

Can deep eutectic solvents be used to mobilise copper from tailings while providing a pathway towards rehabilitation?

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Abstract

*The use of deep eutectic solvents (DESs) has been proposed as a novel technique for environmentally benign mineral processing, with utility in the reprocessing of legacy tailings in a process known as solvometallurgy. These solvents can leach a wide range of metals while having many advantages, such as low volatility, high target metal selectivity, lower production costs and potentially low ecotoxicity. We aimed to determine the capacity of different DESs to mobilise copper (Cu) and other metals from Cu mine tailings. They were: DES 1 (oxaline) – choline chloride + oxalic acid; DES 2 (ethaline) – choline chloride + ethylene glycol; DES 3 – betaine + acetic acid; and DES 4 – betaine + acetic acid + phosphoric acid. In a follow-up experiment, *Plantago lanceolata* seedlings were transplanted to the tailings after DES application in order to determine phytotoxic responses. Our results showed that Cu extraction from tailings increases with DES concentration and pointed to DES 3 and 4 (betaine-based) as more efficient in Cu leaching, although oxaline was more capable of enhancing nutrient availability. Almost all plants died 24 hours after transplanting to DES-treated tailings, except the ones treated with oxaline, which is possibly linked to the remarkable macronutrient mobilisation – an element known to alleviate Cu phytotoxicity. However, only the diluted oxaline (1:128, DES:water) was able to improve plant growth in tailings as it mobilised more nutrients, leading to greater plant biomass and chlorophyll content. Thus it is clear that hazardous effects will depend on the DES formulation, concentration and exposure route, which may promote extreme phytotoxicity by enhancing metal availability in mine tailings. Yet diluted oxaline showed promising beneficial effects in plant health and growth, indicating that some diluted DESs, at concentrations anticipated after application and leaching, may have a role in promoting land rehabilitation.*

Keywords: DES, ecotoxicity, heavy metals, solvometallurgy, phytotoxicity, ecosystem reconstruction, solvent leaching, tailings reprocessing, mine closure

1 Introduction

The processing of mineral ores produces mineral ore concentrate of economic value and a residue considered a valueless waste. The waste byproduct is known as tailings. This is often a suspended slurry comprising variable quantities of non-target metals, which are stored in earthen dams known as tailings storage facilities (TSFs) (Adiansyah et al. 2015; Tibbett 2024). The volume of tailings generated can be almost the same amount as that of the target raw materials processed. For example, a mine that produces 200,000 tonnes of Cu ore material daily might generate the same volume in tailings (Adiansyah et al. 2015). Mine tailings can pose environmental and human health risks due to its extremely high metal content, and its potential to disperse and leach into natural ecosystems, contaminating air, land and water (Degani et al. 2022; Ngole-Jeme & Fantke 2017; Tibbett 2024; Xu et al. 2019). However, mine tailings may also be a secondary source of metals for industrial purposes, which can be recovered by processes known as hydrometallurgy and

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solvometallurgy (Binnemans & Jones 2017). Thus the valorisation of waste or byproducts for the recovery of metals is becoming more relevant and necessary from environmental and economical perspectives (Gaustad et al. 2021).

In the search for new low-energy and more ecologically compatible metallurgical processes, the extraction of metals from mine tailings has emerged as a potential alternative for metal recovery and further removal of metals from the environment (Almeida et al. 2020; Jenkin et al. 2024). Metal removal from tailings may render it less toxic to biota, enabling the establishment of functioning flora and fauna, as this waste material often contains elements and physicochemical attributes entirely different from soils developed from natural pedogenic processes (Tibbett 2024). However, the extraction of metals from low-grade ores can be challenging as they include a variety of metallic sources such as silicates, sulphides, carbonates, phosphates and oxides (Rodriguez et al. 2019a).

A relatively new alternative for efficient metal extraction has been the use of ionic solvents, such as ionic liquids (ILs) and deep eutectic solvents (DESs) (Arrachart et al. 2021). DESs are a form of ionic liquid but are mixtures of compounds that act as hydrogen bond acceptors (HBA), such as choline chloride (ChCl), with hydrogen bond donors (HBD), such as urea or carboxylic acids (Abbott et al. 2003; Abbott et al. 2015). DESs are considered cheaper to produce and can be prepared from natural sources, which tends to make them more biodegradable and generally safer (Arrachart et al. 2021; Liu et al. 2024). These solvents present chemical and physical properties distinct from those of their separate components. For instance, the melting point for a DES comprising ChCl and urea (1:2) is 12°C, while separately, melting points for these compounds are over 100°C (Martinez et al. 2022). Out of the many possible variations of HBA and HBD, the combination of ChCl and carboxylic acids is one of the most used families of DESs (Rodriguez et al. 2019b). Since their introduction in 2001, DESs have been applied to different research fields such as analytical chemistry, organic synthesis, biotechnology and biomass processing (Martinez et al. 2022). Efficient metal leaching by different formulations of DESs has also been demonstrated in several metallic sources, and in the solubilisation of various elements such as As, Au, Co, Cu, Fe, Mn, Pb, Zn, Te and W (Abbott et al. 2015; Almeida et al. 2020; Jenkin et al. 2016; Pateli et al. 2020; Rodriguez et al. 2019a; Rodriguez et al. 2020). DESs are regarded as environmentally safe, biodegradable and chemically stable, and are already produced in large quantities at comparable costs to conventional solvents; attracting attention from academia and the industry alike (Jenkin et al. 2016; Khan et al. 2023; Khandelwal et al. 2016; Li 2022; Martinez et al. 2022).

