Phytoremediation of acid mine drainage using by-product of lysine fermentation

Attila Nagy, Tamás Magyar, Csaba Juhász and János Tamás

ABSTRACT

The main point of this research is to assess the applicability of condensed molasses soluble (CMS), which is an organic by-product of lysine fermentation, as an environmentally friendly complexing agent in rhizofiltration of heavy metal contaminated acid mine drainage (AMD). First, the ecotoxicological properties (growth inhibition, seed germination) of CMS were examined with often applied indicator plant species such as duckweed (*Lemna minor*) and lettuce (*Lactuca sativa*) so as to define the possible applicable CMS concentration. Then the heavy metal accumulation and translocation properties of root accumulator plant species, i.e. common reed (*Phragmites australis*) and sedge (*Carex flacca*), were studied to optimize CMS concentration for rhizofiltration. Due to the CMS application, significant increase in bioaccumulation was detected in the case of every examined heavy metal (As, Cd, Cu, Pb and Zn) at the end of the experiment. Results also showed that CMS increased the heavy metal concentration in shoots, but did not affect the root accumulation characteristics of the plants. Furthermore, CMS treated plants accumulated heavy metals at higher rates in their roots compared to control. The results suggest that CMS is a viable additive and a complexing agent to aid rhizofiltration of heavy metal contaminated AMD.

Key words | *Carex flacca*, complexation, heavy metals, *Phragmites australis*, rhizofiltration

Attila Nagy
Tamás Magyar (corresponding author)
Csaba Juhász
János Tamás
Institute of Water and Environmental Management,
University of Debrecen,
Böszörményi str. 138., 4032 Debrecen,
Hungary
E-mail: *magyar.tamas@agr.unideb.hu*

HIGHLIGHTS

- Condensed molasses soluble (CMS) was tested as an environmentally friendly complexing agent in rhizofiltration of heavy metal contaminated AMD.
- CMS at less than 1 % concentration is recommended based on *Lemna minor* and *Lactuca sativa* phytotoxicological tests.
- Compared to control, CMS significantly increased the bioaccumulation of heavy metals in *Phragmites australis* and *Carex flacca*.
- CMS at less than 1 % concentration is recommended in rhizofiltration of AMD based on the BCF and TLF.

INTRODUCTION

Remediation of soil and water is a huge challenge not only for environmental, but a range of technical and economic reasons. Although several new remediation technologies have been developed in the last 20–30 years, some of these technologies are partly based on traditional methods (e.g. incineration, thermal desorption, solidification, etc.). Traditional remediation technologies are usually expensive and generate secondary wastes (e.g. off-gases, washing liquids) (de Albergaria & Nouws 2016). Phytoremediation is an alternative remediation method, which can be used for the degradation, accumulation or stabilization of contaminants by certain plant species. It is cheap and appropriate for the application to large areas, nevertheless it has critical implications for human health since plants

doi: 10.2166/wst.2020.240

are at the beginning of an exposure route via food intake by animals and humans.

Among different forms of contaminants, a dynamic balance is formed which was determined by the solubility of the trace elements, adsorption capacity, redox potential and pH of the soil (Wang *et al.* 2019). During the rhizofiltration, heavy metals are accumulated or precipitated by the plant roots out of the polluted waters. The plant roots precipitate the metals in the extracellular space or trace elements on the cell wall and are adsorbed, or get in to the cells, located in the cytoplasm and vacuoles, reducing their mobility and thus toxicity (Dushenkov & Kapulnik 2000). The plant species used for rhizofiltration purposes have to tolerate the high metal concentration of the contaminated water for at least a few days. According to Wong (2003), a selection of such plants is essential that are not only tolerant of heavy metals and drought but tolerate scarce nutrient conditions as well. Species that are suitable for rhizofiltration can be quickly grown, have large root masses and root surface areas, their roots are able to capture a lot of metals and a relatively small amount of metal is transported through the roots into the sprouts (Jeevanantham *et al.* 2019). *Eichhornia crassipes*, *Hydrocotyle umbellata*, *Lemna minor* and *Azolla pinnata* are capable of removing many metals dissolved in water. However, the effectiveness of metal removal using aquatic plants is small; the reason is that these plants are relatively small, and have small-surface, slow growing root systems. In contrast, terrestrial plants, in general, form a large amount of biomass and have expansive, fibrous, highly specific surface root systems (Dushenkov & Kapulnik 2000). In addition, several studies (Yadav *et al.* 2011; Srivastava *et al.* 2014) pointed at the applicability of *Phragmites* sp. and the *Carex* sp. in the rhizofiltration technology.

Some plant species acidify the environment with protons during their element intake, mobilizing heavy metals, increasing their bioavailability. However, in the case of some species, an iron hydroxide precipitate may form on the surface of the roots, which may inhibit the metal accumulation (Peverly *et al.* 1995). Successful remediation, by using phytoaccumulation, may be obtained only if the amount of the eliminated heavy metal reaches 200–100 kg ha^{-1} a year. This amount could be realised with uptake of 1–2% metal concentration by the plant tissue. On the other hand, the uptake of heavy metals in soil is related to their bioavailability to plants. Natural and synthetic chelators and complexing agents can be used to increase the uptake and translocation of heavy metals in both hydroponic culture and heavy metal-contaminated soil (Garbisu & Alkotra 2001).

