



Sequential soil washing with mixed biosurfactants is suitable for simultaneous removal of multi-metals from soils with different properties, pollution levels and ages

Zygmunt Mariusz Gusiatin¹ · Maja Radziemska² · Alicja Żochowska¹

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Abstract

In this study, plant (SAP, TAN) and microbial (RAM) biosurfactants were applied to two soils, in single or sequential soil washing. Soil 1 was moderately acidic, with higher content of organic matter and HMs (Cd, Cu, Ni, Pb and Zn), and was aged for 24 months. Soil 2 was alkaline, with lower content of organic matter and HMs, and was aged for 1 month. The stability of the HMs (as I_R), except for Pb, was higher in soil 1 than in soil 2, but the rankings of HM stability were similar: Cu > Ni \approx Pb > Zn > Cd (soil 1) and Cu > Pb > Ni > Zn > Cd (soil 2). The HMs were removed more efficiently from soil 2 than from soil 1, mainly from the readily available fraction and partially from the reducible fraction. Cu (0.3–0.5 times), Ni (0.4–1.1 times) and Zn (0.5–1.0 times) showed the largest increases in stability after soil washing with SAP. With a single washing, SAP and TAN were the least effective for Pb removal (18–31% and 11–35%, respectively), while RAM was the most effective for Cd (69–73%) and Pb (58–63%) removal. Sequential soil washing, especially with SAP followed by RAM, was the best choice for simultaneous removal of multi-HMs.

Keywords Biosurfactants · Soil remediation · Single and sequential soil washing · Metal fractionation and stability · Cluster analysis

Abbreviations

SAP	Saponin
TAN	Tannic acid
RAM	Rhamnolipids
HMs	Heavy metals
I_R	Reduced partition index

Introduction

Soil contamination with heavy metals (HMs) is a global problem; it occurs mainly in highly developed countries like the USA, Australia, China, Germany or Sweden. However,

HM concentrations in soils can also be high in less developed countries (e.g., Pakistan, India, Bangladesh), mainly as a result of using untreated wastewater for crop irrigation (Khalid et al. 2017).

Usually, soils are not contaminated with a single HM, but are co-contaminated with different HMs. Some of the HMs commonly present in soils are nickel (Ni), lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn). Contaminated sites have wide variations in HM concentrations, which affect the level of soil contamination. In polluted soils around the world, Cd concentrations range from 14 to 42 mg/kg; those of Pb range from 302 to 4500 mg/kg; those of Zn, from 393 to 3833 mg/kg; Ni, from 153 to 2603 mg/kg; and Cu, from 235 to 35,582 mg/kg (Khalid et al. 2017). Such variations in HM concentrations can affect the efficiency and feasibility of soil remediation technologies. Therefore, it is reasonable to further develop soil remediation technologies for multi-HM-contaminated soils.

For treatment of HM-contaminated soils, soil washing is still considered both an innovative remediation method (Liu et al. 2017) and, owing to its high efficiency, one of the most cost-effective soil remediation technologies (Khalid et al. 2017). Although EDTA is most commonly used in

✉ Zygmunt Mariusz Gusiatin
mariusz.gusiatin@uwm.edu.pl

¹ Department of Environmental Biotechnology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Słoneczna St. 45G, 10-719 Olsztyn, Poland

² Faculty of Civil and Environmental Engineering, Warsaw University of Life Sciences, Nowoursynowska St. 159, 02-776 Warsaw, Poland

soil washing studies (Bilgin and Tulun 2016, Wei et al. 2016, Naghipour et al. 2017), the use of alternative washing agents, such as plant and microbial biosurfactants, is still attractive.

Rhamnolipids (RAM) are the most intensively studied glycolipid biosurfactant. They are produced by *Pseudomonas aeruginosa* and are composed of one or two rhamnose groups in the hydrophilic moiety, and up to three hydroxy fatty acids (C8–C14) in the hydrophobic moiety (Wan et al. 2015). RAM are widely used in many fields, such as oil recovery, food production, cosmetics, and pharmaceuticals (Liu et al. 2018). In environmental remediation, they can act as ligands and have a strong affinity for HMs due to their carboxyl groups. Up to now, most studies on the use of RAM have involved Cu, Cd, Ni, Pb or Zn removal from soils via soil washing (at varying pH, concentrations of RAM or washing times) or soil flushing (at different concentration of RAM, flushing time or flow rate) (Mulligan and Wang 2006; Juwarkar et al. 2007; Venkatesh and Vedaraman 2012; Wan et al. 2015).

Saponin (SAP) is a kind of glycoside and, thus, is a secondary plant metabolite. As a nonionic biosurfactant, SAP has diverse physicochemical properties that have been successfully used in a number of commercial applications, including soil washing. Previous studies indicate that, for removal of HMs from soil, SAP needs to be used at acidic pH and relatively high concentrations (~3%), and for relatively short extraction times (3–6 h) (Hong et al. 2002; Gusiatiin and Klimiuk 2012).

Tannic acid (TAN) is a naturally occurring plant polyphenol that is a hydrolyzable tannin. Although TAN is able to form complexes with HMs because it has numerous phenolic hydroxyl groups (Friman et al. 2004), its applications in soil washing have not been completely investigated. So far, TAN has been found useful as a washing agent only for soils contaminated with a mixture of Cu, Pb and Zn or with As, and the operational conditions for HM removal via soil washing with TAN are similar to those applied with SAP (Gusiatiin et al. 2014; Gusiatiin 2014).

Most available studies on the use of RAM, SAP or TAN as washing agents have focused on optimizing the removal of one or two HMs from soils, not mixtures of three or more HMs. When assessing the effectiveness of microbial and plant biosurfactants for soil washing, not only the operational conditions for removal of HMs from different soils need to be taken into consideration, but also the level of HM pollution in soil (because of the limited number of active sites that biosurfactants have available for complexing HMs) and the age of the contaminated soil (due to changes in HM distribution over time). Kulikowska et al. (2015) found that the efficiency of Cu, Ni and Zn removal with a solution of humic substances from sewage sludge compost decreased as soil age increased, while the efficiency of Pb and Cd removal

did not depend on soil aging. When testing synthetic washing agents, some authors found that a combination of acid and chelator could remove HMs with higher efficiency than any single acid or chelator (Yang et al. 2012). Thus, soil washing with combinations of biosurfactants that have different affinities for specific HMs could be important, especially for treatment of multi-HM-contaminated soils. At present, data on the use of RAM, SAP and TAN for removing multi-HMs from soils with different pollution levels and ages are limited, as is the use of these biosurfactants in combination for removing HMs via sequential soil washing.

Thus, the aim of this study was to compare the efficiency of simultaneous removal of multi-HMs (Cd, Cu, Ni, Pb and Zn) from different soils (based on soil properties, total HM content and aging time) with RAM, SAP and TAN, and to determine the effect of these biosurfactants on HM redistribution, in terms of HM mobility and stability. Moreover, the effect on HM removal of sequential soil washing with the same or different classes of biosurfactant was determined to select the most effective sequence of washing solutions for simultaneous multi-HM removal.

Materials and methods

Soils

In this study, two soils spiked with HMs were used for soil washing with biosurfactants. The soils were collected from the surface level of soil (depth 0–30 cm) in Warmia and Mazury Province, north-eastern Poland. Then, the soils were air dried and ground to pass through a 1-mm sieve. Next, each soil (2 kg) was spiked with a mixture of five HMs: Cd, Cu, Ni, Pb and Zn. To obtain different total HM concentrations in soils, the following weights of HM salts (Sigma-Aldrich) were dissolved in distilled water and added to the soils: 0.1372 g $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 3.6602 g $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$, 2.4658 g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 6.3940 g $\text{Pb}(\text{NO}_3)_2$ and 9.0963 g $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (soil 1); and 0.0549 g $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.4641 g $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$, 0.9863 g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 1.5985 g $\text{Pb}(\text{NO}_3)_2$ and 3.6385 g $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (soil 2). After spiking, the soils were aged at room temperature and maintained at 60–65% of their maximum water holding capacity. As it can be assumed that HMs that are present at lower concentrations in soil require a shorter time to achieve equilibrium than HMs that are present at higher concentrations, the length of aging differed depending on the soil. Soil 1, which had higher HM concentrations, was allowed to age for 24 months, whereas soil 2, with lower HM concentrations, was allowed to age for 1 month. During the aging process, the containers were weighed each week and the appropriate amount of water was added if necessary. For

homogenous HM distribution, the soil was gently mixed once a week. After aging, the soils were dried, crushed and kept in tightly closed plastic containers for further experiments.