Despite being largely considered as 'eco-friendly' or environmentally benign due to their composition of salt and organic compounds, DESs have seldom been assessed in terms of ecotoxicity – with most findings being reported for microorganisms (Ferreira et al. 2022). Overall, the current literature suggests that DESs are as safe as initially expected, depending on which organisms are being exposed to a particular DES formulation (Hayyan et al. 2013). The assumption of its low toxicity is often related to its individual components, however, these may interact, depending on their nature and proportion (Kudlak et al. 2015; Macário et al. 2018), and it has been suggested that the behaviour of DESs should be treated as closer to that of a single compound instead of a mixture (de Moraes et al. 2015). For example, DES cytotoxicity was observed in brine shrimp larvae at much higher levels than that of its individual components, indicating a synergistic toxic effect from the mixture (Hayyan et al. 2013), and similar effects were seen in mammalian cells exposed to a DES comprising ChCl and oxalic acid (Radošević et al. 2015). Other formulations can also be hazardous, such as observed in DESs based on ethylene glycol or 1-propanol as the HBD, which were toxic to the marine bacteria *Aliivibrio fischeri*, depending on concentrations and exposure time (Macário et al. 2018).

As for the inherent phytotoxic effects of DESs, little research has been done (Ferreira et al. 2022), with most trials running for short time periods (e.g. seven days) and mainly assessing stress-related enzymes, chlorophyll contents, germination and the initial seedling development of model ecotoxicological species (e.g. wheat, cress, garlic), all to varying degrees of toxicity responses and always under direct exposure, e.g. using liquid medium or hydroponics (Radošević et al. 2015; Rodrigues et al. 2021; Samori et al. 2019; Wen et al. 2015). However, DES phytotoxicity potential has yet to be explored in solid matrices, substrates and soils that largely sustain plant growth in natural environments, especially because these solvents might

mobilise nutrients (e.g. P, Mg, Ca, Cu, Zn) as well as toxic metal(loid)s (As, Cd, Pb), generating effects that can range from beneficial to extremely toxic.

Considering the tailor-made nature of DES production, many combinations may be generated, making toxicity assessments quite challenging – especially since ecotoxicological tests have been mostly performed on microorganisms and cell cultures (Chen & Mu 2021; Ferreira et al. 2022). Indeed, results are quite variable and, despite the aforementioned results, in some cases almost no effects are observed in microorganisms exposed to multiple DESs formulations (Cardellini et al. 2015; Hayyan et al. 2013).

Despite some indications of the inherent toxic effects from direct exposure to DESs in microorganisms, mammalian cells and plants (Ferreira et al. 2022; Hayyan et al. 2015; Khan et al. 2023), little is known about the potential risk these solvents pose to biota when applied in soils or metal-rich matrices such as tailings. To the best of our knowledge, phytotoxic effects from mine tailings after DES applications have yet to be addressed. Thus we set out to: (1) determine the potential of different DESs in Cu recovery/leaching from tailings originated at Philex Mining Corporation's Padcal copper-gold mine based in the Philippines; (2) determine other metals and plant nutrients possibly mobilised by DES application; and (3) assess plant growth in the leached tailings to verify the prospects for future rehabilitation. We hypothesised that DESs are efficient in leaching Cu and other elements from tailings but are possibly phytotoxic at high concentrations due to excess metal availability, depending on the DES formulation and dilution.

2 Materials and methods

To address the potential of Cu extraction from tailings and the possible toxicity effects to plant development, we screened different DES solvents and their Cu leaching potential, followed by two phytotoxicity trials. Tailings were obtained from TSF1 at Philex's Padcal mine. Metal concentrations were determined by inductively coupled plasma optical emission spectroscopy (using a Perkin Elmer Avio 500), after digestion with 50% HNO₃ (2 hours, DigiPrep block digestion system) and filtration; standards and quality control solutions were made from certified stocks (NIST) (Table 1).

Table 1 Total metal concentrations in tailings obtained from TSF1 at the Philex mine (Philippines) after nitric acid digestion. Values are averages ($n = 3$) $\pm$ standard error. Average values in US soils are shown for comparison (Shacklette & Boerngen 1984)

Element	Total concentration (mg kg ⁻¹)	Limit of detection (mg kg ⁻¹)	Average US soils (mg kg ⁻¹)
Cu	1,440 $\pm$ 56	0.02	17
Al	18,565 $\pm$ 903	0.20	47,000
Ca	4,676 $\pm$ 231	0.45	9,200
Fe	17,192 $\pm$ 823	0.10	18,000
K	5,551 $\pm$ 187	0.50	15,000
Mg	18,292 $\pm$ 167	0.10	4,400
Mn	355 $\pm$ 8	0.01	330
P	633 $\pm$ 17	0.10	260
Zn	27 $\pm$ 1	0.03	48

2.1 DES preparation and screening

Ten solvents with different combinations were prepared on a hot magnetic stirrer (50°C): DES 1 (oxaline) – choline chloride (1 M) + oxalic acid (1 M); DES 2 (ethaline) – choline chloride (1 M) + ethylene glycol (2 M); DES 3 – betaine (1 M) + glacial acetic acid (4 M); DES 4 – betaine (1 M) + glacial acetic acid (4 M) + 1% (weight) phosphoric acid; and DES 5 – calcium chloride (1 M) + ethylene glycol (1 M). DESs 1 and 2 are classified as type III; DESs 3 and 4 as type III or type V (Abranches & Coutinho 2022); and DES 5 as type IV (Smith et al. 2014). Another five DESs were then prepared using the same compounds, but with H₂O₂ (0.6 mL at 35%) added to further enhance Cu extraction, and these were named as DES 1A, 2A, 3A, 4A and 5A.

