^{-1} DW) or Pb (500 mg.Kg^{-1} DW)

Element/organ	Amendment	GSH	GSSG	GSHt	GSSG/GSH	PC
Roots						
Control soil	OVC	64 ± 5 a	7 ± 1 a	78 ± 4 a	0.11 ± 0.02 a	101 ± 8 a
	SV	72 ± 6 a	9 ± 2 a	90 ± 8 a	0.13 ± 0.03 a	88 ± 10 a
	LVL	85 ± 7 b	8 ± 1 a	101 ± 7 a	0.09 ± 0.01 a	92 ± 7 a
Zinc	OVC	124 ± 5 c	11 ± 3 a	146 ± 11 b	0.09 ± 0.01 a	613 ± 45 e
	SV	132 ± 10 c	20 ± 3 b	172 ± 10 c	0.15 ± 0.03 ab	594 ± 37 e
	LVL	161 ± 11 d	25 ± 4 b	211 ± 14 d	0.16 ± 0.03 b	628 ± 28 e
Lead	OVC	195 ± 15 e	94 ± 8 d	383 ± 22 f	0.48 ± 0.07 cd	123 ± 11 b
	SV	226 ± 17 f	101± 10 d	428 ± 43 f	0.45 ± 0.02 c	137 ± 7 b
	LVL	209 ± 11 ef	114 ± 12 d	437 ± 44 f	0.55 ± 0.08 d	147 ± 26 b
Leaves						
Control soil	OVC	47 ± 2 a	5 ± 1 a	57 ± 4 a	0.11 ± 0.01 a	56 ± 5 a
	SV	54 ± 3 a	6 ± 1 a	66 ± 3 a	0.11 ± 0.02 a	62 ± 6 ab
	LVL	56 ± 4 a	5 ± 2 a	66 ± 5 a	0.09 ± 0.02 a	58 ± 7 a
Cadmium	OVC	82 ± 10 b	7 ± 3 ab	96 ± 12 b	0.09 ± 0.02 a	104 ± 8 d
	SV	94 ± 7 bc	8 ± 1 b	110 ± 7 b	0.08 ± 0.01 a	112 ± 7 d
	LVL	148 ± 11 d	15 ± 3 c	170 ± 12 c	0.10 ± 0.02 a	115 ± 12 d
Zinc	OVC	211 ± 14 e	20 ± 3 d	251 ± 15 d	0.09 ± 0.03 a	88 ± 4 bc
	SV	220 ± 17 e	26 ± 2 d	272 ± 18 d	0.12 ± 0.03 a	94 ± 10 cd
	LVL	206 ± 9 e	27 ± 3 d	260 ± 14 d	0.13 ± 0.04 a	90 ± 11 cd
Lead	OVC	112 ± 11 c	103 ± 8 e	383 ± 22 f	0.48 ± 0.07 cd	123 ± 11 b
	SV	165 ± 15 d	123 ± 11 e	428 ± 43 f	0.45 ± 0.02 c	137 ± 7 b
	LVL	143 ± 18 d	114 ± 12 d	437 ± 44 f	0.55 ± 0.08 d	147 ± 26 b

Plants were cultivated in the absence of vermicompost (0VC), in the présence of 20% solid vermicompost (SV) or irrigated with liquid vermicompost leachate (5%). Data are the means of 5 replicates ± S.E. For a specific organ and a given parameter, means followed by the same letters were not different according to the Tukey test at $P = 0.05$. ND : not detected

Cd-exposed plants presented the lowest total glutathione value among stressed plants. The GSSG/GSH ratio (Table 7) remained stable in control plants and in plants exposed to Cd or Zn but it drastically increased in Pb-exposed plants reaching high values especially in the leaves ($P < 0.001$). The phytochelatin (PC) concentrations culminated in the roots of Cd-treated plants: their concentrations were six times higher than in the roots of control plants and more than two times higher than in those of Zn-treated plants. Vermicompost had no obvious impact on PC content in the roots of control or HM-treated plants, except a slight increase induced by LVL in the Zn-treated plants. PC concentrations were lower in the leaves than in the roots and once again were maximal in Cd-treated plants.