The physicochemical characteristics of the spiked soils are presented in Table 1. Although the soils had the same texture, they differed in pH and organic matter content. Soil 1 had higher conductivity than soil 2, and in soil 1, all HMs exceeded Polish limits for industrial areas (15 mg Cd/kg, 600 mg Cu/kg, 500 mg Ni/kg, 600 mg Pb/kg, 2000 mg Zn/kg), whereas in soil 2, only Pb was above the limit.

Table 1 Selected physicochemical properties of spiked soils (values are given as means, $n=3$, \pm standard deviation)

Characteristic	Unit	Soil 1	Soil 2
Texture	–	Sandy clay loam	
pH _{KCl}	–	5.9 \pm 0.3	7.7 \pm 0.2
Conductivity	mS/cm	1.6 \pm 0.1	0.1 \pm 0.0
Organic matter	%	5.67 \pm 0.21	4.02 \pm 0.10
Cation exchange capacity	cmol/kg	38.8 \pm 1.3	39.7 \pm 0.9
Before spiking			
Cd	mg/kg	0.2 \pm 0.02	0.0
Cu	mg/kg	12.0 \pm 2.3	1.6 \pm 0.06
Ni	mg/kg	17.9 \pm 1.7	4.8 \pm 0.2
Pb	mg/kg	13.5 \pm 1.3	0.9 \pm 0.01
Zn	mg/kg	20.3 \pm 1.9	6.1 \pm 0.2
After spiking			
Cd	mg/kg	46 \pm 1.4	16 \pm 0.8
Cu	mg/kg	1019 \pm 19.3	305 \pm 10.4
Ni	mg/kg	534 \pm 2.9	166 \pm 8.5
Pb	mg/kg	4133 \pm 140	854 \pm 16.4
Zn	mg/kg	2278 \pm 41.4	683 \pm 1.4

Table 2 Selected characteristics of biosurfactant solutions (values are given as means, $n=3$, \pm standard deviation)

Characteristic	Unit	SAP	TAN	RAM
Concentration	% (w/v)	3	3	3
Total organic carbon (TOC)	g/L	12.7 \pm 0.6	14.7 \pm 1.1	24.5 \pm 0.5
Critical micelle concentration (CMC)	g/L	0.9 \pm 0.1	12.7 \pm 0.3	0.022 \pm 0.001
pH	–	5.58 \pm 0.12	4.24 \pm 0.15	6.82 \pm 0.09
Conductivity	mS/cm	29.0 \pm 0.1	1.5 \pm 0.1	25.1 \pm 0.2
Surface tension	mN/m	38.7 \pm 0.6	41.5 \pm 0.9	22.4 \pm 0.5
Interfacial tension ^a	mN/m	6.0	4.5	4.5
Density	g/mL	1.098	1.085	1.076
Cd	mg/L	0 \pm 0.0	0 \pm 0.0	0.03 \pm 0.0
Cu	mg/L	0.95 \pm 0.09	1.07 \pm 0.12	0.51 \pm 0.06
Ni	mg/L	0.18 \pm 0.04	0 \pm 0.0	0.18 \pm 0.07
Pb	mg/L	0.33 \pm 0.01	0.04 \pm 0.01	0 \pm 0.0
Zn	mg/L	0.56 \pm 0.08	0.04 \pm 0.0	0.24 \pm 0.06

^aAdopted from Urum and Pekdemir (2004)

Biosurfactants

Three commercially purchased biosurfactants (SAP, TAN and RAM) were tested as soil washing agents. SAP (product no. 84510) is nonionic biosurfactant in the form of a powder, with a sapogenin content of 8–25% (Sigma-Aldrich). TAN (product no. 16201) is a natural polyphenolic compound that is in the form of a solid with a molecular weight of 1701.2 g/mol, a light yellow to tan color, a faint odor, and a purity of 90.8%.

RAM is a 25% aqueous solution that contains a mixture of anionic rhamnolipids of type R1 (C₂₆H₄₈O₉) and type R2 (C₃₂H₅₈O₁₃) that is sold under the trade name of JBR425. It is a dark brown, viscous liquid. JBR425 is produced from sterilized and centrifuged fermentation broth, which has had all protein removed (Jeneil Biosurfactant Co LLC, USA). All three biosurfactants were used without further purification.

Based on previous experiments, it was estimated that the optimum concentration of plant biosurfactants (SAP, TAN) for removal of HMs from soil is 3% (Gusiatin et al. 2014). RAM was used at the same concentration as SAP and TAN. The 3% solution was prepared by dissolving 30 g of SAP or TAN, or 120 g of RAM in 1 L of distilled water. Selected properties of the biosurfactants are given in Table 2. Although these biosurfactants were used at the same % concentration, the concentration of total organic carbon was higher with RAM than with the plant biosurfactants. The RAM solution was neutral, whereas the plant biosurfactant solutions were acidic. In contrast to the TAN solution, the SAP and RAM solutions were saline, which is likely due to the purity of these biosurfactants. RAM were more efficient at decreasing the surface tension of water than SAP and TAN. The biosurfactant solutions should not be an additional source of soil contamination with HMs because the total concentrations of HMs in the solutions were low;

expressed as the sum of all HMs, they were SAP, 2.02 mg/L; TAN, 1.15 mg/L; RAM, 0.95 mg/L.

Soil washing

Both soils were washed in batch conditions. 1 g of contaminated soil (m) was weighed and placed in 50 mL capacity polyethylene tubes, and then 40 mL of biosurfactant solution (V) at a concentration of 3% was added. The samples were shaken at 150 rpm at ambient temperature in a mechanical shaker for 24 h. At the end of soil washing, the suspensions were centrifuged at 8000 rpm for 20 min and the supernatants were filtered. In the liquid samples, the total HM concentrations were measured. In the solids previously dried at 60 °C, HM fractions were determined.

In addition, sequential soil washing (1° and 2°) was performed with the following combinations:

- the same class of biosurfactant in the 1° and 2° washings: SAP–SAP, TAN–TAN and RAM–RAM,
- different class of biosurfactant in the 1° and 2° washings: SAP–RAM and TAN–RAM.

The operational conditions (m/V, biosurfactant concentration, washing time) were the same in the 1° and 2° soil washings.

Analytical methods

Soil texture was determined based on particle size distribution (Mastersizer 2000 laser particle size analyzer, Malvern Instruments, UK). The pH and electrical conductivity in soil were determined at 1:2.5 ratio (w/v) in 1 M KCl and distilled water extracts, respectively, using a pH-meter (HI 221) and a conductivity meter (HI 8733). In addition, the pH and electrical conductivity were measured directly in extracts after soil washing with biosurfactants.

Soil organic matter content was determined by sample combustion at 550 °C in a muffle furnace (Carbolite ESM-9920). The cation exchange capacity (CEC) of the soils was assayed with Kappen's method (Ostrowska et al. 1991).

In the original SAP, TAN and RAM solutions, total organic carbon (TOC) was measured using a Shimadzu Liquid TOC-VCSN analyzer. Surface tension was measured using a K100 tensiometer (Krüss, Germany) employing the Wilhelmy plate method. Total HM concentrations in soil were determined with flame atomic absorption spectrometry (FAAS) (Varian, AA280FS) after soil digestion in a mixture of 35–38% HCl and 65% HNO₃ in a microwave oven (MARSXpress, CEM USA). Soil digestion was performed in polytetrafluoroethylene vessels with 1 g of dried soil (at 105 °C), 9 mL of HCl and 3 mL of HNO₃. A one-stage microwave program (170 °C, 800 W, 30 min) was used.