The potential of these 10 DESs to extract Cu was tested by adding 10 mL of solvent into 5 g of tailings, under agitation for seven days (500 rpm, 30°C), in duplicates. The concentrations of Cu and other metals in the extraction solutions were analysed by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific iCAP Q, single quadrupole ICP-MS with a Cetac 560 autosampler), and standard results were presented. The most efficient DESs in Cu removal were DESs 1, 1A and 5A; leaching between 40–50 mg L⁻¹ Cu (approximately 5–7% of total Cu in the sample), with a pattern trend observed for Fe (Figures 1a, 1b). Based on these results, DESs 1 to 4 were selected for the following ecotoxicological assays. Despite the high Cu mobilisation by DESs with H₂O₂ these were shown to result in extreme phytotoxicity during a short germination trial (data not shown). Therefore, we decided to not move forward with these solvents.

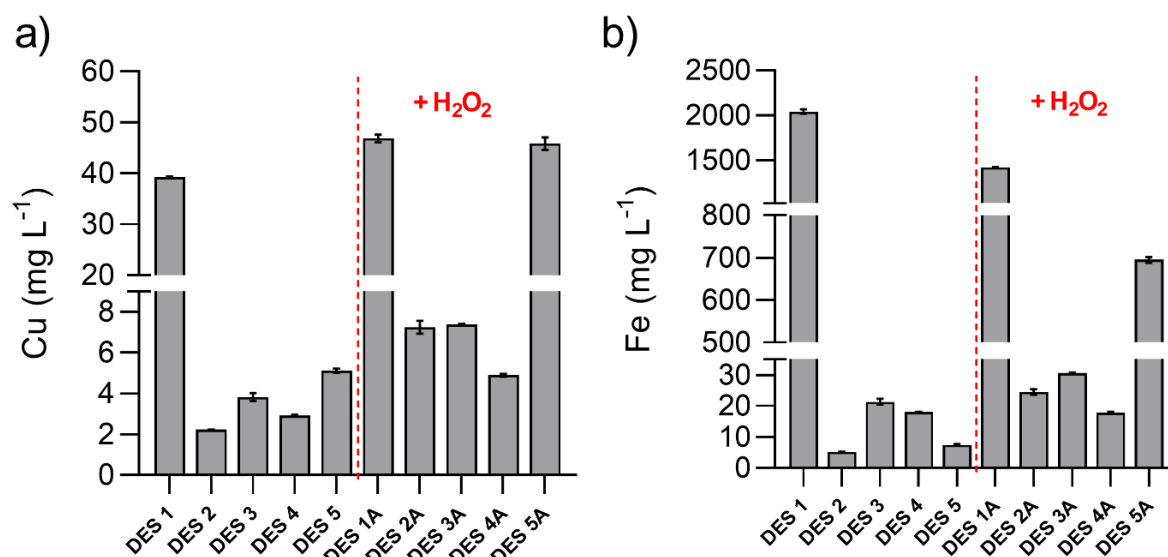


Figure 1 Cu (a) and Fe (b) concentrations (mg L⁻¹) in extraction solutions from tailings treated with DESs with and without H₂O₂ addition (n = 2, standard error). Values are averages and standard errors from duplicates. DES 1 – choline chloride + oxalic acid; DES 2 – choline chloride + ethylene glycol; DES 3 – betaine + acetic acid; DES 4 – betaine + acetic acid + phosphoric acid; DES 5 – calcium chloride + ethylene glycol; DES 1A-5A – same formulations but with H₂O₂ addition

2.2 Tailings treatment and phytotoxicity assay

After a preliminary germination screening, DESs with H₂O₂ displayed extreme phytotoxicity and therefore were removed from further experiments. The first four DESs described previously (1, 2, 3 and 4) were then selected for the phytotoxicity assay and applied at different dilution rates with deionised water, at 1 (pure), 1/2, 1/4, 1/16, 1/32 and 1/128 ratios, equivalent to 1,000, 500, 250, 62.5, 31.25 and 7.8 mM, respectively. Plastic pots containing 200 g of fresh tailings received 20 mL of DESs 1, 2, 3 and 4 at different dilutions, while control pots received only deionised water (n = 4). Pots were left to stand for three days at room temperature before being flushed twice with deionised water, and 50 mL of each leachate solutions was collected for metal determination via ICP-MS.

After one week, six-month-old ribwort (*Plantago lanceolata*) seedlings were transplanted to each pot. Plants were grown for 22 days, with photographs being taken of each seedling at day 1 and then at day 22 to determine the amount of green cover (GC, in cm²), using the Canapeo App (www.canapeoapp.com). Photographs of shoots were taken from above the pot, at the same height for all plants and all covering the same area (623.7 cm², A4 sheet of paper). The percentage of GC was then converted to cm² by the equation $GC_{area} = (623.7/100) \times GC\%$. The GC was selected as a toxicity endpoint instead of the dry biomass, and this was due to all seedlings having similar sizes but clearly displaying various degrees of toxicity, which were better captured by determining the area of non-necrotic (green) foliar tissues.

2.3 Diluted oxaline and nutrient mobilisation

Based on the results from DES 1 (choline chloride + oxalic acid) at the highest dilution of 1/128, which appeared to be less toxic in comparison to others, we decided to test its effects on plant biomass, chlorophyll content and nutrient mobilisation from the tailings. Pots with 200 g of tailings received either 20 mL of diluted DES 1 or deionised water (control) and were left to react for 10 days before being flushed with 50 mL of deionised water (n = 4). These leachates were collected for Cu, Mg and P determination via ICP-MS. On the following day, 10 seeds of *P. lanceolata* were sown into each pot, and transparent plastic covers with a small opening were placed on top of each treatment in order to avoid effects from DES volatilisation on control plants. Seeds germinated in all pots and were thinned down to only one seedling per pot and left to grow for 10 weeks. During the last week, chlorophyll contents (SPAD-units) were estimated by a chlorophyll meter (Minolta SPAD 502 Plus). At harvest, shoots were separated from roots and dried at 65°C for one week before dry weight was assessed. Roots were washed under tap water and stored in 50% ethanol. For morphological parameters (area, length and diameter), roots were scanned and analysed using WinRhizo[®] software.