Rhizofiltration can be facilitated by complexation as well. According to Strawn *et al.* (2015), the complexation ability of the bivalent transition metals changes in the order of $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+}$. EDTA is the most commonly used synthetic chelator for enhancing phytoextraction, but it is reported to have toxic effects on soil microbial (Mühlbachová 2011) and soil enzymatic activities (Epelde *et al.* 2008), as well as on cultivated plants (Neugschwandtner *et al.* 2012), may persist for several weeks or months and can contaminate groundwater due to its non-biodegradable nature (Rizwan *et al.* 2016). Instead of EDTA, using complexing agents with non-toxic organic, natural, plant or of microbiological origin, in addition to providing micronutrients, seems to be an appropriate solution for rhizofiltration (Rizwan *et al.* 2016).

The primary objective of the research is to evaluate the complexing effect of the lysine fermentation by-product condensed molasses soluble (CMS) used as an environmentally friendly complexing agent in the phytoremediation of the heavy metal-contaminated acid mine drainage (AMD) and optimize the qualitative and quantitative parameters of the by-product and the applied dose, by the accumulation properties of the increased heavy metal tolerant and accumulation plant species. AMD was modelled by using a flotation sludge and deionized water, which contains added CMS in different concentrations. With the extract of the suspension, ecotoxicological and bioaccumulation tests were carried out. Duckweed (*Lemna minor*) and lettuce (*Lactuca sativa*) as often applied indicators were used for ecotoxicological tests, growth inhibition and seed germination studies in order to identify the possible application dose of CMS for bioaccumulation tests. The bioaccumulation tests were carried out with common reed (*Phragmites australis*) and sedge (*Carex flacca*) plants, where the CMS induced heavy metal accumulation and translocation properties, were investigated to assess the applicability of CMS in complex induced rhizofiltration.

MATERIALS AND METHODS

The contaminated matrix used for modelling AMD

The contaminated matrix originated from the reservoir of a flotation sludge of an abandoned Pb-Zn mine, which is strongly contaminated with heavy metals (Szárazvölgyi reservoir) (Tamás & Kovács 2003). The examined matrix was flotation sludge with heavy metal contents from Szárazvölgyi reservoir near to Gyöngyösoroszi (Hungary). The contaminated area is located at the southern part of Western Mátra, in the valley of Toka-brook, North Hungarian region, Hungary (Figure 1).

The flotation sludge was composed of fine-grained sand based on the grain-size distribution. The primary minerals of the flotation sludge were quartz and feldspar and their dark colour was from pyrite and marcasite. These oxidation partials occurred under surface conditions, so as a final product, limonite ($2Fe_2O_3{\cdot}3H_2O$) and jarosite ($KFe_3^{3+}(OH)_6(SO_4)_2$) were detected (Tamás & Kovács 2003). The contamination of the used flotation sludge is a result of the impact of particulate emissions from the zinc smelter and is characterized by high accumulation of heavy metals such as Cd, Pb and Zn (Table 1).

The complexing agent

CMS, which is a biodegradable, environmentally friendly by-product of lysine fermentation, was applied as a complexing agent since it is a stable, non-moldy substance as a result of the higher salt content, and can be utilized as a nutrient source because of the high macro and micro element content (Table 2). On the other hand, CMS is produced in large quantities and has high organic material content, therefore its potential use in rhizofiltration can be one of the solutions for the waste management of CMS.

Table 1 | Table EC, pH and total element content of applied flotation sludge (element content was measured by field portable X-ray fluorescence spectrometry)

Parameter	**Average value ± SD**
pH in H_2O, [–]	5.2 ± 0.43
pH in 1 M KCl, [–]	4.81 ± 0.36
EC, [mS/cm]	4.08 ± 0.29
Fe, [mg kg^{-1}]	27,800 ± 760
Zn, [mg kg^{-1}]	2,780 ± 110
Mn, [mg kg^{-1}]	1,340 ± 500
Pb, [mg kg^{-1}]	885 ± 50
Ni, [mg kg^{-1}]	385 ± 140
Cu, [mg kg^{-1}]	265 ± 100
As, [mg kg^{-1}]	206 ± 45
Cr, [mg kg^{-1}]	*
Cd, [mg kg^{-1}]	*
Hg, [mg kg^{-1}]	*

*Below detection limit.

Acid mine drainage

The AMD was modelled by deionized water and heavy metal containing flotation sludge in the concentration of 1 m/m% and CMS in the concentration of 0.2 V/V%, 0.3 V/V%, 0.5 V/V%, 1 V/V%, and 2 V/V%. In the case of phytotoxicological test, there was one treatment where only CMS (100%) was used as an extracting agent for

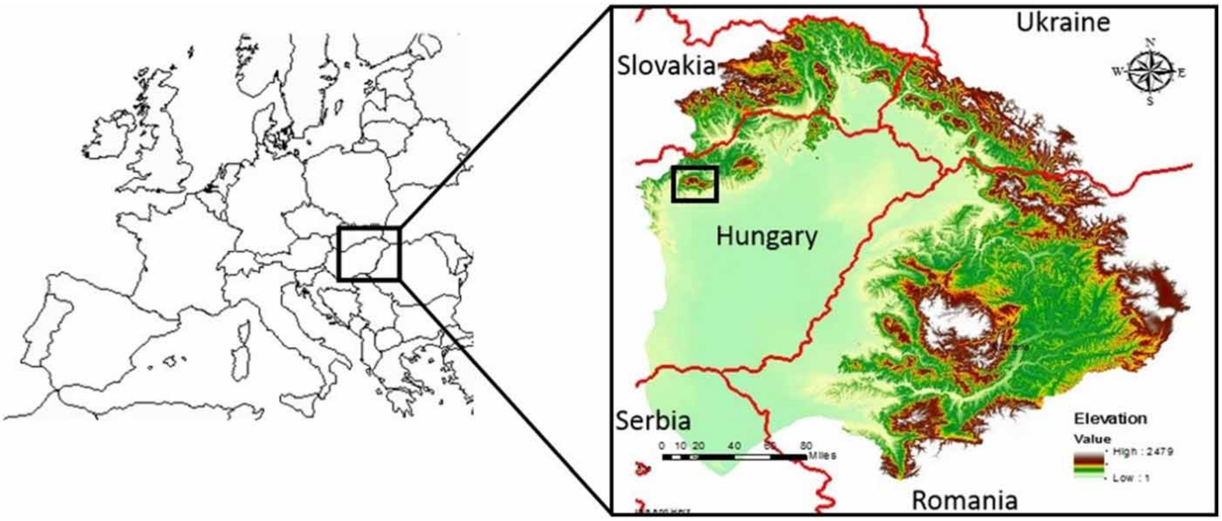

Figure 1 | The location of contaminated area (Szárazvölgyi reservoir, Hungary).