After cooling, the extracts were filtered through Whatman 40 filter papers (pore size 8 μm) into 100-mL glass flasks and filled to the mark with distilled water. In biosurfactant solutions and supernatants after soil washing, the HM concentrations were measured directly after sample dilution with distilled water.

For HM fractionation in soil, a modified BCR sequential extraction procedure was used (Pueyo et al. 2008). Four operationally defined fractions were determined for each HM using specific extracting agents and extraction conditions: F_1 , exchangeable and acid soluble (0.11 M CH₃COOH, m/V = 1 g/40 mL, 16 h); F_2 , reducible (0.5 M NH₂OH·HCl, m/V = 1 g/40 mL, 16 h); F_3 , oxidizable (30% H₂O₂, m/V = 1 g/10 mL; ×2, water bath at 85 °C; 1 M CH₃COONH₄, m/V = 1 g/50 mL, 16 h); and F_4 , residual (using the same procedure as for total HMs).

All analyses were performed in triplicate. The accuracy and precision of total HM analysis using the FAAS method were checked using CRM 142 R reference material. The results of the BCR sequential extraction procedure were checked by comparing the sum of HM concentrations in the F_1 , F_2 , F_3 and F_4 fractions with the total HM concentration. The values of Cd, Cu, Ni, Pb and Zn recovered were satisfactory, ranging from 92 to 107%. TraceCERT® Cd, Cu, Ni, Pb and Zn standards for AAS (Fluka) were used for preparation of the calibration curves. The purity of the reagents was of analytical grade or better.

Calculations

Total HM concentration in soil/total HM concentration removed from soil (TM):

$$TM = \frac{(C_L \times f)}{m} \times V, \quad (1)$$

where C_L is the HM concentration in extract after soil digestion or in supernatant after soil washing (mg/L), f is the dilution coefficient (–), V is the volume of extract or supernatant (L), and m is the dry mass of soil (kg).

Reduced partition index (I_R) to assess the stability of HMs in soil before and after soil washing (Gusiatiin and Klimiuk 2012):

$$I_R = \frac{\sum_{i=1}^k i^2 F_i}{k^2}, \quad (2)$$

where i is the index number of the extraction step, progressing from 1 (with the weakest extractant) to 4 (with the strongest extractant), F_i is the relative content of an HM in the i th fraction (as a decimal value), and k is the total number of HM fractions in the BCR procedure.

The experiments were performed in triplicate to evaluate the reproducibility of the results. Differences between HM stabilities and removal of HMs with sequential soil washing were analyzed with Duncan's test with $p < 0.05$ considered significant. Cluster analysis (CA) was used to separate the HMs in soils into meaningful groups (clusters) based on their I_R before and after a single soil washing. All statistical analyses were performed using Statistica 13.1 (Dell, Inc.).

Results and discussion

HM distributions in soils

The distributions of HMs as determined with the modified BCR procedure are shown in Fig. 1. The HMs had different distribution patterns in soil 1 and soil 2. In soil 1, the content of all HMs was lower in the exchangeable fraction ($F1$) and higher in the reducible ($F2$) and oxidizable ($F3$) fractions than in these fractions in soil 2. Although HMs at low concentrations are usually more strongly bound to soil than HMs at high concentrations (Han et al. 2003), this was not the case in the present study: the HMs in soil 1, which were present at higher concentrations, were more strongly bound than those in soil 2. This was confirmed by the values of the reduced partition index (I_R) (Fig. 1). The differences in HM distributions and their bonding strength are caused by two factors: soil properties and soil aging. Firstly, soil 1 contained more organic matter than soil 2 (Table 1), which facilitated greater HM distribution into the oxidizable ($F3$) fraction. Moreover, soil 2 was alkaline (Table 1), which facilitated HM distribution into carbonates, increasing their content in the $F1$ fraction. Secondly, the length of time that the soils were aged after spiking was different, i.e., 24 months for soil 1 and only 1 month for soil 2. With aging time, HMs slowly redistribute into more stable fractions.

Cd is highly mobile because it has a high affinity with carbonates and may coprecipitate with carbonate minerals. Tang et al. found that, although soils spiked with different concentrations of Cd (48.2–813 mg/kg) had been aged for 3 years, the percentages of total Cd in the $F1$ and $F2$ fractions were still very high, with a mean value of 98% (Tang et al. 2018). In the present study, the total percent content of Cd in these two fractions was the same. Ghayoraneh and Qishlaqi (2017) found that 70% of total Cd in soils from around a Zn/Pb smelter (northwest Iran) was in the $F1$ and $F2$ fractions. Those authors wrote that increasing the pH of soil rich in Fe–Mn oxides and clay minerals increases the content of Cd in the $F2$ fraction, whereas decreasing the pH increases its content in the $F1$ fraction. In the present study, however, the soil aged for 24 months at pH 5.9 had a lower content of Cd in the $F1$ fraction than the soil aged 1 month at pH 7.7. This indicates that the difference in soil aging

times had a greater influence on Cd distribution than the difference in soil pH.

In the case of Zn in soil 1, more was present in the $F2$ fraction than the $F1$ fraction. Sorption of Zn onto Fe–Mn oxides is probably the most important of the factors that influence its distribution in soil (Kabala and Singh 2001). In contrast, in soil 2, Zn was predominantly in the $F1$ fraction. In alkaline soils, Zn is associated with carbonates ($ZnCO_3$) (Ghayoraneh and Qishlaqi 2017).

As with Cd and Zn, the percent content of Ni in the $F1$ fraction was relatively high. Tewari et al. (2010) reported that the addition of soluble Ni (as an inorganic salt) increased its content in the $F1$ and $F2$ fractions in acidic soil; in the $F1$, $F2$ and $F4$ fractions in neutral soil; and in the $F2$ and $F4$ fractions in alkaline soil. In spiked soil cultivated for 3 years, Ni content in the $F1$ fraction was still high (60% of 100 mg/kg) (Kuziemska et al. 2014).

Cu is specifically adsorbed or fixed in soils and, thus, has lower mobility than other HMs (Alloway 1995). In soil 1, which had a higher organic matter content, the percent content of Cu in the $F3$ fraction was higher than that in soil 2 (10.1% vs. 4.8% of total Cu), which had a shorter aging time and lower organic matter content (4.0%). This can be attributed to the high constant of complexation between Cu and soil organic matter, as the HM forms strong associations with oxygen and sulfur atoms on the surface of organic matter (Evans 1989). In addition, Cu had the largest percent content in the $F4$ fraction of all the HMs. This is due to the fact that Cu is easily chemisorbed on or incorporated in clay minerals (Ghayoraneh and Qishlaqi 2017).

The majority of Pb was associated with Fe–Mn oxides. Pb has a very strong affinity for Fe–Mn oxides and, as a result, inner sphere sorption complexes are formed (Scheinost et al. 2001). Thus, in the present study, Pb had the smallest changes in distribution between soils, despite the differences in total Pb concentrations and aging times. Similarly, Kulikowska et al. (2015) did not observe significant changes in the content of Pb in the reducible fraction as aging time increased from 1 to 24 months (83% of total Pb in this fraction after aging for 1 month, and 82% after aging for 12 and 24 months). Moreover, Ghayoraneh and Qishlaqi (2017) reported that the distribution pattern of Pb in soil was similar, with the largest share in the reducible fraction, regardless of the source of the Pb (native or anthropogenic).