2.4 Statistical analyses

A two-way analysis of variance (two-way ANOVA) was carried out for the GC and leachate element results using two factors, solvent type and concentration, and Tukey's test was used to discriminate the significant differences ($p < 0.05$). When necessary to attain normality, data was transformed by $\log(x)$ or $\log(x+1)$. Linear regressions were carried out for each DES to assess the effects of increasing solvent concentrations in mobilising metals in the tailings (leachate concentrations). To verify metal mobilisation patterns among the different DESs, a principal component analysis (PCA) was carried out on the average results obtained with the least toxic treatment (1/128 dilution), including 16 variables (metals in leachates) but further reduced to 10 variables by removing highly correlated variables and improving variability explanation. Prior to analyses, data was transformed by $\log(x+1)$ and z-score normalised (Legendre & Legendre 2012). In the second experiment, one-way ANOVA was carried out to determine differences ($p < 0.05$) between results from the control and the diluted DES 1 (1/128) samples. All analyses were carried out in R software (ggplot2, ggthemes, ExpDes.pt, Rcmdr), except for the PCA, which was performed using PRIMER-e v7.

3 Results

Two days after DES application in the tailings, 50 mL of each leached solution was collected from the bottom of the pots after flushing with deionised water. A linear regression was calculated for the Cu concentrations detected in these leachates in relation to the solvent concentration, including pure deionised water (Figure 2). All regressions were significant ($p < 0.01$) and Cu extraction increased with DES concentrations, with the lowest R^2 found in the model with DES 1 (0.46, Figure 2a) and the highest found for DES 3 (0.94, Figure 2c) and DES 4 (0.91, Figure 2d), indicating that Cu removal is better explained by the gradient concentration of these latter solvents. The maximum Cu extracted by pure DES solutions (no dilution) was in the order of DES 4 (112 mg L^{-1}) > DES 3 (99 mg L^{-1}) > DES 1 (59 mg L^{-1}) > DES 2 (6.3 mg L^{-1}).

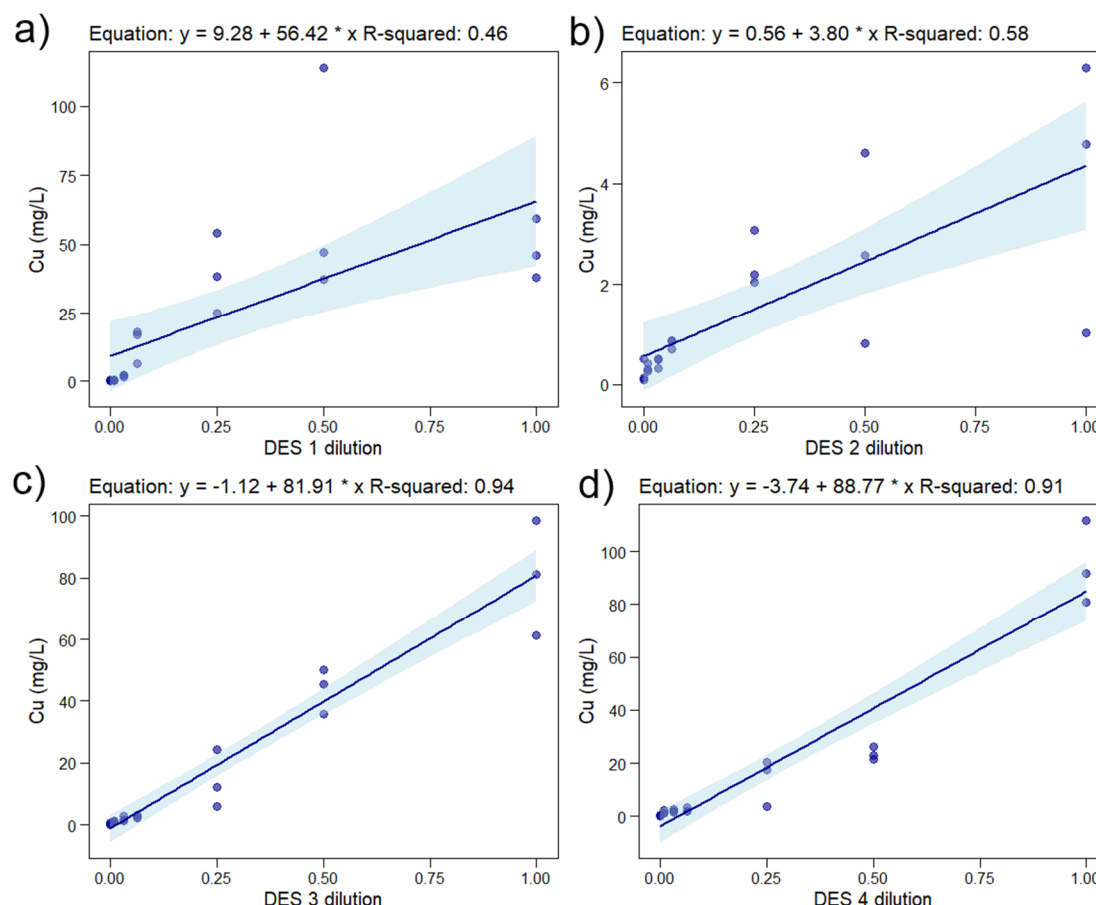


Figure 2 Linear regressions for Cu concentrations in leached solutions ($n = 3$) from tailings treated with DESs at different dilution rates, from 0 (control with water) to 1 (pure DES). All regressions were significant ($p < 0.01$). (a) DES 1: choline chloride + oxalic acid; (b) DES 2: choline chloride + ethylene glycol; (c) DES 3: betaine + acetic acid; (d) DES 4: betaine + acetic acid + phosphoric acid

On average, undiluted DESs 3 and 4 were responsible for the highest Cu removal from tailings (Figure 3a). However, DES 1 showed the highest removal capacity for other elements, especially Mg, Fe, K and V (Figure 3), while DES 2 had the lowest overall metal extraction potential, with leachate concentrations being markedly lower than the other DESs, except for K (Figure 3d).