Total phenolic compounds, flavonoids, salicylic acid, and proline concentration

Total phenolic compound concentrations (Fig. 5A) were lower in the roots than in the leaves. In the absence of vermicompost (OVC), the root total phenolic compounds did not significantly increase in plants exposed to HM. SV increased the root total phenolic compounds in plants exposed to Zn and Pb while LVL increased them in all stressed plants. At the leaf level, total phenolic compounds increased in control plants exposed to LVL ($P < 0.01$). In stressed plants, the leaf total phenolic compounds increased in plants cultivated in the absence of vermicompost (OVC) and even to a higher extent in plants exposed to LVL.

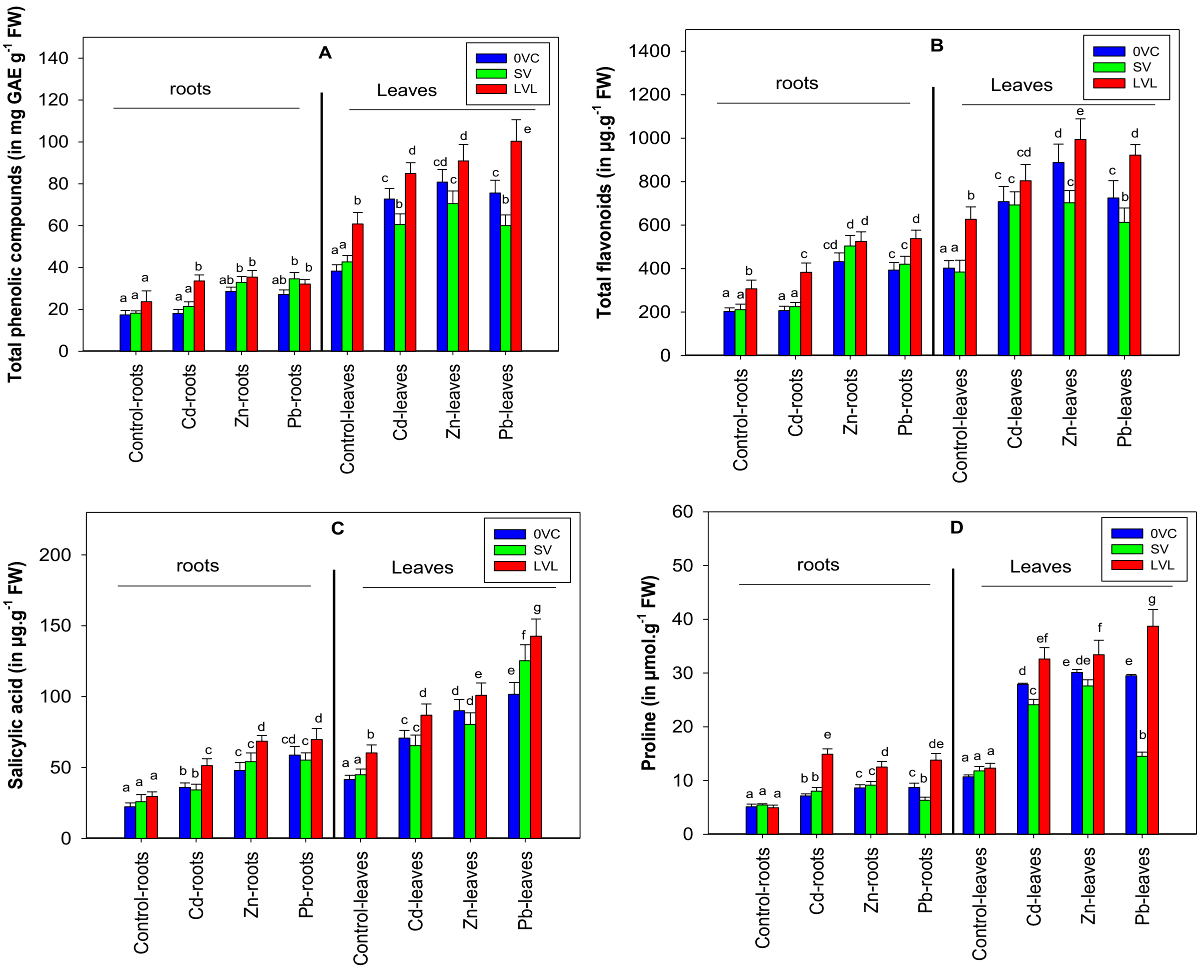

Fig. 5 Total phenolics compounds (in mg GAE g^{-1} FW) (A), total flavonoids (µg.g^{-1} FW) (B) salicylic acid (µg.g^{-1} FW) (C) and proline concentration (in µmol.g^{-1} FW) (D) in roots and leaves of *Zygopyllum fabago* growing for 10 weeks in non-polluted control soil, or in soil contaminated with cadmium (10 mg.Kg^{-1} DW), zinc (250 mg.Kg^{-1} DW) or lead (500 mg.Kg^{-1} DW) in the absence of vermicompost (0VC), in the presence of solid vermicompost 20% (SV) or irrigated with liquid vermicompost leachates 5% (LVL). Each value is the mean of six replicates and vertical bars are standard errors. Values sharing a similar letter are not significantly different at $P = 0.05$ according to the Tukey test

Flavonoids increased in the roots and in the leaves of control plants exposed to LVL treatment ($P < 0.001$; Fig. 5B). Heavy metals in the absence of vermicompost induced an increase in root flavonoids in Zn- and Pb-treated plants but had no impact on Cd-treated ones. At the leaf level, flavonoid concentrations increased in all stressed plants, the maximal values being recorded for stressed plants receiving the LVL treatment. Salicylic acid (SA; Fig. 5C) also increased in roots and leaves of stressed plants as well as in the leaves of control ones. SA concentrations always showed maximal values in plants treated with LVL. The highest endogenous SA concentrations were observed in Pb-treated plants.