Because HMs in the $F1$ fraction are loosely bound to soil and they can be bioavailable and highly toxic, the content of HMs in this fraction determines their mobility. Thus, the most mobile HMs in both soils were Cd, Zn and Ni. In contrast, the least mobile HMs were Cu and Pb. The fraction with the second highest content of HMs was the $F2$ fraction, which contained 80–85% of the Pb, 54–61% of the Cu, 29–51% of the Ni, 29–48% of the Zn and 22–37% of the Cd in each soil. Cu and Ni had

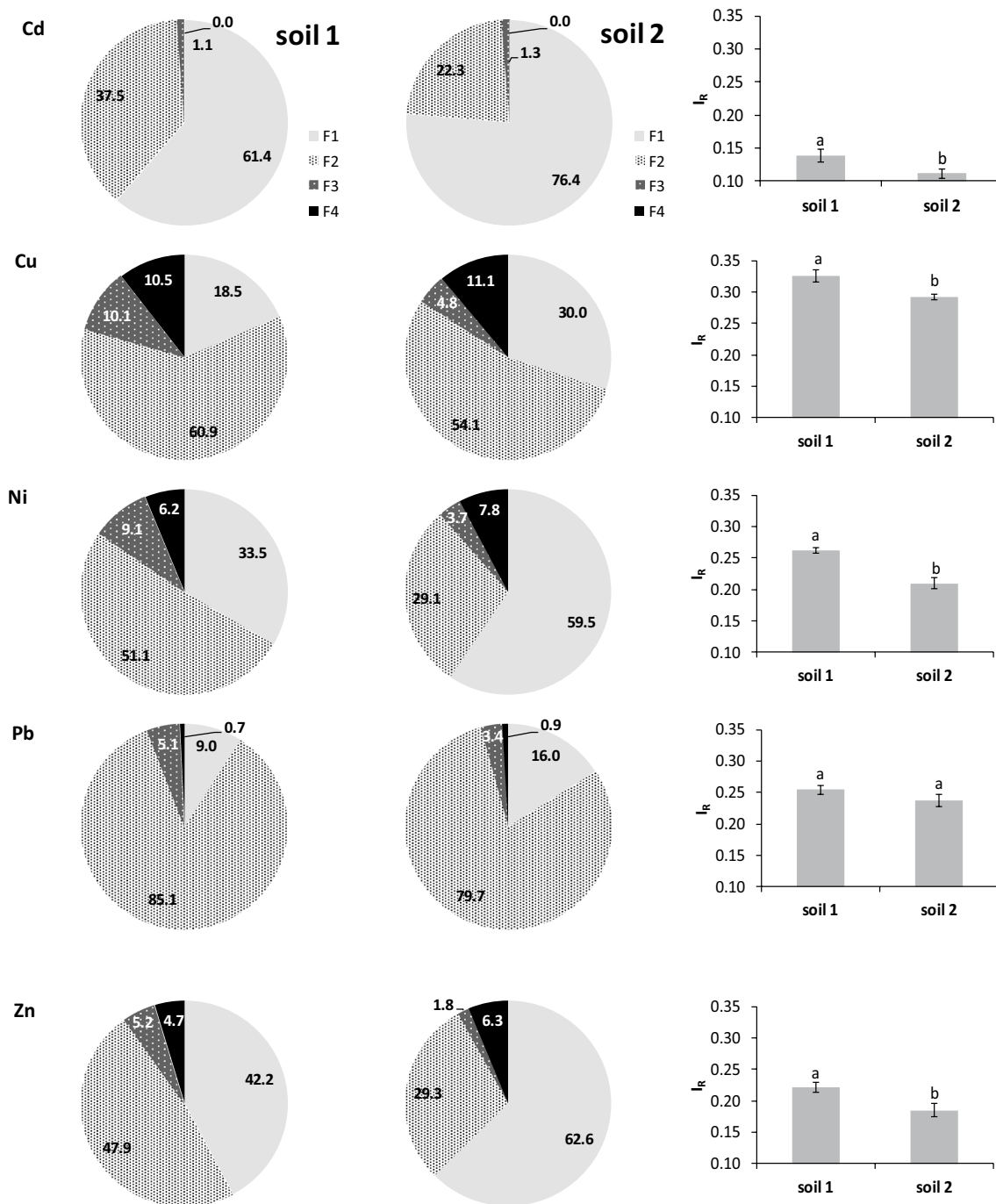


Fig. 1 HM distribution in contaminated soils (as % content of individual fractions) and their stability (as reduced partition index, I_R). For each HM, values of I_R under the same letters do not differ significantly at $p < 0.05$

the highest percent content in the $F3$ fraction, and their content in this fraction was higher in soil 1 than in soil 2. The percent content of HMs in the $F4$ fraction was not high, with Cu, Ni and Zn having the highest content in

this fraction. Low levels of HMs in the $F4$ fraction are typical of anthropogenic contamination of soil. Based on the I_R values, the rankings of HM stabilities in both soils

were nearly the same: $Cu > Ni \approx Pb > Zn > Cd$ (soil 1) and $Cu > Pb > Ni > Zn > Cd$ (soil 2).

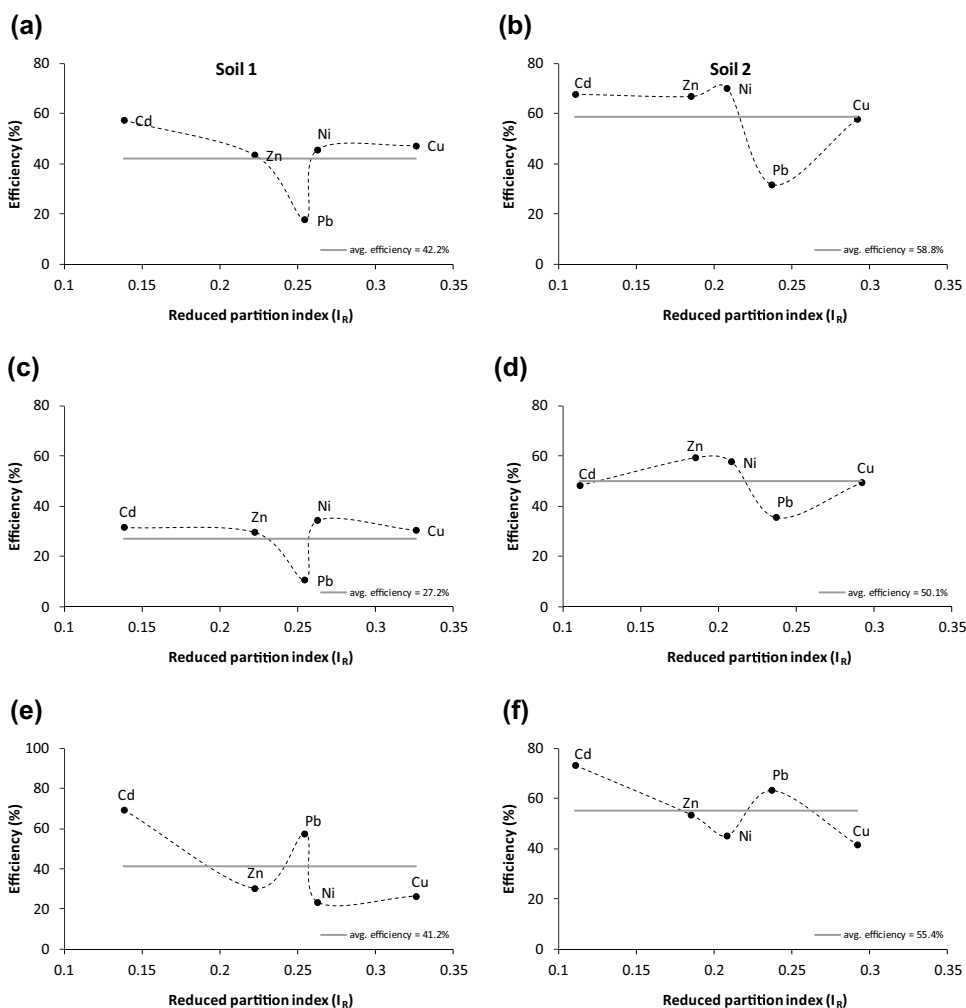
The effect of single soil washing with biosurfactants on HM removal

In Fig. 2, the efficiency of HM removal with SAP, TAN and RAM in relation to HM stability in soil (as I_R) is shown. Theoretically, an HM with a lower I_R should be removed more efficiently than one with a higher I_R . In the present study, this relationship was true for a given HM in different soils, but not for different HMs in the same soil. This suggests that removal of multi-HMs from a given soil was affected by factors other than just their stability (based on the I_R).

The results clearly indicate that the removal of HMs varied depending on the type of HM, its concentration in soil, and the class of biosurfactant used for soil washing. All HMs were more efficiently removed from soil 2, which had a lower total HM concentration and lower I_R , than from soil 1, irrespective of the kind of biosurfactant that was used.