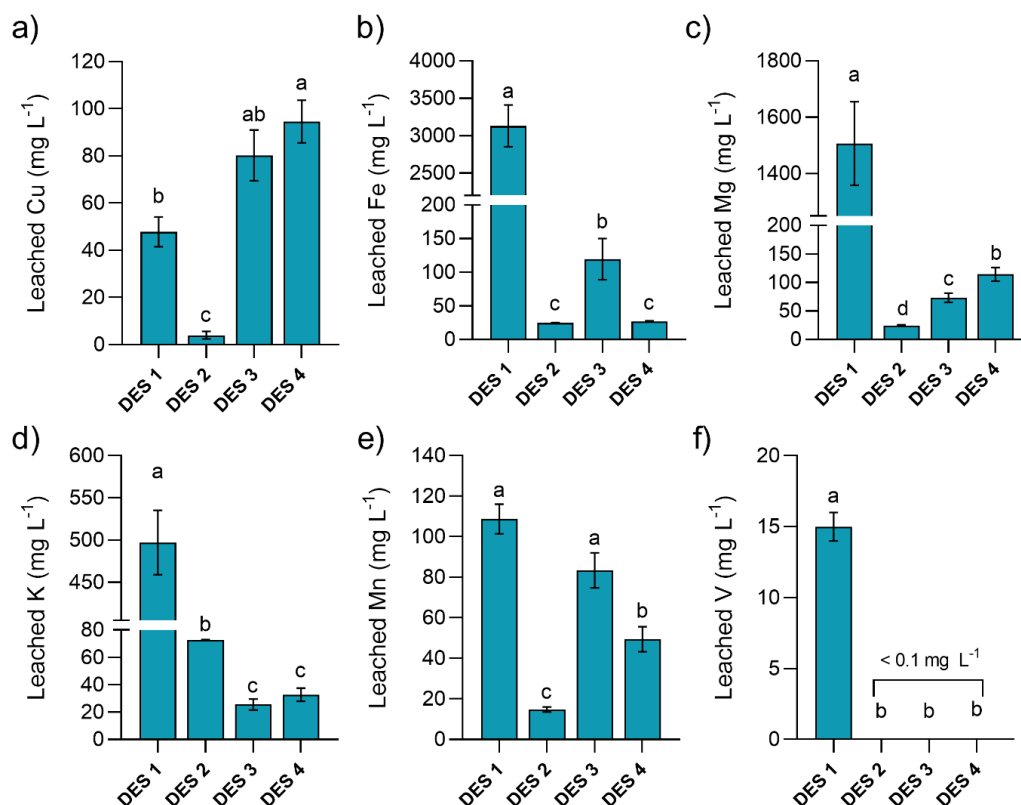


Figure 3 Cu (a), Fe (b), Mg (c), K (d), Mn (e) and V (f) concentrations in leachates from tailings treated with four different undiluted DESs (n = 3, ± standard error). Different letters represent significant differences (ANOVA, Tukey's test, p < 0.05). DES 1: choline chloride + oxalic acid; DES 2: choline chloride + ethylene glycol; DES 3: betaine + acetic acid; DES 4: betaine + acetic acid + phosphoric acid

The most diluted DES also presented divergent metal mobilisation capabilities. Diluted DESs 3 and 4 also led to higher Cu concentration in leachates, albeit almost 100 times lower than the pure treatments, at 1–2 mg L⁻¹ Cu (Figure 4a). Similarly, diluted DES 1 also mobilised more Mg, Mn and K than the former two solvents, however, diluted DES 2 also mobilised considerably more of these elements; all of which were much inferior to their concentrated treatments (Figures 4c, 4d, 4e).

The PCA of leachate concentrations for the diluted solvents shows a clear separation of DES 1 and 2 from DES 3 and 4, and from control (Figure 5), explaining 86.1% of the variability. We have excluded V from the PCA, as it had correlated strongly with Cu without adding much to the explained variability. Other strong correlations are seen among Ca, Mg, K and Mn concentrations, all of which are associated with DES 1 and DES 2, while the other two solvents are correlated with higher Cu and Zn. This shows that even at a high dilution rate, these solvents still present considerable differences between one another in terms of metal mobilisation capacities and compared to pure water.

Figure 6 shows how GC is greatly reduced under DESs treatments, even after 24 hours of exposure, in which the diluted DES1 (1/128) was the only solvent to not significantly reduce GC. Based on these results and how diluted DES1 is associated with higher nutrient availability (Figure 5), another small experiment was carried out to assess the potential of this solvent in supporting plant growth in metal contaminated tailings. This treatment was compared against a control with deionised water. After 10 weeks of growth the dry weight of *P. lanceolata* seedlings was around threefold higher than those in control conditions (Figure 7a), while chlorophyll contents presented a 3.4-fold increase (Figure 7c). Analysis from the leachates collected before sowing also showed higher Cu, P and Mg mobilisation by diluted DES 1 compared to deionised water (Figures 7d, 7e, 7f), with almost five times higher P (0.98 mg L⁻¹) and Mg (44.8 mg L⁻¹) concentrations. Similar patterns were also observed for K and Ca in leachates.