In contrast to flavonoids and SA, proline concentration (Fig. 5D) did not increase in control plants receiving the LVL treatment. However, it increased in all stressed plants, and to a higher extent in the leaves than in the roots, with the noticeable exception of Pb-exposed plants cultivated in the SV treatment. For all stressed plants, the highest proline concentration was observed in LVL-treated plants.

Discussion

Zygophyllum fabago is well adapted to HM polluted environment

The present work demonstrates that the Syrian bean-caper *Zygophyllum fabago* is a good candidate for phytomanagement of moderately polluted agricultural soils. This perennial shrub has a deep root system and is able to cope with marginal areas under various climatic conditions including highly desertic areas (Nagiloo and Claβen-Bockhoff 2020). Studies performed in nutrient solutions with fully available HM already indicated that *Z. fabago* is able to survive to deleterious doses of Cd, Zn or Pb. The mean concentrations of accumulated Cd recorded in the present study were in the same range as those previously reported for leaves (Lefèvre et al. 2005) and roots (Lefèvre et al. 2016) for plants exposed to 10 µM $CdCl_2$, which corresponds to a dose 10 times lower than the dose used in our spiked soils. In the present work, Cd bioavailability in the substrate was *c.a.* 5% in the absence of vermicompost. In contrast, a similar level of Zn was reported in roots and shoots in our present study on soil substrate, and in nutrient solutions using similar doses (Lefèvre et al. 2010, 2014, 2016) thus suggesting that bioavailability is not a major limiting factor for Zn absorption. On heavily polluted mine tailings containing up to 12,000 mg.Kg^{-1} DW Zn, Conesa et al. (2006) reported Zn accumulation ranging from 530 to 750 µg.g^{-1} DW in the shoots, corresponding to 138–196% of the values recorded in our plants growing on a soil which contained 40 times less Zn. This suggests that *Z. fabago* efficiently regulates Zn homeostasis (Párraga-Aguado et al. 2016) which may be related to the fact that Zn, in contrast to Cd and Pb, is an essential element for plant metabolism. Lead accumulation was in the same range as values recorded by López-Orenes et al. (2014, 2018) and were much lower than values recorded for Zn and this may be related to the very low bioavailability commonly reported for this element (Table 4; Lambrechts et al. 2011a, b).