Zn removal differed the most between soils, with 23.4%, 28.8% and 22.5% more removed from soil 2 than soil 1 with SAP, TAN and RAM, respectively. Regardless of the concentration of Pb in soil, SAP and TAN removed this HM less effectively than RAM. In more contaminated soil, SAP was the most effective for Cd, Ni and Cu removal, while TAN was most effective for removal of all HMs, except for Pb. In the soil with the lower HM concentrations, both plant biosurfactants also effectively removed Zn. In contrast, RAM effectively removed Cd and Pb but was less effective for Cu and Ni removal than TAN and SAP. RAM removed Zn from both soils with lower or similar efficiencies to SAP and TAN, respectively. The efficiency of Pb removal with RAM was 2.3–4.5 times higher than removal of this HM with SAP and 0.8–4.5 times higher than its removal with TAN. In terms of the efficiency of removal of individual HMs, the biosurfactants can be ranked in the following order: for Cd in both soils and Pb in soil 1, $RAM > SAP > TAN$; for Pb in soil 2, $RAM > TAN > SAP$; for Cu and Ni in both soils, $SAP > TAN > RAM$; for Zn in soil 1, $SAP > TAN \approx RAM$; and for Zn in soil 2, $SAP > TAN > RAM$.

Fig. 2 Efficiency of HM removal from soils with biosurfactants as a function of HM stability in soil (I_R): **a, b** SAP; **c, d** TA; **e, f** RAM



In the present study on soil contaminated with multi-HMs, RAM removed Cd and Pb more effectively than the other HMs. Anionic RAM with a negative charge (from a dissociated carboxyl group in micelles) can form ionic bonds with HMs, creating stabilizing forces in the RAM–HM complex that are stronger than those in the HM–soil complex (Açikel 2011). Wan et al. (2015) found that Pb can be complexed more readily with RAM than Cd.

Slizovskiy et al. (2011) found that the effectiveness of RAM in removal of HMs from soil decreased in the following order: Pb (68%) > Cu (56%) > Cd (43%) > Zn (39%). Juwarkar et al. (2008) used RAM synthesized from distillery wastewater by *P. aeruginosa* BS2 and found that the effectiveness of HM removal was as follows: Cd = Cr > Pb = Cu > Ni. This can be attributed to the fact that the stability constants of RAM complexes with Cu, Pb and Cd are higher than those of its complexes with Zn and Ni (Ochoa-Loza et al. 2001). Although RAM–Cu complexes have a high stability constant, Cu removal from both soils in the present study was much lower than Pb and Cd removal, which is in agreement with previous studies that have shown a high affinity of RAM for Pb and Cd. The relatively low Cu removal may be due to the presence of other HMs, particularly Pb, at higher total concentrations than Cu. The efficiency of Cu removal with RAM can be high when soil is not co-contaminated with other HMs. Venkatesh and Vedarman applied a 2% RAM solution to soil spiked with only Cu and were able to remove 74% of the HM, even though its initial concentration was nearly 5000 mg/kg (Venkatesh and Vedarman 2012).

SAP and TAN were less effective at removing Pb than RAM, but with regard to removing the other HMs, SAP and TAN were either more effective, or similar in effectiveness to RAM. The effectiveness of the plant biosurfactants differed more with soil 1 (more contaminated) than soil 2 (less contaminated). Overall, SAP was more effective than TAN for removal of Cd, Cu, Ni, Zn, but less effective for removal of Pb, especially in soil 2. TAN is phenolic organic acid with numerous hydroxyl groups associated with the aromatic ring, forming a trihydroxy-benzoic structure. With phenolic compounds like TAN, the presence of suitably oriented hydroxyl groups in the structure of the ligand can be essential for the formation of complexes between the phenolic groups and HMs (Karamać and Karamać 2009).

The ability of SAP to remove more HMs than TAN is related to the content of carboxylic groups in the two substances, with more than ten times more carboxylic groups in SAP than TAN (Gusiatin 2014). SAP can remove a large amount of Cd from soils because it contains an exchangeable secondary H⁺ cation that can be exchanged for HM cations (Hong et al. 2002). Thus, these H⁺ cations were easily exchanged with HMs that had a high share in the mobile fraction, i.e., Cd, Ni, Zn, which was especially

noticeable in soil 2. In soil 1, the share of Pb in the mobile fraction was the lowest, so SAP and TAN removed less of this HM than of the others. In addition, an excessive concentration SAP could have led to the formation of Pb precipitates and decreased the removal of this HM (Zhang et al. 2018).

Zhan et al. (2012) stated that SAP can effectively remove HMs from soil at concentrations far above its critical micelle concentration (CMC) of 0.1%. In the present study, at 3% SAP, the effectiveness of Cu, Cd, Pb, and Zn removal was 44%, 95%, 83%, and 20%, respectively. The effectiveness of Pb removal was higher in Zhan et al. (2012) than in the present study due to the lower Pb content (179.0 mg/kg) and to soil properties that improved soil washing performance in their study, i.e., a low CEC (5.7 cmol/kg) and a low organic matter content (1.3%). They reported that the mechanisms involved in HM removal with SAP were not only ion exchange, but also micelle formation and complexation of HMs with carboxyl groups (Zhan et al. 2012).

In the present study, all biosurfactants were used at concentrations above their CMC, which means that micelles were responsible for HM removal. When biosurfactant concentrations are above the CMC, the proposed mechanism of HM removal from soil includes several steps (Chen et al. 2008; Tang et al. 2017). First, under the conditions of reduced interfacial tension that are present above the CMC, biosurfactant molecules from dissociated micelles can readily adsorb at a soil–water interface. At this stage, the biosurfactant hydrophilic anions interact with cationic HMs, while the nonionic hydrophilic polar groups of the biosurfactants may react with soil constituents. As a result, a monolayer of biosurfactant coverage is achieved. The adsorbing biosurfactant molecules continually form ion pairs with HM ions in a head-to-head and tail-to-tail fashion, mainly by ion exchange of H⁺ cations between HMs in soil and biosurfactants and HM complexation with carboxyl and hydroxyl groups of biosurfactants. Because, the interfacial tension of biosurfactants is lowest at concentrations above CMC, the formation of HM-biosurfactant complexes is facilitated by reduced adhesion between HM and soil particles. Next, orientational rearrangement of biosurfactant molecules at the soil–water interface leads to self-assembly of HM-biosurfactant complexes by aggregation of hemimicelles on the top of the monolayer. Finally, micellar HM–biosurfactant complexes are desorbed into bulk solution (Chen et al. 2008). Under conditions of reduced biosurfactant surface tension, micellar HM–biosurfactant complexes are easily released into solution, and their re-adsorption in soil is prevented. In this study, although all the biosurfactants effectively reduced surface and interfacial tension (Table 2), their ability to remove individual HMs differed.

The effect of single washing with biosurfactants on HM distributions and their stability in soils

In the present study, the effectiveness of the biosurfactants for HM removal was analyzed based on the changes in the concentrations of HMs in the fractions. The fractions were grouped according to the BCR procedure, based on the availability of the HMs in that fraction: ready available (RA) comprises only the *F1* fraction; potentially labile (PL) consists of the *F2* and *F3* fractions, whose mobility can increase when soil properties (pH, redox) change; and non-available (NA) comprises only the *F4* fraction, in which HMs are strongly bound to the crystalline structures of minerals in soil matrices (Cao et al. 2018).