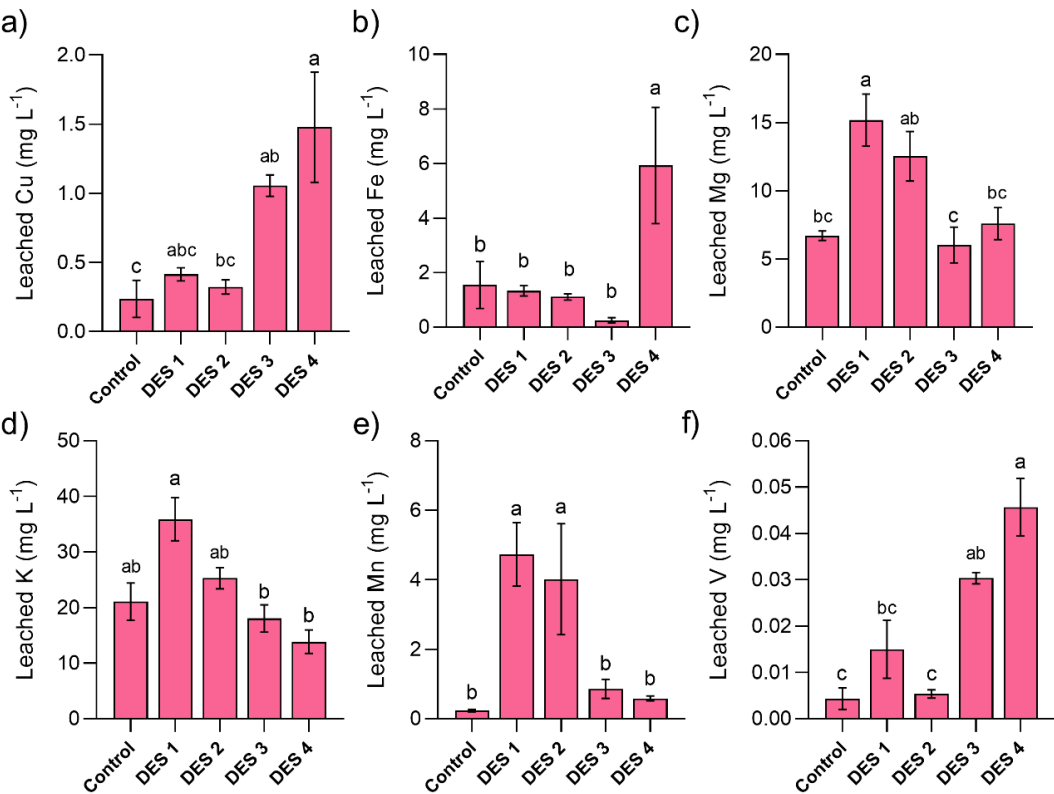


Figure 4 Concentrations of Cu (a), Fe (b), Mg (c), K (d), Mn (e) and V (f) in leachates from tailings treated with DESs at dilutions of 1/128 (n = 3, ± standard error). Different letters represent significant differences (ANOVA, Tukey's test, p < 0.05). DES 1: choline chloride + oxalic acid; DES 2: choline chloride + ethylene glycol; DES 3: betaine + acetic acid; DES 4: betaine + acetic acid + phosphoric acid

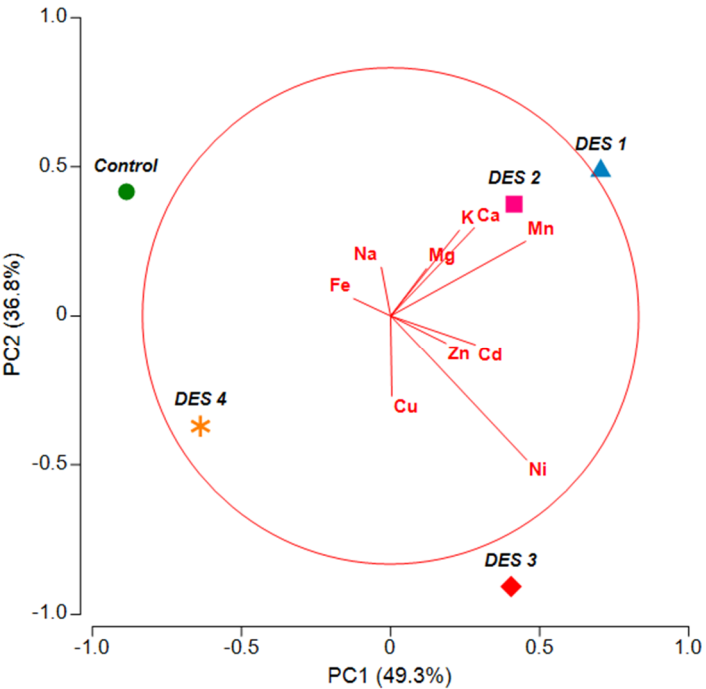


Figure 5 Principal component analysis for 10 variables (metal concentrations, mg L⁻¹) in leachates obtained after DES treatments at a dilution of 1/128 (DES:water). DES 1: choline chloride + oxalic acid; DES 2: choline chloride + ethylene glycol; DES 3: betaine + acetic acid; DES 4: betaine + acetic acid + phosphoric acid