Table 2 | Chemical and physical parameters of CMS

Parameters	Average value ± SD
Density, [g cm^{-3}]	1.2
Dry matter content, [g dm^{-3}]	54
Residue on ignition, [g dm^{-3}]	37.9
Material can be filtered out, [g dm^{-3}]	70
Chemical oxygen demand (Mn), [g dm^{-3}]	99
Chemical oxygen demand (Cr), [g dm^{-3}]	418.0 ± 9%
Biochemical oxygen demand (BOD_5), [g dm^{-3}]	60
Total N-content, [g dm^{-3}]	69.2 ± 2%
Ammonia-type nitrogen content, [g dm^{-3}]	27.7 ± 2%
Carbon content, [g dm^{-3}]	89.7
Total phosphate (P_2O_5), [g dm^{-3}]	1.77
Phosphate in the filtrate (P_2O_5), [g dm^{-3}]	0.2 ± 2%
Total sulphate, [g dm^{-3}]	254
Sulphate in the filtrate, [g dm^{-3}]	198
Chloride content, [g dm^{-3}]	6.1
Potassium (K_2O), [g dm^{-3}]	9.43
Sodium (Na), [g dm^{-3}]	3.71
Calcium (Ca) [g dm^{-3}]	1.34
Magnesium (Mg), [g dm^{-3}]	0.77
Iron (Fe), [g dm^{-3}]	0.12
pH, [-]	2.26

the contaminated matrix. The suspensions of CMS + AMD + mine tailings were extracted for 48 h to establish the chemical equilibrium and, after that, the experiments were carried out with the extract of suspensions.

Phytotoxicological tests

Optimization of the quantitative parameters of CMS complexing agent applied during the rhizofiltration was carried out using duckweed (*Lemna minor*) reproduction test and lettuce (*Lactuca sativa*) laboratory seedling test. In both tests, root lengths of seedlings and duckweeds were measured. *Lemna minor* is a widely used, standard test species (OECD 2002). Carlson *et al.* (1991) revealed that the root growth of lettuce was found to be more sensitive to heavy metals than other species. The heavy metal content had a stimulating effect on lettuce (Beltrami *et al.* 1999). Cadmium did not have any significant effect on the concentration of Mn, Fe, Cu, Zn in roots, while it decreased the concentration of Fe Cu Zn in sprouts and increased the amount of Mn. Raised Mn concentration promoted the reduction of Cd toxicity (Ramos *et al.* 2002). The pH and the electrical conductivity of the different treatments were also measured in the CMS AMD extracts to analyze the effect of pH and electrical conductivity of each treatment on the root length of the duckweed and lettuce seedlings.

Duckweed (*Lemna minor*) reproduction test and growth inhibition test

Duckweeds living at polluted sites are able to accumulate a significantly greater amount of each heavy metal (Pb, Cd, Cr, Zn, Cu, Hg) than those that originated from non-polluted sites (Mukherjee *et al.* 2004). From an ecotoxicological testing point of view, to detect the effect of CMS on metal uptake more intensively, the investigated duckweeds are originated from a non-polluted habitat.

Lemna minor was kept in a similar condition as its natural habitat for 8 weeks. After that, the growth inhibition test was carried out and lasted for 7 days according to temperature and light and other environmental requirements of OECD Guideline (2002). The selected 15 duckweeds in monoculture without overlay were placed into Erlenmeyer vessels, filled with 100 mL AMD extract of 0.2%, 0.3%, 0.5%, 1%, 2% and 100% concentrated CMS under natural illumination and at room temperature (22 ± 0.5 °C). The control group was placed into fresh water. Nutrients for plants were provided by the extracting agent.

At the end of the experiment, the roots were measured separately, then the significance of differences was assessed between the average root lengths of each treatment. Visible signs of toxicity, chlorosis, necrosis, root destruction and population disintegration were considered based on imaging technology, and using image classification to make the segmentation to distinguish the green and chlorotic frond surfaces. Visible toxic effects and root lengths were compared between treated and control populations. Between average root lengths, results of each treatment by Tukey's variance analysis were used to assess the significance of differences; furthermore, descriptive statistics (mean, standard deviation) were applied. The root lengths showed a normal distribution and their histograms followed the well-known Gaussian curve.

Lettuce (*Lactuca sativa*) seed germination test

Well germinating lettuce seeds were placed into Petri vessels, onto filter-paper and treated by different CMS concentrated AMD extracts, which is in accordance with the experimental method of Ratsch & Johndro (1986).

Table 3 | Toxicological qualification of the seedlings

Mean root length as a percentage of the control	Qualifications based on development
0–5%	full inhibition
6–50%	extreme inhibition
51–90%	slight inhibition
91–120%	non-inhibition
>120%	stimulating

Twenty seeds were germinated in a filter-paper with 10 mL AMD extract of 0.2%, 0.3%, 0.5%, 1%, 2% and 100% concentrated CMS at room temperature (22 ± 0.5 °C). The control group was placed into fresh water. Nutrients for plants were provided by the extracting agent.