The tested biosurfactants removed HMs more effectively from the RA fraction than from the PL and NA fractions in both the soil with high HM concentrations and the soil with low HM concentrations. RAM were more effective than the other two washing agents for removing Cd from the RA and PL fractions in both soils. As a result, RAM removed Cd with the highest efficiency (Fig. 2). In contrast to washing with SAP and RAM, washing with TAN caused the Cd concentration in the PL fraction in washed soil to be higher than that in unwashed soil. This suggests that, during soil washing with TAN, Cd is partially redistributed from the *F1* to the *F2* fraction. Wan et al. found that a 1% RAM solution removed Cd from the RA fraction in soil co-contaminated with Pb and lindane with an efficiency of 75% (Wan et al. 2015). Hong et al. (2002) found that, in soils containing 701–1484 mg/kg of Cd, most of which was in the RA fraction, 3% SAP almost completely removed Cd from that fraction. Zhan et al. (2012) confirmed that SAP decreased Cd content in RA in soil below detection limits, whereas it decreased Cd content in the PL fraction more efficiently (> 80%) than in the present study (by 25–36%). In the present study, Cd was not found in the NA fraction in soils either before or after washing with biosurfactants (Fig. 3a, b). Although TAN had acidic pH, it removed Cd from the RA fraction less effectively than SAP, especially in soil 1. This can be attributed to the stronger affinity of TAN for other HMs (e.g., Pb), which were present at higher concentrations.

In soil 1, SAP and RAM removed Cu from the RA fraction more effectively than TAN, whereas in soil 2, SAP and TAN removed the most Cu from this fraction (117 and 119 mg/kg, respectively) (Fig. 3c, d). In both soils, SAP decreased the Cu concentration in the *F2* and *F3* fractions (i.e., the PL fraction) more than TAN and RAM did. The large amount of Cu removed from these two fractions with SAP was the reason why total Cu removal from both soils with SAP was so high. Gusiatin and Klimiuk (2012) reported that 3% SAP efficiently removed Cu from the *F1* and *F2* fractions, and even from the *F3* fraction in soils that

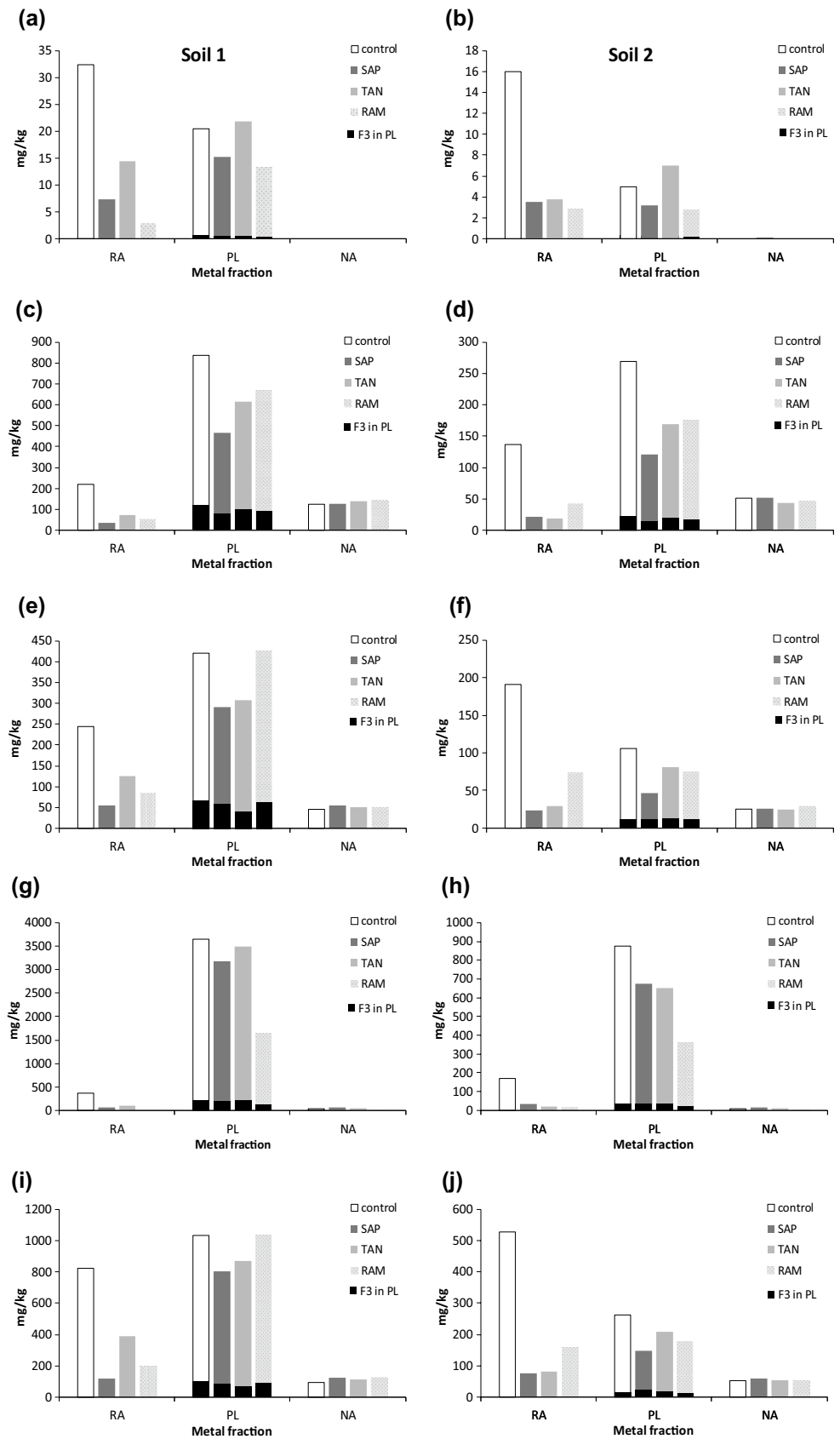
differed in texture (loamy sand, loam and silty clay). Gusiatin et al. (2014) observed that SAP and TAN removed Cu with similar efficiency from the *F1* fraction in industrial soil co-contaminated with Cu, Pb and Zn; however, SAP removed substantially more Cu from the *F2* fraction in this soil than TAN did.

SAP was the most efficient biosurfactant for removing Ni from the RA and PL fractions in both soils and, thus, had the highest efficiency of total Ni removal of the three biosurfactants (Fig. 2). In the soil with the higher total Ni content, RAM only removed Ni from the RA fraction, whereas in the soil with the lower total Ni content, RAM also partially removed Ni from the PL fraction. Of the three biosurfactants, TAN was the least efficient at removing Ni from the RA fraction in soil 1 and from the PL fraction in soil 2 (Fig. 3e, f).

It is worth mentioning that all three biosurfactants almost completely removed Pb from the RA fraction (Fig. 3g, h). However, the biosurfactants differed in their ability to remove Pb from the PL fraction. Soil washing with RAM decreased the Pb concentration in the PL fraction by 55% (soil 1) and 59% (soil 2). In contrast, SAP and TAN decreased the Pb concentration in the PL fraction in soil 1 by 13 and 5%, respectively, and they decreased the Pb concentration in this fraction in soil 2 by 23 and 26%, respectively. These decreases in Pb content in the PL fraction are due to removal of Pb from iron oxides and the results suggest that RAM more effectively releases Pb from these oxides than plant biosurfactants. Akintunde et al. (2015) confirmed that the Fe concentration in soil was decreased about 60% by RAM. Neilson et al. (2003) found that, although RAM did not effectively remove Pb from heavily contaminated (3780–23,900 mg/kg) aged soils, they were more effective than carboxymethyl-beta-cyclodextrin for removing Pb bound to amorphous iron oxides. In the present study, SAP showed little ability to remove Pb from the reducible fraction. These results are similar to those of previous studies. Deng et al. (2015) reported that carbonate-bound, organics-bound and sulfide-residual Pb could be effectively removed from soil by SAP. Gusiatin et al. (2014) found that less than 10% of Pb in the *F2* fraction was removed with a 3% SAP solution. Hong et al. (2002) also used SAP and observed that Pb was more efficiently removed from the exchangeable and carbonate fractions than from the Fe–Mn oxide and organic fractions. RAM and SAP may differ in their ability to remove Pb because the biosurfactants differ in their ability to form complexes with the HM, as the stability constant ($\log K$) of Pb–SAP chelate in aqueous solution is lower ($\log K[\text{Pb}] = 3.91$) than that of Pb–RAM chelate ($\log K[\text{Pb}] = 8.58$) (Ochoa-Loza et al. 2001; Tang et al. 2017).