Accumulated HM did not compromise plant survival but however induced an obvious growth inhibition in plants cultivated in the absence of vermicompost (Fig. 1). A decrease in plant growth should not necessarily be regarded as a symptom of injury since growth inhibition allows stressed plants to affect more energy to resistance mechanisms. Lefèvre et al. (2009) reported that Cd inhibited stem elongation and axillary bud development in *Z. fabago*. Modification of structural development may be related to uneven distribution of these elements in various organs but also within organs as reported by Lefèvre et al. (2014; 2016) and to an impairment of physiological functions. In the absence of vermicompost, all HM decreased net photosynthesis, stomatal conductance and *Chl*b content (Fig. 3; Table 5). Accumulated HM also decreased the leaf WC and the shoot Ψ_w comparatively to plants cultivated on non-polluted control soil (Table 5). The recorded decrease in *A* values correlated with a decrease of g_s, so that photosynthesis inhibition might be due to stomatal limitations. Heavy metals are known to interfere with calcium channels in guard cells and thus compromise stomatal regulation (Riyazuddin et al. 2022). It has to be mentioned, however, that stomatal conductance was recorded after 10 weeks of treatment and the fact that leaves presented a decrease in water content suggests that such stomatal closure was not instantaneous and could have been preceded by consistent water losses. Lefèvre et al. (2005) provided evidences that *Z. fabago* may adopt various strategies depending on the duration of the stress to cope with Cd toxicity.

Heavy metals also induced secondary oxidative stress in plants cultivated in the absence of vermicompost, as indicated by an increase in both MDA and carbonyl residues (Fig. 4), especially in Cd-treated plants. In *Z. fabago*, cadmium is known to bind to phytochelatins, especially at the root level (Lefèvre et al. 2014, 2016), as suggested in Table 7. Synthesis of PC may be regarded as a protective mechanism against free Cd accumulation but it occurs from the antioxidant precursor glutathione and PC oversynthesis may lead to the depletion of protecting GSH if glutathione synthesis itself is not increased (Thakur et al. 2022). We suggest that this occurred in our Cd-treated plants since HM-induced increase in total glutathione (GSHt; Table 7) was far lower for Cd-treated plants than for plants exposed to Zn or Pb. This hypothesis is strengthened by the recorded decrease in DPPH radical scavenging activity recorded in Cd-treated roots. Despite the HM-induced growth inhibition, the plants cultivated in the absence of vermicompost were able to remove 2.77, 10.75 and 0.13 mg of Cd, Zn and Pb respectively from the substrate. Moreover, plant culture in the column reduced the volume of leachate and decreased the Cd and Zn concentration in these leachates comparatively to CWP (Table 6). This reinforces the putative interest of *Z. fabago* for phytostabilization and phytoextraction purposes, at least for Cd and Zn since 95% of the removed elements were present in the plants while less of 5% were removed by the leachate.

Solid vermicompost contributes to stress avoidance through a decrease in HM bioavailabilities

Amendments could be used to improve the behavior of plants used in phytomanagement and may act on soil properties but also directly on plant physiological behavior (Lambrechts et al. 2011b; Kafle et al. 2022; Khosropour et al. 2022; Oladoye et al. 2022; Shen et al. 2022). Data start to be available regarding the putative interest of SV for phytomanagement of polluted areas (Wang et al. 2022; Zuhair et al. 2022; Sengupta et al. 2023). In contrast, using LVL provided valuable information regarding the plant agricultural performances (Arthur et al. 2012; Čabilovski et al. 2023) or responses to salt and drought stress (Benazzouk et al. 2018, 2019, 2020; Cicek et al. 2022; Voko et al. 2022) but to the best of our knowledge was not until now tested for phytoremediation purposes.