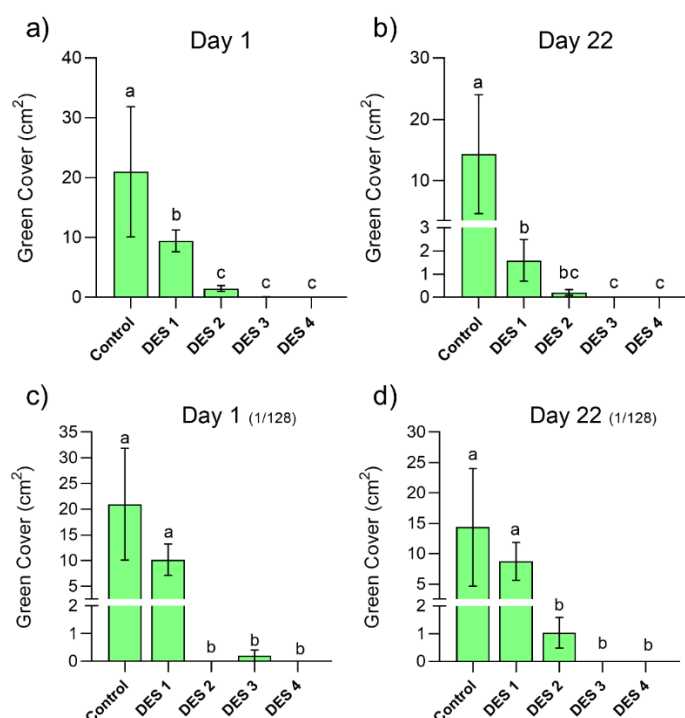


Figure 6 Shoot green cover (cm²) of *P. lanceolata* seedlings after 1 and 22 days of growth on tailings treated with different DESs. Bars are averages with standard errors (n = 3) for pure DES treatments at day 1 (a) and day 22 (b), and for diluted DES (1/128) at day 1 (c) and day 22 (d). Different letters represent significant differences (ANOVA, Tukey's test, p < 0.05). DES 1: choline chloride + oxalic acid; DES 2: choline chloride + ethylene glycol; DES 3: betaine + acetic acid; DES 4: betaine + acetic acid + phosphoric acid

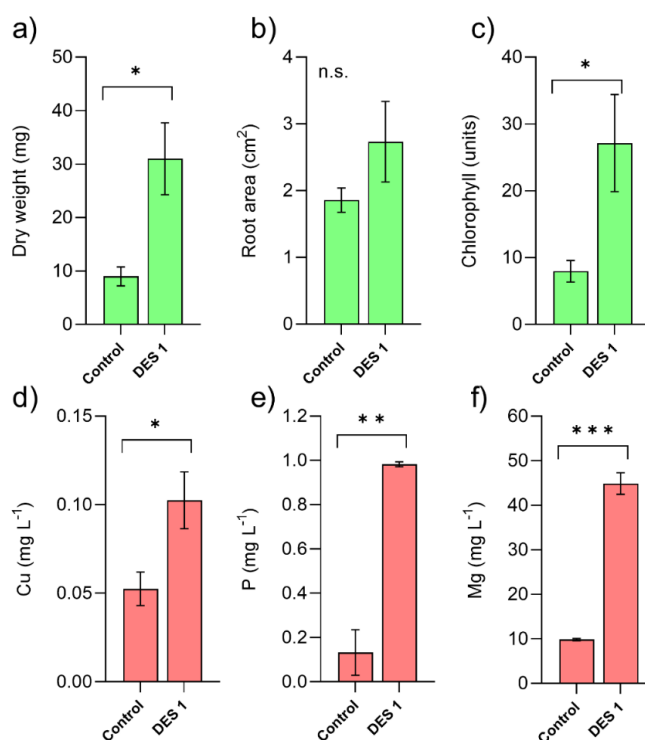


Figure 7 Effects of diluted DES 1 (1/128) applied in tailings on *P. lanceolata* growth and nutrient mobilisation (n = 4, ± standard error): (a) total dry weight; (b) total root area; (c) chlorophyll content; and leachate concentrations of (d) Cu, (e) P and (f) Mg. Asterisks represent significant differences (ANOVA, * p < 0.05, ** p < 0.01, *** p < 0.001). Control: deionised water, DES 1: oxaline

4 Discussion

4.1 Copper extraction

As we hypothesised, Cu leaching from tailings increased with DES concentrations, with all four DES formulations increasing Cu mobilisation in comparison to pure water, but to variable degrees. Both DESs 3 and 4 (betaine-based), showed the highest Cu recovery potential, followed by DES 1 (oxaline), although only the latter was efficient in also mobilising other divalent cations such as Mg and Mn. A betaine-based DES (with ethylene glycol + malic acid) has been demonstrated as particularly effective in Cu solubilisation from oxides, especially at temperatures of 50 and 60°C (Dlugosz et al. 2024). Efficient CuO solubilisation in oxaline was also reported by Pateli et al. (2020), although the authors also noted that this particular DES was more effective for Zn and Fe oxides: indeed, here we also observed the highest Fe concentration in leached solutions from tailings treated with this DES (Figure 3b).

DES 2 (ethaline), comprising ChCl + ethylene glycol, had the lowest efficiency in leaching Cu even when applied in full concentration ($< 10 \text{ mg L}^{-1}$). Ethaline is known to have a combination of properties, including high solubility of several neutral and charged species: indeed, high efficiency in Cu recovery was demonstrated for sources like sulphate- or sulphide-based minerals, but not as much for Cu oxides (Anggara et al. 2019; Aragon-Tobar et al. 2024). In any case, metal leaching from mine tailings by DESs appears to take up to 14 days for maximum extraction in comparison to other metal sources (Drogobuzhskaya et al. 2024). Therefore, we assume a large metal pool remained relatively available in the tailings when *P. lanceolata* seedlings were transplanted three days after DES application and flushing.