In the present study, TAN removed Pb much less effectively than in Gusiatin et al. (2014). This difference may be due to the conditions in the previous study, in which the pH

Fig. 3 Concentrations of HMs in ready available (RA), potentially labile (PL) and non-available (NA) fractions in soils before and after single washing with biosurfactants: **a, b** Cd, **c, d** Cu, **e, f** Ni. RA indicates the exchangeable and acid soluble fraction, the PL fraction comprises the reducible (F2) and organic (F3) fractions (indicated in black), and NA indicates the residual fraction



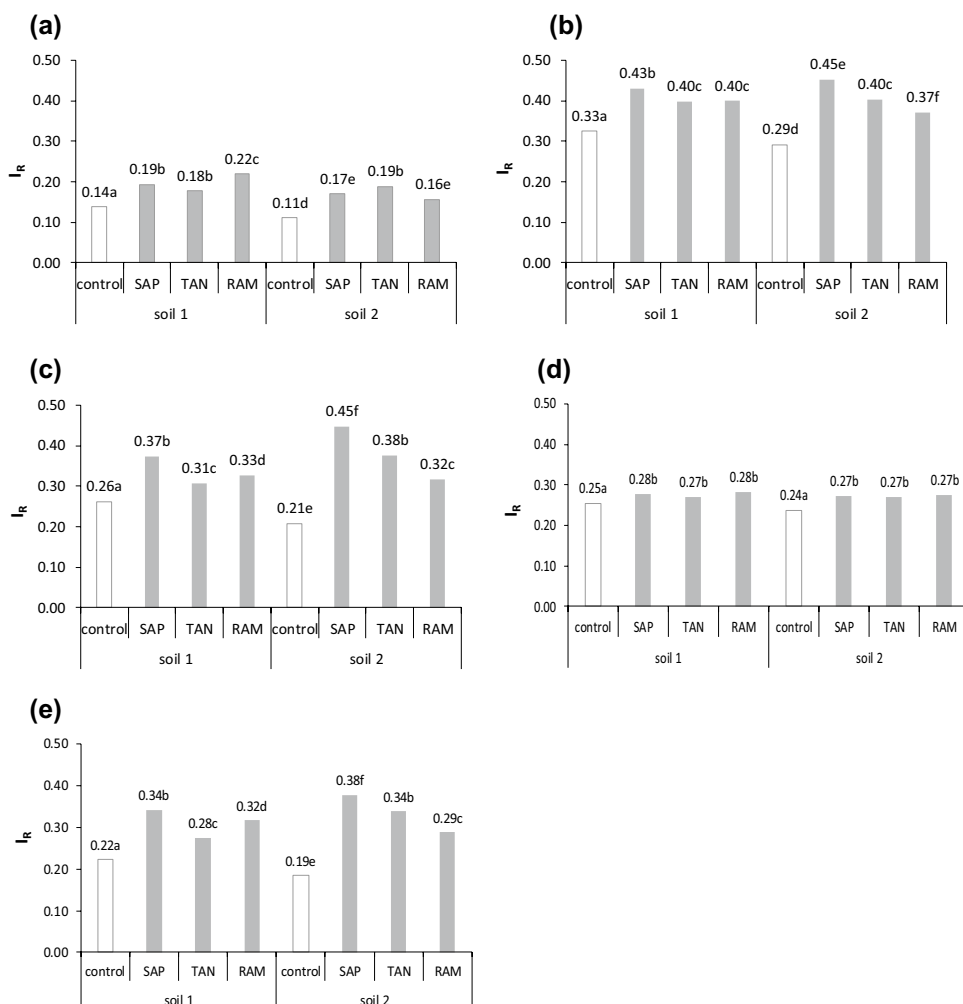
of the washing solution was lower than that in the present study (3.44 vs. 4.24), and the soil had a very low organic matter content (0.21%) and a low cation exchange capacity (15 cmol/kg). In the present study, although the total concentrations of Zn and Ni differed, they had similar distribution patterns (Fig. 1). For this reason, removal of these HMs from the RA and PL fractions proceeded in a similar fashion. Except for Cd, the investigated HMs could not be removed from the NA fraction during a single washing with the tested biosurfactants. In fact, the concentrations of Ni, Pb and Zn in the NA fraction increased slightly during washing with SAP, and the concentrations of Cu, Ni, Pb and Zn in this fraction increased during washing with TAN and RAM.

As shown in Fig. 4, soil washing with SAP, TAN and RAM significantly increased the stability of all the HMs in the soils ($p < 0.05$). This increase was due to effective HM removal from the RA fraction, partial removal from the F2 fraction (part of the PL fraction), minimal changes in HM concentration in the F3 fraction, and some increase in HM concentration in the F4 fraction, which is the most important with regard to the stability of HMs. The stability of most of

the HMs increased more in soil 2 than in soil 1, which had higher total concentrations of the HMs. In both soils, the increase in Cu, Ni and Zn stability was greater than that of the other HMs, and SAP increased the stability of Cu, Ni and Zn more than TAN and RAM did.

Hierarchical cluster analysis also indicated that SAP, TAN and RAM affected HM stability (Fig. 5). Dendrograms were created on the basis of the I_R of the HMs, and these dendrograms differed between washed and unwashed soils. In unwashed soil 1, the HMs were grouped into one cluster (Pb and Ni) and three singletons (Zn, Cu and Cd). The smaller distance between the members of the cluster reflects the fact that, although the fractionation of Pb and Ni differed somewhat, they had similar stability. After soil 1 was washed with biosurfactants, Zn, Ni and Pb showed the greatest changes in stability. After washing this soil with SAP or RAM, Zn and Ni were most similar in terms of stability; after washing this soil with TAN, Zn and Pb were most similar (Fig. 5a). In unwashed soil 2, in contrast, Zn and Ni were grouped into a cluster, and Pb, Cu and Cd were in singletons. After washing soil 2 with SAP or TAN, Ni and

Fig. 4 The effect of single soil washing with SAP, TAN and RAM on HM stability (as I_R): **a** Cd, **b** Cu, **c** Ni, **d** Pb, **e** Zn. Values of I_R followed by the same letters do not differ significantly at $p < 0.05$