The present study demonstrates that both solid vermicompost (SV) and liquid vermicompost leakage (LVL) may improve phytomanagement of HM-polluted soil by *Zygophyllum fabago* but that the two types of compounds are acting through different mechanisms in the soil-plant system. Solid vermicompost decreased HM bioavailabilities while LVL clearly increased them (Table 4). The SV-induced decrease in bioavailability was also recorded for CWP columns containing no plants, confirming that the reduced bioavailability was a direct effect of vermicompost itself and not an indirect consequence of SV on plant development. The concentrations of the leachates in Cd and Zn were strongly reduced (Table 6) in SV-containing columns. The total volume of leachate was also decreased but in columns containing plants only and not in CWP demonstrating that the decrease in leaching should be regarded as a consequence of SV-induced increase in plant growth and transpiration stream. Indeed, SV increased both E and g_s values in Cd and Zn-treated plants compared to OVC treatment (Fig. 2), and the high water-holding capacity frequently reported for SV (Khosropour et al. 2022) probably only plays minor role in preventing leaching (Fig. 2).

The major mechanisms of HM immobilization in polluted soils are heavy metal adsorption, co-precipitation, complexation and ion-exchange adsorption. The main functional groups commonly found on the surface of vermicompost are O-H, C-H, C=C, C=O, $Si-O_2$ and C-O (Zhang et al. 2019). According to Pan et al. (2022), vermicompost also provides polysaccharide and mucus secretion from earthworm which may contribute to HM retention. Vermicomposts contain humic acids which have a high complexation capacity for HM since they contain carboxylic and phenolic groups as complexation sites (Rong et al. 2020). According to Ondrasek et al. (2018), humic acids behave as highly complexed and polymerized organics and play crucial roles in Cd and Zn retention due to their large negative charges.

As a consequence of reduced HM bioavailability, HM concentrations significantly decreased in all plant organs (Fig. 3) and this may explain the increase in dry weight induced by SV for all organs of plants growing on spiked soils (except roots exposed to Pb) (Fig. 1). Since the recorded increase in plant growth was higher than the recorded decrease in HM accumulation, the total amount of HM removed by the plant significantly increased, especially if we consider the amounts of HM translocated to the shoots (Table 6). This confirms our first tested hypothesis and demonstrates that in the precise case of *Z. fabago*, SV may contribute both to phytostabilization through a decrease in HM mobility down the profile, and to phytoextraction by indirectly improving the total amount of HM translocated to the shoots.

It is tempting to speculate that all beneficial impacts of SV on physiological functions of plants growing on spiked soil should be regarded as the consequences of a lower rate of HM accumulation inside the plant tissues. A higher net photosynthesis in Cd- or Zn treated plants exposed to SV may result from an increase in stomatal conductance but this was not anymore the case for Pb-stressed plants which exhibited an improved photosynthesis in the absence of any g_s increase. Conversely, the decrease in HM accumulation did not lead to an obvious decrease in oxidative stress since OVC- and SV-exposed plants growing on spiked soils presented almost similar levels of MDA and carbonyl residues (Fig. 5). This might suggest that antioxidative strategies are saturated in HM-treated plants of *Z. fabago* already for endogenous doses of HM corresponding to those encountered in SV treated plants and similar values recorded for DPPH-scavenging activity in both types of plants reinforce this hypothesis. According to Landorfa-Svalbe et al. (2022) and Khosropour et al. (2022), HM-induced decrease in chlorophyll concentration is one of the main reasons for the diminished photosynthetic activity and vermicompost can increase chlorophyll contents in stressed plants. According to Lefèvre et al. (2014), Z. *fabago* can trigger protective mechanisms to avoid photosynthesis inhibition through upregulation of several proteins involved in the photosynthetic apparatus and C-metabolism.