The lengths of the roots were measured on the 10th day. The average length of the seedlings was expressed as a percentage of the control sample (Table 3).

Tukey's variance analysis has been used for laboratory seed germination tests in order to detect significance of differences among treatments. The germination ability of each treatment was evaluated and expressed as a percentage of the control sample. Furthermore, descriptive statistics (mean, modus, median, deviation) were applied taking into account 0 values as well.

Heavy metal bioaccumulation test in aqueous media – testing CMS for rhizofiltration

Based on the quantitative results of CMS, originated from toxicological tests, small-scale model experiments have been carried out with common reed and sedge. These plant species are well-known from their heavy metal accumulation ability (Soltz & Greger 2002). The reason for choosing common reed (*Phragmites australis*) and sedge (*Carex flacca*) is that common reed is suitable for the remediation of soils contaminated with heavy metals (Tordoff *et al.* 2000), and sedge is found to possess an innate tolerance to heavy metals (Matthews *et al.* 2004). The aim of our experiments was to investigate the applicability of the chosen two plant species for rhizofiltration purposes. The effect of the extracting agent on plant heavy metal uptake was also examined. Furthermore, the non-phytotoxical concentration range which resulted from the maximal heavy metal uptake on the investigated plant species was determined.

Accumulation experiments were carried out by using common reed (*Phragmites australis*) and sedge (*Carex flacca*) with AMD extract of 0.2%, 0.3%, 0.5%, and 1% concentrated CMS under natural illumination and room temperature for 6 weeks. The control group was placed into AMD extract without CMS. Nutrients for plants were provided by the extracting agent.

The pH and electric conductivity were measured on the 2nd, 4th and 6th weeks by potentiometric methods. The heavy metal concentration of the matrix and the plant parts (roots, shoots) of common reed and sedge were determined by ICP-OES on the 6th week. Tukey's variance analyses were used to determine the differences between heavy metal accumulation properties caused by CMS.

In order to compare the accumulation properties of the common reed and sedge treated by AMD extracts with CMS at different concentrations, bioconcentration and translocation factors were calculated. Bioconcentration factors (BCF) were calculated as follows (Renoux *et al.* 2001) (Equation (1)).

$$\mathrm{BCF} = \frac{\mathrm{Metal}_{(\mathrm{plant})}}{\mathrm{Metal}_{(\mathrm{matrix})}} \tag{1}$$

$\mathrm{Metal}_{(\mathrm{plant})}$ is the total metal concentration in the plant tissues at the end of the 6th week, and $\mathrm{metal}_{(\mathrm{matrix})}$ is the total metal concentration of applied AMD extract at equilibrium. Translocation factors (TLF) were also calculated to analyse the movement of metals from the root to the shoot (Equation (2)).

$$\mathrm{TLF} = \frac{\mathrm{Metal}_{(\mathrm{root})}}{\mathrm{Metal}_{(\mathrm{sprout})}} \tag{2}$$

$\mathrm{Metal}_{(\mathrm{root})}$ is the total metal concentration of the roots, and $\mathrm{metal}_{(\mathrm{sprout})}$ is the total metal concentration in the sprouts.

RESULTS AND DISCUSSION

Duckweed (*Lemna minor*) growth inhibition test

Before assessing the use of CMS in complex induced rhizofiltration, a *Lemna minor* growth inhibition test and a *Lactuca sativa* seed germination test were carried out in order to optimize the quantity of the CMS complexing agent. During the experiment, the duckweed population was constant, however CMS in 1% and 2% concentrations in AMD caused more than 20% chlorosis of frond surfaces as an indication of possible increased toxic metal accumulation in duckweed (Figure 2). Furthermore, the duckweed

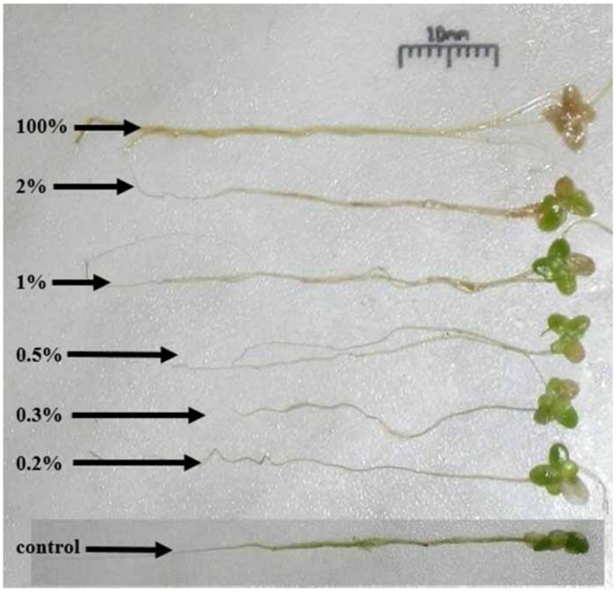

Figure 2 | Symptoms of duckweed in case of AMD extracts with different CMS treatments.

Table 4 | Symptoms of duckweed samples in case of AMD extracts with different CMS treatments

CMS concentration in AMD extract	Chlorosis area, [%]	Root lengths, [mm]
Control	No chlorosis	41.7^a
0.2%	No chlorosis	38.3^a
0.3%	12% chlorosis	35.5^a
0.5%	17% chlorosis	35.55^a
1%	21% chlorosis	32.8^b
2%	27% chlorosis	30.9^b
100%	100% chlorosis and necrosis	30.6^b

There was no significant difference between the same alpha indices based on variance analysis ($p < 0.05$).

population necrotized when CMS was used in 100% concentration for extraction, possibly due to the high acidity (pH = 2.43). When CMS was applied in 0.3%, 0.5%, 1% and 2% concentrations, increasing chlorosis was observed on the roots and also on new fronds as a function of decreasing CMS concentration (Table 4). In AMD extracts with CMS concentration lower than 2%, acidity became neutral and slightly alkaline (pH = 7.02–7.35) with slight changes. However, the EC changed from 6.51 to 2.68 with the CMS concentration changes (1% to 0.2%).