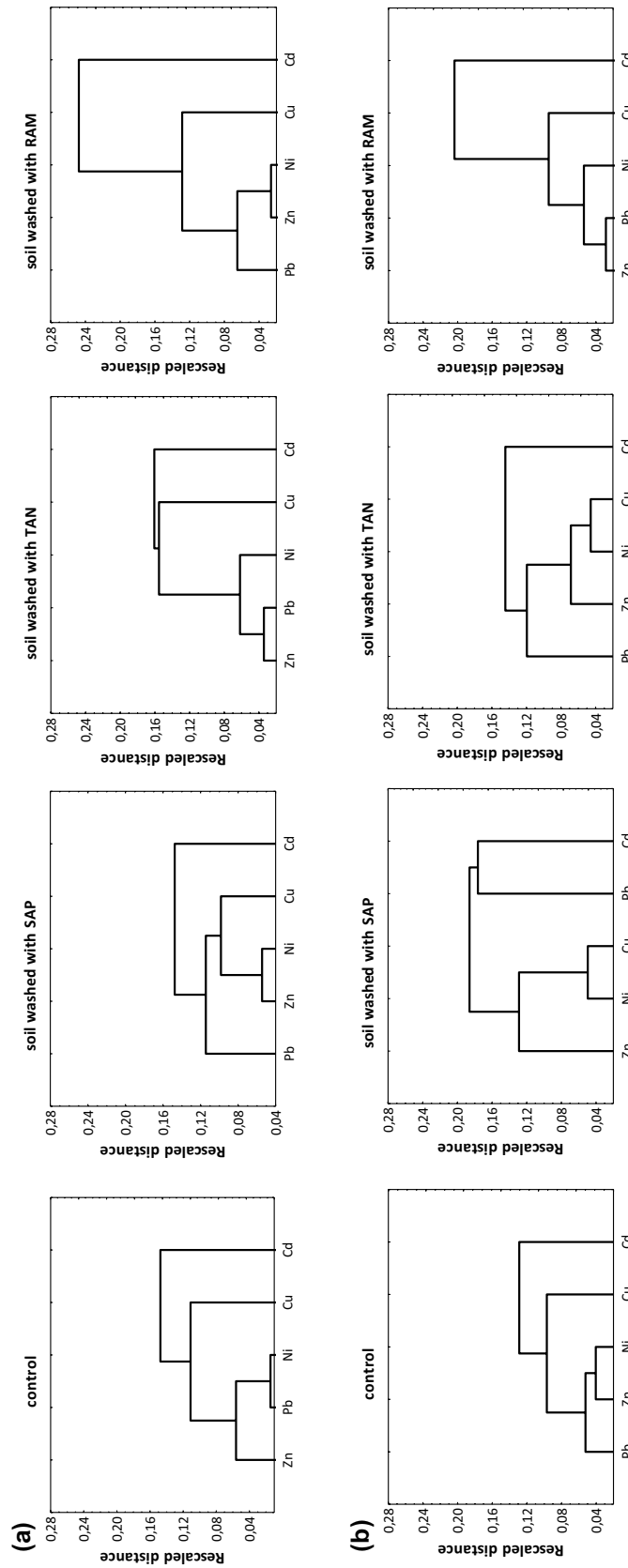


Fig. 5 Dendrograms from hierarchical cluster analysis of HM stability (as f_R) in unwashed soils and soils washed with biosurfactants: **a** soil 1, **b** soil 2

Cu were grouped into a cluster, and after washing this soil with RAM, Zn and Pb were in a cluster (Fig. 5b).

The effect of sequential soil washing with biosurfactants on HM removal

The results of HM removal from soils by sequential soil washing with biosurfactants belonging to the same class or different classes are presented in Fig. 6. With all the combinations of biosurfactants used, sequential soil washing was more effective for removal of all HMs than single soil washing (Fig. 2). This is in agreement with previous studies (Wei et al. 2016; Kulikowska et al. 2015; Gusiatin and Radziemska 2018).

With regard to most HMs (i.e., Zn, Ni, Cu), sequential soil washing with plant biosurfactants (SAP-SAP or TAN-TAN) removed more HMs than sequential soil washing with microbial biosurfactants (RAM-RAM). In contrast, Pb removal with RAM-RAM washing was much more effective than with SAP-SAP or TAN-TAN washing. RAM-RAM washing was also more effective than TAN-TAN washing for removing Cd from soil 1 and soil 2.

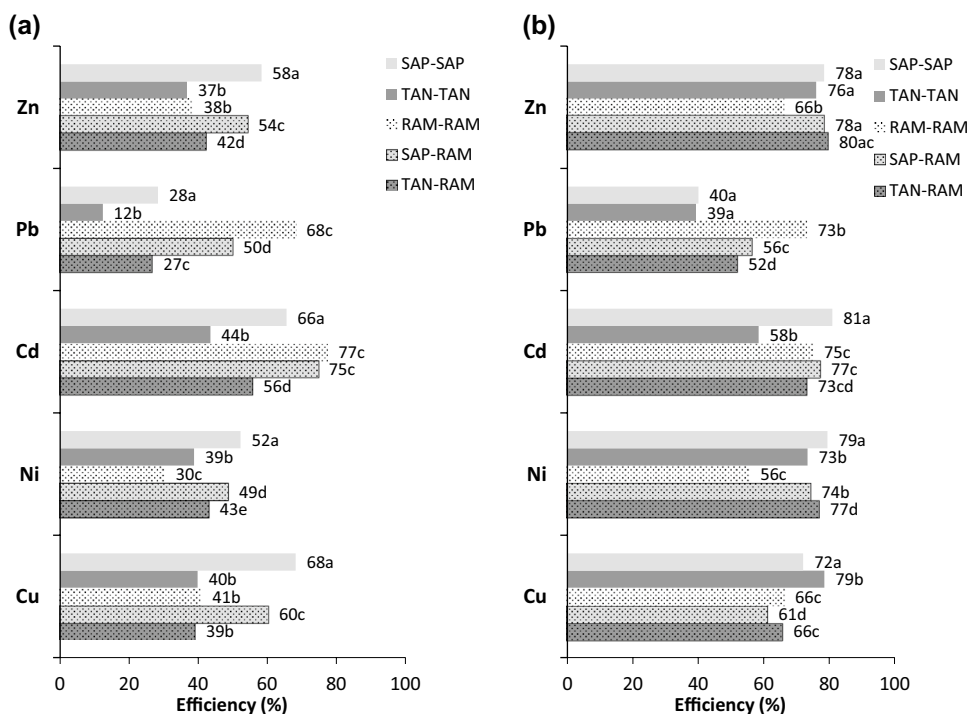
In the present study, sequential soil washing with different classes of biosurfactants was beneficial for simultaneous removal of multi-HMs. With the TAN-RAM sequence, the efficiency of Ni, Cd, Pb and Zn removal from both soils was higher than the efficiency of their removal with TAN-TAN. For removal of Cu, Zn and Ni, SAP-RAM washing had slightly lower or similar efficiency to washing with SAP only. However, for removal of Pb from both soils and Cd

from soil 1, SAP-RAM was more efficient than SAP-SAP. Sequential soil washing with SAP-RAM and TAN-RAM removed more Zn and Ni than sequential washing with RAM-RAM.

Sequential soil washing can first remove the more easily extracted HM fractions (e.g., exchangeable) and then remove the more difficult-to-extract fractions (e.g., carbonate and reducible) (Gusiatin and Klimiuk 2012). In the case of soil contaminated with multi-HMs, the HMs are not always completely removed from the exchangeable fraction after the first washing because the HMs compete for the active sites on the biosurfactant. This competition limits the number of active sites on the surface of the biosurfactant that are available for simultaneous removal of HMs. Moreover, after each washing cycle, a new equilibrium distribution of contaminants is established (Ye et al. 2014). Therefore, adding fresh biosurfactant solution in successive washings provides new active sites for HM complexation and partially can enable removal of HMs that are in the more difficult-to-remove fractions.

Kulikowska et al. (2015) noticed that, even with three-step soil washing with humic substances, one class of extractant may not be sufficient for removing HMs from soils heavily contaminated with multi-HMs. Thus, as with other washing agents, sequential extraction using extractants other than humic substances may be needed to remove multi-HMs from soil. Sequential soil washing with the use of different biosurfactants in each washing step can increase the efficiency of removal of different HMs due to complementary HM complexation caused by differences in the affinity of HMs for a given biosurfactant. For example, Beiyuan et al.

Fig. 6 HM removal efficiencies after sequential soil washing with different combinations of biosurfactants: **a** soil 1, **b** soil 2. For a given HM, efficiency values followed by the same letters do not differ significantly at $p < 0.05$



(2018) observed that, under various soil washing schemes, the complementary action of EDTA and EDDS led to better Cu and Pb removal with a mixture of these washing agents than with EDDS alone.

Conclusions

Application of SAP, TAN or RAM gave better results for treatment of soil that was less contaminated with HMs and aged for a shorter period of time than for treatment of soil that was more contaminated with these metals and aged for a longer time. When an individual HM had a lower stability (as I_R) in one soil than in the other, more of that HM was removed from the soil in which the I_R was lower. However, when comparing different HMs in the same soil, this association between I_R and HM removal was not always observed. This suggests that the affinity of a given biosurfactant for a particular HM was more important than the stability of that HM. Regardless of the soil properties, soil age and level of soil contamination with HMs, SAP was most effective for Cu, Ni and Zn removal, whereas RAM was most effective for Cd and Pb removal. TAN was less effective for Cu, Ni and Zn removal than SAP, but more effective than RAM. All biosurfactants effectively removed the HMs from the ready available fraction and partially removed these metals from the potentially labile fraction (including the mainly reducible fraction). Although SAP, TAN and RAM did not completely remove the HMs, they changed their distribution and substantially increased their stability in soil.

The results of the present study suggest that, for treatment of multi-HM-contaminated soils, and especially those co-contaminated with Pb, sequential soil washing with plant biosurfactants and then with microbial rhamnolipids could remove metals more efficiently than a single washing or sequential washing employing the same class of biosurfactant in each step.

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