Besides its impact on HM bioavailability, SV may have various beneficial impacts on different soil properties. Indeed, it improves biological activity in polluted (Karaca and Chalabee 2023) and increases bacterial diversity (Wang et al. 2021; Zuhair et al. 2022). Vermicompost is also a rich amendment containing high amounts of available essential elements (Santana et al. 2018; Palansooriya et al. 2023). It may thus help to counteract nutrient deficiencies (especially N and K) found in polluted tailings where *Z. fabago* is growing on a soil with very low levels of fertility (Conesa et al. 2006; Párraga-Aguado et al. 2022; Kabbas et al. 2014; López-Orenes et al. 2018). In the present study, however, an artificially-polluted agricultural soil was used and contained sufficient amounts of available nutrients. It is therefore unlikely that the positive effect of SV on plant growth was the result of additional nutrient supply.

Liquid vermicompost leachate contributes to stress tolerance through reinforcement of endogenous protecting mechanisms

Liquid vermicompost leachate (LVL) increased HM bioavailability in spiked soils both in the presence and in the absence of plants (Table 4). As a consequence, an increase in HM concentration in leachate from the column (C_l) was observed for all elements (Table 6). However, the volume of leakage was lower for columns containing Cd- or Pb-spiked soils and plants treated with LVL than in similar columns maintained in the absence of vermicompost. LVL increased root growth, even in the absence of HM. In the presence of HM, its positive impact was by far higher on root growth than on shoot growth, and shoot DW of LVL-treated plants were lower than shoot DW of SV-exposed ones. In HM-containing columns, more than 95% of the removed HM were present in the plants (Table 6) and less than 5% were recovered in leachates. LVL did not increase %L for Cd or Zn comparatively to OVC columns and this confirms our second hypothesis that stimulating root growth contributed to limit HM leaching down the profile despite the LVL-induced increase in their mobility.

It is noteworthy that HM concentrations increased in the shoots of LVL-treated plants but decreased at the root level. An increase in both DW and HM accumulation in the shoots comparatively to the shoots of OVC plants clearly implies that LVL acts directly at the plant level and increases its level of tolerance to accumulated HM. It is obvious that LVL-treated plants exposed to Cd or Pb presented a decrease in MDA and carbonyl residues content despite the recorded increase in HM and exhibited a concomitant increase in the antioxidant capacity (Fig. 4). LVL contains high concentrations of polyphenol having the ability to chelate heavy metals ions. Compounds such as flavonoids and phenolic acids play a role in ROS scavenging (Kosem et al. 2022; Feng et al. 2024). These compounds are present in LVL and their direct absorption by stressed plants could therefore not be ruled out as suggested by the increase in total phenolic compounds (Fig. 5A) and flavonoids (Fig. 5B) recorded in plants exposed to LVL. An increase in total phenolic compounds and flavonoids was recorded for the leaves of LVL-treated control plants but it was clearly higher in the presence HM-treated plants which paradoxically presented a lower transpiration rate (Fig. 2C). Moreover, OVC and SV-treated plants were also able to trigger phenolic compounds and flavonoids accumulation. This leads us to hypothesize that a significant proportion of these compounds present in LVL-treated plants exposed to HM resulted from a *de novo* synthesis by the plant rather than from absorption of exogenous compounds provided by LVL. This hypothesis is in agreement with the data of Kosem et al. (2022) who showed that the profile of individual phenolic compounds found in LVL-treated plants of *Ocimum basilicum* exposed to severe water stress differed from the profile of these individual compounds found in LVL This is also valid for glutathione: although LVL increased GSHt in roots of Cd-treated plants and in leaves of Cd- and Pb-stressed ones (Table 7), GSH was not detected in LVL (Table 3) and its accumulation was therefore the consequence of the activation of endogenous synthesis.