AMD extracts with CMS concentrations higher than 0.5% showed significantly shorter root lengths compared to the control sample (Table 4). However, the average root lengths of AMD + CMS extracts treatments with 0.2 to 0.5% concentration were not significantly different but 4.3–7.05% shorter than the control, which could be the result of the increased heavy metal concentration due to the CMS. Preliminary studies proved the duckweeds to have an excellent heavy metal absorption capacity (Rahmani & Sternberg 1999), thus the inhibited growth was possibly caused by the higher element accumulation in the plants. The higher element accumulation is originated from the possible trace element content increase due to the complexing effect of the CMS in AMD extracts.

Since the chlorosis was below 20% and there were no significant differences among the root lengths, the optimal concentration for CMS application should be less than 1% in AMD extract based on the duckweed test.

Lettuce (*Lactuca sativa*) seed germination test

Parallel to the *Lemna minor* test, the effect of CMS induced complexation on heavy metal uptake was also assessed within *Lactuca sativa* seed germination test in the same treatments (with the same extracts) as in the

Table 5 | Lettuce (*Lactuca sativa*) seed germination ability

CMS concentration in AMD extract	Seed germination ability	Root lengths, [mm]	Root lengths, [% to control]	Inhibition
Control	100%	40.25^d	–	–
0.2%	85%	32.6^c	81%	slight inhibition
0.3%	75%	26.5^{bc}	66%	slight inhibition
0.5%	65%	22.2^b	55%	slight inhibition
1%	60%	7.42^a	19%	extreme inhibition
2%	0%	–	0%	full inhibition
100%	0%	–	0%	full inhibition

There was no significant difference between the same alpha indices based on variance analysis ($p < 0.05$).

duckweed test. Based on the results, the continuously increasing CMS concentration decreases the seed germination ability of lettuce in the AMD extract compared to the control treatment (100% of the control samples were germinated). It can be observed that CMS in concentration higher than 2% resulted in no germination. CMS in 1%–0.5% concentration showed a moderate effect on the germination ability (60–65%), whereas 0.3% and 0.2% were found to have a slight effect (75–85%) on germination ability (Table 5).

The differences in seedling development are also detected for each AMD extract with different CMS treatments. The length of the seedlings was shortened with increasing concentrations of CMS (Figure 3).

AMD extracts with different CMS treatments resulted in significantly different root lengths. It can be observed that CMS in concentration higher than 1% resulted in 81% shorter root length compared to control, while CMS in 0.3–0.5% concentration showed a moderate effect on root length (34–45% decrease), whereas 0.2% was found to have slight effect (19% decrease) in root length. Consequently, 0.2%, 0.3% and 0.5% CMS AMD treatments were categorized in 'slight inhibition'; therefore the optimal CMS concentration range which could be used for rhizofiltration purposes was found to be below 1% (Table 5). The changes of root lengths were shown to be in strong correlation with the EC ($r^2 = 0.969$, $p < 0.05$), which represents the toxicity of increasing heavy metal concentrations.

Based on the lettuce seed germination test, it can be stated that the optimal concentration of CMS is less than 1% in AMD extract and can be used for rhizofiltration purposes.

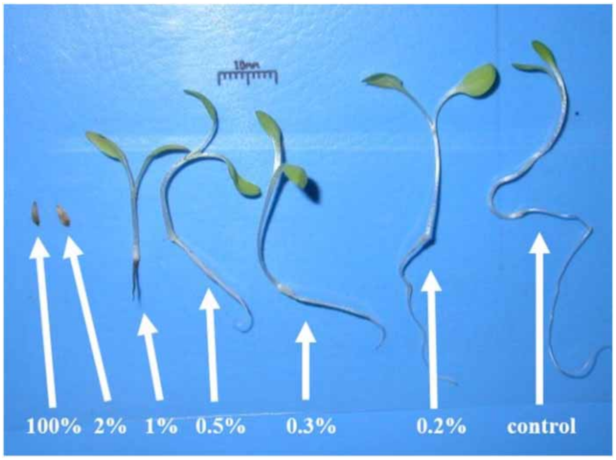

Figure 3 | The development of lettuce seed samples treated by AMD extracts with different CMS concentration.

Table 6 | Parameters of AMD extracts with different CMS concentration

Parameter	Concentration in AMD	2nd week	4th week	6th week
Electric conductivity, [mS cm^{-1}]	1%	7.34	7.36	7.32
	0.5%	3.74	3.51	3.53
	0.2%	2.86	2.92	2.88
	Control	2.10	2.08	2.02
pH, [-]	1%	6.81	6.80	6.81
	0.5%	7.09	7.05	7.06
	0.2%	7.12	7.10	7.13
	Control	5.88	6.05	5.93

Heavy metal bioaccumulation test – testing CMS for rhizofiltration

Based on the phytotoxicity tests, CMS in 0.2%, 0.5% and 1% concentrations were tested in bioaccumulation tests. As was described earlier, the pH of the treatments with CMS is neutral and did not differ to a large extent among treatments. There were also no notable changes in time as well (Table 6), thus decreasing pH had no significant effect on the solubility of heavy metals, and therefore the increasing concentration of dissolved heavy metals (Table 7) was clearly the result of complexing ability of CMS. The electrical conductivity differed within the treatments, which is in relation to the element concentrations of the extracts, but there were no notable temporary changes in EC. The EC in the control sample is due to the moderately or slightly acidic conditions.