4.2 Phytotoxicity and metal excess

Some DESs can strongly impact early developmental stages in plants, possibly affecting cellular membrane permeability and causing cell destruction (Khan et al. 2023). Here, application of betaine-based DESs 3 and 4 in tailings killed all seedlings within 24 hours after transplanting, regardless of dilution (Figures 4a, 4c). On the other hand, DES 1 (oxaline) promoted the largest GC of all DESs assessed. Similarly, in cress plants (*Lepidium sativum*) directly exposed to DESs in filter papers, betaine + citric acid (34 mM) was also more detrimental to shoot development than ChCl + citric acid (34 mM), suggesting an inherently higher toxicity of betaine-based DESs (Samori et al. 2019).

Direct exposure of wheat seedlings to oxaline (seven days) was considered to be non-phytotoxic by Radošević et al. (2015), although shoot and root impairments were observed at concentrations as low as 3.7 mM: half of our most diluted oxaline solution (7.8 mM). Such results suggest a certain bias towards reporting the benign/non-toxic effects of DESs where toxicity is found at a particular concentration, but it is overall described as 'low toxicity' or 'relatively harmless' (Rodrigues et al. 2021). In the ecotoxicity trials carried out by Lapeña et al. (2020), different model (test organisms) microorganisms were exposed to various DES mixtures, none of which presented effect concentration at 50% (EC_{50}) values higher than 1 g L^{-1} ; thus they were not considered as toxic to the environment. However, it is clear that toxicity exists and depends on the DES concentration: as we showed here, oxaline led to phytotoxicity when applied at pure concentration (1 M), equivalent to 133 g L^{-1} , but not at the dilution of 1/128, equivalent to 1 g L^{-1} . But most importantly, the 'environment' is not itself factored into most ecotoxicological assays, which usually involve direct exposure to DESs in artificial settings such as petri dishes and pure growth media. DESs interact with the environment and may promote indirect toxicity, such as seen here in tailings, where excessive amounts of metals are mobilised and become available for plants or other organisms. Further research on DES effects in more natural settings and for longer time frames are still necessary.

4.3 Oxaline and nutrient availability

Betaine-based DESs 3 and 4 promoted the highest Cu availabilities, which may have led to Cu phytotoxicity. Copper is a tightly regulated micronutrient maintained at lower root and shoot concentrations compared to other nutrients, and is found naturally in soils below 60 mg kg^{-1} (Alloway 2013). Thus Cu tailings on their own

can be highly phytotoxic (Degani et al. 2022), and enhanced Cu availability by DES application was already expected to promote detrimental effects such as rhizotoxicity, oxidative stress, cell damage and plant death (Kumar et al. 2021; Soltangheisi et al. 2024).

Despite considerable Cu leaching, DES 1 was responsible for the highest mobilisation of other metals such as Mg, Mn and K (at pure concentrations), with similar patterns being observed in the most diluted treatment (1/128 dilution). The PCA with all four diluted DESs clearly shows a higher correlation between DES 1 with nutrient cations, which are ordinated opposite to the control, and betaine DESs. These cations are nutrients known to have an antagonistic relationship with excess Cu (Kumar et al. 2021), and are capable of counteracting its toxicity, decreasing Cu-induced oxidative stress, improving root growth, stimulating antioxidant enzymes and possibly preventing Cu from binding to toxic action sites at plasma membrane surfaces (Juang et al. 2021). The effects of diluted DESs partly indicate how toxicity could be diminished in situ after pure DESs are applied to the tailings and metals are leached gradually to a point at which low concentrations remain; enough to provide nutritional benefits but not phytotoxicity.

DES 1 (oxaline) was particularly efficient in the mobilisation of Mg, a macronutrient that is known to improve tolerance to elevated Cu (Chen et al. 2013) as well as other metals such as Mn and Al (De Oliveira et al. 2022; Rengel et al. 2015). Aluminium, in particular, was also present at high levels in these tailings (Table 1) and was possibly highly mobilised to toxic ranges after DES applications as the lack of 2:1 clays and organic matter to provide adsorption sites for Al ions increases its bioavailability in the rhizosphere (Li et al. 2022). Magnesium is the central atom in the chlorophyll molecule and highly required for proper plant physiology, chlorophyll synthesis and ribosome aggregation (Ahmed et al. 2020; Hawkesford et al. 2023). Therefore, the reason for higher chlorophyll contents in plants grown in tailings treated with diluted oxaline in comparison to control (water-treated) plants (Figure 7) is probably due to the enhanced Mg availability promoted by this DES, while the overall higher biomass could be linked to more available macronutrients (e.g. P, K and Ca) in this condition. Considering that plant performance is one of the main indicatives of successful rehabilitation in post-mining environments (Tibbett 2024), clearly the application of concentrated DESs is unable to provide proper conditions for vegetation establishment. However, our results with diluted DESs are more promising, not by leaching toxic elements but by enhancing nutrient availability and fertility.

5 Conclusion

All concentrated DESs promoted phytotoxicity when applied to Cu tailings and, despite its lower Cu leaching capacity, the DES based on choline chloride and oxalic acid (oxaline) was less harmful than the betaine formulations. Although we have not assessed the phytotoxicity of DESs via direct contact it is clear that these solvents are not as 'environmentally benign' as is often claimed, and their hazardous effects depend on the DES formulation, concentration and exposure route. Here we show extreme phytotoxicity from tailings treated with concentrated DESs, which is possibly associated with high metal mobilisation and consequential plant toxicity. Despite not having the exorbitant amounts of metals contained in mine tailings, soils might produce similar effects if in contact with these solvents, becoming toxic to plants as well as other soil organisms. However highly diluted DESs, likely representative of post-leaching concentrations after DES treatment, may improve plant growth in tailings by enhancing nutrient availability. This shows the potential use of DESs in solvometallurgy and metal recovery, leading to some diluted DESs improving plant nutrition and growth, and early pedogenesis potentially promoting land rehabilitation. Further ecotoxicological work should focus on DESs effects applied in other substrates such as sand or soil to further elucidate these interactions.

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