Liquid vermicompost leachate also contains high concentration of phytohormones (Table 3) and our previous work already demonstrated that these molecules may strongly influence plant response to abiotic stress (Benazzouk et al. 2020). A high auxin concentration may explain the increase in root growth which was also observed in control plants growing on non-polluted substrate (Fig. 1A). Salicylic acid (SA) is another plant hormone present in LVL and it reached its maximal endogenous concentration in Pb-treated plants (Fig. 5C). High endogenous levels of SA have been correlated with increased HM tolerance in plants (Freeman et al. 2005). According to López-Orenes et al. (2014), Pb exposure induces an increase in SA in *Z. fabago* and this is confirmed by the Pb-induced SA increase recorded in the OVC-treated plants (Fig. 5C). Our data however suggest that additional SA provided by LVL may reinforce tolerance of *Z. fabago* to Pb toxicity. Salicylic acid promotes carotenoid synthesis in Syrian beancaper (López-Orenes et al. 2014) and the recorded increase in carotenoid content induced by LVL treatment (Table 5) suggests that additional SA afforded by LVL was able to efficiently stimulate carotenoid synthesis protecting chloroplasts against free radical formation and explaining, at least partly, the recorded increase in net photosynthesis despite the maintenance of stomatal closure. Salicylic acid was also reported to influence oxidation of coniferyl alcohol and ferulic acid in Pb-stressed plants of *Z. fabago* (López-Orenes et al. 2020). This process increases the strength and rigidity of the cell walls and would stimulate the immobilization of toxic metal ions in the cell wall and avoid their entry in symplasm. Lead could also interact with thiol groups and PC could be involved in Pb chelation but in our study, Pb had only a minor impact on PC concentration (Table 7) and LVL had no significant effect on their biosynthesis, which could be due to the energy cost associated with sulfate reduction and PC synthesis (López-Orenes et al. 2014, 2021).

Besides its crucial role in oxidative stress management, SA may also directly influence the plant water status in HM-treated plants. In *Z. fabago*, López-Ornes et al. (2020) demonstrated that SA induced accumulation of ABA and 12-oxo-phytodienoic acid (OPDA). Both compounds induce stomatal closure and this could explain the low g_s values recorded in LVL-treated plants (Fig. 2B), although the increase in net photosynthesis (Fig. 2A) lead to an increase in WUE in plants receiving the liquid amendment (Fig. 2D). Proline is an important osmoprotecting solute allowing osmotic adjustment and maintenance of a favorable water potential gradient. It is noteworthy that proline concentration culminated in LVL-treated plants and reached maximal values in response to Pb toxicity (Fig. 5D). Similarly, the lowest root and leaf Ψ_s values were recorded in LVL-treated plants (Table 5). However, proline was not detected in LVL (Table 3). According to López-Orenes et al. (2014), SA increased proline synthesis in Pb-treated plants of *Z. fabago* and the highest proline accumulation observed in LVL-treated plants might be explained by the presence of SA in the liquid vermicompost leachate.

Conclusions

It is concluded that solid vermicompost and liquid vermicompost leachate had positive impacts on heavy metals phytostabilization and phytoextraction by the Syrian beancaper *Zygophyllum fabago*. The two types of amendments have distinct effects on the plant physiological behavior but some of their properties may appear as complementary and are not mutually exclusive.

Solid vermicompost reduced HM bioavailability and decreased HM accumulation in the shoots. However, the recorded increase in plant growth was higher than the recorded decrease in HM concentration leading to an increase in the total amount of HM removed from the substrate and thus an improvement of phytoextraction efficiency. In contrast, liquid vermicompost leachate increased HM bioavailability, HM accumulation in the shoot, and root growth. It also increased plant tolerance to accumulated pollutants, mainly through an improvement of oxidative stress management allowing the plant to remain alive and grow despite a high shoot HM content. Consequently, LVL-treated plants also increased the total amount of HM removed from the substrate but did not increase HM leaching.

Differences among heavy metal behavior were however recorded for Cd, Zn and Pb. It could therefore be interesting to test the impact of vermicompost on a polluted substrate simultaneously contaminated by those three pollutants. Similarly, a combined application of solid vermicompost and liquid vermicompost leachate would be interesting to study. Field experiments are thus still required to analyze the use of various forms of vermicompost on *Zygophyllum fabago* for phytostabilization and phytoextraction on HM-polluted sites.

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Data availability The data that support the finding of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This article does not contain any studies involving humans or animals performed by any of the authors.

Consent to participate This article does not contain any studies involving humans.

Consent to publish This article does not contain any studies involving humans; hence no consent to publish was obtained.

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