The increasing element content in the extracts is attributed to the complexing ability of CMS on the heavy metals; consequently the solubility and bioavailability of these heavy metals are increased (Table 7) in parallel with the increasing CMS concentration.

During the experiments visible chlorosis or necrosis were not detected on sedge and common reed. Based on the results, common reed and sedge accumulated significant amounts of heavy metals in shoots and rhizomes in the case of treatment with CMS in concentration higher than 0.2%, compared to the element content of the control plants (Table 8). With the increasing concentration of CMS, both of the investigated plant species accumulated heavy metals in increasing concentrations, confirming the accumulation effect of CMS due to complexation and eventually on element uptake.

Despite the Pb content being measurable only in the case of AMD extract treated with CMS in 1% concentration, the Pb content of other CMS-AMD extracts might not have been negligible, since there were considerable lead amounts in the plant tissues in the case of most treatments. Molasses have previously been reported to bind to many metal ions,

Table 7 | Heavy metal concentrations of AMD extracts with different CMS treatments

	As	**Cd**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
CMS concentration in AMD	**[mg kg^{-1}]**							
Control	*	0.010	0.017	0.144	*	*	*	0.842
0.2%	0.016	0.012	0.061	0.141	0.177	*	*	1.87
0.5%	0.042	0.011	0.121	0.327	2.52	0.011	*	3.53
1%	0.12	0.03	0.214	6.67	10.5	0.01	0.151	4.52

*Below detection limit (ICP-OES).

Table 8 | Heavy metal concentration (mg kg^{-1}) in common reed and sedge in the case of AMD extracts with different CMS treatments

		Phragmites australis		***Carex flacca***	
Elements	**CMS concentration in AMD extract**	**rhizome**	**shoot**	**rhizome**	**shoot**
Zn	Control	162^{d}	64.75^{c}	71^{d}	38^{c}
	1%	$1,228^{c}$	526^{b}	$1,262^{c}$	224^{b}
	0.5%	909^{b}	523^{b}	697^{b}	213^{b}
	0.2%	779^{a}	157^{a}	194^{a}	132^{a}
Cu	Control	17.45^{d}	8.7^{a}	4.81^{d}	3.61^{c}
	1%	214^{c}	10.2^{b}	61.1^{c}	18.3^{b}
	0.5%	97.3^{b}	10.2^{b}	30.7^{b}	9.42^{a}
	0.2%	32.8^{a}	9.25^{ab}	17^{a}	8.56^{a}
Pb	Control	46.5^{a}	9.92^{a}	5.63^{d}	*
	1%	148^{c}	15.25^{a}	236^{bc}	18.4^{c}
	0.5%	58.3^{ab}	*	268^{b}	9.21^{ab}
	0.2%	53^{a}	*	36.9^{a}	6.72^{a}
Cd	Control	0.59^{d}	0.29^{b}	0.87^{a}	*
	1%	12.26^{bc}	1.5^{a}	10.91^{b}	1.01^{b}
	0.5%	9.83^{abc}	*	11.2^{b}	0.34^{a}
	0.2%	6.74^{ab}	*	1.17^{a}	0.39^{a}
As	Control	*	*	*	*
	1%	33.1^{ab}	2.3^{b}	260^{c}	7.19^{a}
	0.5%	26.5^{a}	1.71^{b}	66.1^{b}	11.4^{b}
	0.2%	30.2^{a}	6.09^{a}	15.8^{a}	5.09^{a}

*Below detection limit (ICP-OES). There was no significant difference between the same alpha indices based on variance analysis ($p < 0.05$).

and the Cu and Pb-binding capacities of molasses appear to be much higher than those for other ions (Hatano *et al.* 2016). Furthermore, molasses may serve as regulators in plant stress tolerance (Hatano & Yamatsu 2018). Besides, Peverly *et al.* (1995) declared that the Fe content on the surfaces of the reed rhizomes had increased due to $Fe(OH)_2$ precipitation formation in the rhizosphere. This layer may lead to immobilization, therefore the precipitation of certain metals (e.g. Cu, Pb) and it may protect the plant against the over-accumulation of metals (Batty *et al.* 2000).

Common reed and sedge were found to be root accumulators, since significant element uptake was measured in both of the common reed and sedge root and shoot, but the elements are allocated in roots in significantly higher rates (Table 9), which is in accordance with the findings of Soltz & Greger (2002), which described that the translocation of heavy metals through the roots into the shoots is inhibited for common reed and sedge. The importance of this property is that the common reed does not let toxic metals enter to the food chain, in contrast with other plant species, which can accumulate a large quantity of toxic metals into their shoots.

In accordance with Baltodini *et al.* (2004) the heavy metal (Cu, Pb, Cd, As, Zn) translocation through rhizomes into the shoots was reduced in case of both examined plants. Higher translocation factors of common reed for some heavy metals (Pb, Cu) assume more significant translocation inhibition compared to sedge regardless of different CMS concentrations. The Cu accumulation in common reed was three to four times higher than in the sedge, which suggests that the common reed accumulates Cu more intensively than sedge. On the other hand, sedge accumulated more than eight times higher Cd from extracts with CMS in 1% than common reed, although in other treatments the differences were negligible in the case of the Cd other heavy metals. Furthermore, the tendency of Cd accumulation is in accordance with the increasing CMS concentration. In the case of other metals and treatments, there were smaller differences between the accumulation properties of the examined plants, although sedge accumulated more As than common reed. The calculated bioconcentration factors showed the concentrations of heavy metals accumulated in plants are 200–2,000 times higher than in applied AMD + CMS extracts. Despite that, the CMS concentration has significant and trend-like effect on the accumulated heavy metals, trend like changes in BCF due to different CMS concentrations can only be detected in the case of Zn and As in sedge. Trend like changes in TLF can only be observed in the case of Zn,

Table 9 | Bioconcentration and translocation factors of sedge and common reed

		Bioconcentration factor		**Translocation factor**	
Elements	**CMS concentration in AMD extract**	***Phragmites australis***	***Carex flacca***	***Phragmites australis***	***Carex flacca***
Zn	Control	*	*	2.50	2.12
	1%	388	329	2.33	5.63
	0.5%	406	258	1.74	3.27
	0.2%	500	174	4.96	1.47
Cu	Control	*	*	1.88	1.2
	1%	502	371	20.9	3.34
	0.5%	1,853	332	9.54	3.26
	0.2%	683	420	3.77	1.98
Pb	Control	*	*	3.47	*
	1%	1,046	1,685	14.9	12.8
	0.5%	*	*	*	29.1
	0.2%	*	*	*	5.49
Cd	Control	*	*	*	*
	1%	463	401	8.17	10.8
	0.5%	937	1,068	34.0	33.3
	0.2%	548	127	*	2.99
As	Control	*	*	*	*
	1%	295	2,227	14.4	36.2
	0.5%	668	1,835	15.5	5.79
	0.2%	2,326	1,339	4.96	3.10

*Values below the detection limit, not possible to calculate.

Cu, As in sedge and in Cu in common reed. The reason is possibly that not only the element content of the plant tissues was increased, but also the element concentrations of the extracts.

CONCLUSIONS

Based on the duckweed (*Lemna minor*) growth inhibition and the lettuce (*Lactuca sativa*) seed germination tests, the application of CMS is optimal at less than 1% concentration in AMD extract for complex-induced rhizofiltration technology.

According to the results, the bioaccumulation of As and Zn by *Carex flacca*, Cd and Pb by *Phragmites australis*, Cu by both plant species was significantly more efficient with CMS at 1% concentration than in the case of CMS at 0.5%. However, in other cases (e.g. As and Zn by common reed and Cd and Pb by sedge) the bioaccumulation was not considerably different whether 1% or 0.5% CMS concentration was applied in AMD extracts. Furthermore, treatments with 1% of CMS only performed better whether common reed or sedge is applied for the rhizofiltration, which might suggest the effect of plant specific characteristics on metal uptake rather than the effect of CMS. For better understanding the co-effects of CMS and plant specific characteristics of heavy metal uptake require further studies.

Therefore, considering and summarizing the bioaccumulation properties of both plant species, as well as the phytotoxicity results and the fact that the applied AMD was contaminated with several heavy metals (i.e. Zn, Cu, Cd, Pb and As) at the same time, CMS at less than 1% concentration is recommended to use as a complexing agent in rhizofiltration.

The applicability of CMS in rhizofiltration has waste utilization aspects as well. Whereas CMS contains a large amount of heterogeneous organic molecules with high molecular weight, and requires large equipment investments and time to treat it in a conventional wastewater treatment approach, rhizofiltration can offer environmentally friendly utilization of the CMS.

ACKNOWLEDGEMENT

The research was financed by the Higher Education Institutional Excellence Programme (NKFIH-1150-6/2019) of the Ministry of Innovation and Technology in Hungary, within the framework of the 4th thematic programme of the University of Debrecen.

REFERENCES

Baltodini, D., Alfani, A., Tommasi, P. D., Bartoli, G. & De Santo, A. V. 2004 Assessment of macro and microelement accumulation capability of two aquatic plants. *Envíromental Pollution* **130**, 149–156. doi: 10.1016/j.envpol.2003.12.015.

Batty, L. C., Baker, A. J. M., Wheeler, B. D. & Curtis, C. D. 2000 The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis* (Cav.) Trin ex. Steudel. *Annual Botany* **86**, 647–653. doi: 10.1006/anbo.2000.1191.

Beltrami, M., Rossi, D. & Baudo, R. 1999 Phytotoxicity assessment of Lake Orta sediments. *Aquatic Ecosystem Health and Management* **2**, 391–401. doi: 10.1016/S1463-4988(99)00036-6.

Carlson, C. L., Adriano, D. C., Sajwan, K. S., Abels, S. L., Thoma, D. P. & Driver, J. T. 1991 Effects of selected trace metals on germinating seeds of six plant species. *Water Air Soil Pollution* **59**, 231–240.

de Albergaria, J. T. V. S. & Nouws, H. P. A. 2016 *Soil Remediation: Applications and New Technologies*. CRC Press, Boca Raton, FL, USA, p. 174.

Dushenkov, S. & Kapulnik, Y. 2000 Phytofiltration of metals. In: *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment* (Y. Raskin & B. D. Ensley, eds). John Wiley and Sons Inc., New York, NY, USA, pp. 89–106.

Epelde, L., Hernández-Allica, J., Becerril, J. M., Blanco, F. & Garbisu, C. 2008 Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. *Science of the Total Environment* **401**, 21–28. doi: 10.1016/j.scitotenv.2008.03.024.

Garbisu, C. & Alkotra, I. 2001 Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology* **77**, 229–236. doi: 10.1016/S0960-8524(00)00108-5.

Hatano, K. & Yamatsu, T. 2018 Molasses melanoidin-like products enhance phytoextraction of lead through three Brassica species. *International Journal of Phytoremediation* **20** (6), 552–559. doi: 10.1080/15226514.2017.1393397.

Hatano, K., Kanazawa, K., Tomura, H., Yamatsu, T., Tsunoda, K. & Kubota, K. 2016 Molasses melanoidin promotes copper uptake for radish sprouts: the potential for an accelerator of phytoextraction. *Environmental Science and Pollution Research* **23**, 17656–17663. doi: 10.1007/s11356-016-6904-x.

Jeevanantham, S., Saravanana, A., Hemavathy, R. V., Senthil Kumar, P., Yaashikaa, P. R. & Yuvaraj, D. 2019 Removal of toxic pollutants from water environment by phytoremediation: a survey on application and future prospects. *Environmental Technology & Innovation* **13**, 264–276. doi: 10.1016/j.eti.2018.12.007.

Matthews, D. J., Moran, B. M. & Otte, M. L. 2004 Screening the wetland plant species *Alisma plantago-aquatica, Carex rostrata* and *Phalaris arundinacea* for innate tolerance to zinc and comparison with *Eriophorum angustifolium* and *Festuca rubra* Merlin. *Enviromental Pollution* **134** (2), 343–351. doi: 10.1016/j.envpol.2004.07.023.

Mühlbachová, G. 2011 Soil microbial activities and heavy metal mobility in long-term contaminated soils after addition of EDTA and EDDS. *Ecological Engineering* **37**, 1064–1071. doi: 10.1016/j.ecoleng.2010.08.004.

Mukherjee, S., Bhattacharyya, P. & Duttagupta, A. K. 2004 Heavy metal levels and esterase variations between metal-exposed and unexposed duckweed *Lemna minor*: field and laboratory studies. *Environment International* **30**, 811–814. doi: 10.1016/j.envint.2004.01.006.

Neugschwandtner, R. W., Tlustos, P., Komárek, M., Száková, J. & Jakoubková, L. 2012 Chemically enhanced phytoextraction of risk elements from a contaminated agricultural soil using *Zea mays* and *Triticum aestivum*: performance and metal mobilization over a three year period. *International Journal of Phytoremediation* **14**, 754–771. doi: 10.1080/15226514.2011.619231.

OECD Guideline 2002 *Lemna sp*. Growth Inhibition Test, OECD guidelines for testing of chemicals, Draft Guideline July 2002. ref. No. 221.

Peverly, J. H., Surface, J. M. & Wang, T. 1995 Growth and trace metal absorption by *Phragmites australis* in wetlands constructed for landfill leachate treatment. *Ecological Engineering* **5**, 21–35. doi: 10.1016/0925-8574(95)00018-E.

Rahmani, G. N. H. & Sternberg, S. P. K. 1999 Bioremoval of lead from water using *Lemna minor*. *Bioresource Technology* **70**, 225–230. doi: 10.1016/S0960-8524(99)00050-4.

Ramos, I., Esteban, E., Lucena, J. J. & Garate, A. 2002 Cadmium uptake and subcellular distribution in plants of *Lactuca sp.* Cd-Mn interaction. *Plant Science* **162**, 761–767. doi: 10.1016/S0168-9452(02)00017-1.

Ratsch, H. C. & Johndro, R. 1986 Comparative toxicity of six test chemicals to lettuce using two root elongation test methods. *Environmental Monitoring* **6**, 267–276. doi: 10.1007/BF00396794.

Renoux, A., Tyagi, R. & Samson, R. 2001 Assessment of toxicity reduction after metal removal in bioleached sewage sludge. *Water Research* **35**, 1415–1424. doi: 10.1016/S0043-1354(00)00400-0.

Rizwan, M., Ali, S., Rizvi, H., Rinklebe, J., Tsang, D. C. W., Meers, E., Ok, Y. S. & Ishaque, W. 2016 Phytomanagement of heavy metals in contaminated soils using sunflower: A review. *Critical Reviews in Environmental Science and Technology* **46**, 1498–1528. doi: 10.1080/10643389.2016.1248199.

Soltz, E. & Greger, M. 2002 Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland species growing on submerged mine tailing. *Environmental and Experimental Botany* **47**, 271–280. doi: 10.1016/S0098-8472(02)00002-3.

Srivastava, J., Kalra, S. J. S. & Naraian, R. 2014 Environmental perspectives of *Phragmites australis* (Cav.) Trin. Ex. Steudel. *Applied Water Science* **4**, 193–202. doi: 10.1007/s13201-013-0142-x.

Strawn, D. G., Bohn, H. L. & O'Connor, G. A. 2015 *Soil Chemistry*, 4th edition. Wiley Blackwell, Chichester, UK, p. 392.

Tamás, J. & Kovács, E. 2003 Characterisation of heavy metal polluted site for a phytoremediation project. In: *New Results in the Trace Element Research: 10th International Trace Element Symposium* (I. Pais, ed.). Szent István University, Budapest, Hungary, pp. 318–333.

Tordoff, G. M., Baker, A. J. M. & Willis, A. J. 2000 Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* **41**, 219–228. doi: 10.1016/S0045-6535(99)00414-2.

Wang, Z., Liu, X. & Qin, H. 2019 Bioconcentration and translocation of heavy metals in the soil-plants system in Machangqing copper mine, Yunnan Province, China. *Journal of Geochemical Exploration* **200**, 159–166. doi: 10.1016/j.gexplo.2019.02.005.

Wong, M. H. 2003 Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* **50**, 775–780. doi: 10.1016/S0045-6535(02)00232-1.

Yadav, B. K., Siebel, M. A. & van Bruggen, J. J. A. 2011 Rhizofiltration of a heavy metal (Lead) containing wastewater using the wetland plant *Carex pendula*. *Soil, Air, Water* **39** (5), 467–474. doi: 10.1002/clen.201000385.

First received 14 November 2019; accepted in revised form 5 May 2020. Available online 18 May 2020