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Removal of heavy metals present in groundwater from a northern Mexico mining community using *Agave tequilana* Weber extracts

Remoción de metales pesados presentes en aguas subterráneas de una comunidad minera del norte de México utilizando extractos de *Agave tequilana* Weber

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Abstract

Groundwater samples from a mining community in North of Mexico were studied. Concentration of metals above the maximum allowable concentration from Mexican regulation were found. Spherical agglomeration technique (SAT) was used to remove metals (Pb, Cu, Cr, Ni, Zn, Mn, Cd). Two precipitating agents were tested: NaOH and Ca(OH)$_2$. Also, *Agave tequilana* Weber extract as hydrophobicizing agent was employed to avoid metal redisolution. High metal removal proved SAT effectiveness under pH ranging between 9-11 and extract doses from 0.3 g extract/g pollutant. Better removal percentages were reached when using Ca(OH)$_2$ as precipitating. Reported removal efficiency in that case yielded removal percentages as high as 99% removal for Pb under the three pH tested. The heavy metal evaluation index before treatment was 2354.91, but decreased to levels as low as 4.7 after SAT application.

Keywords: metal redisolution, hydrophobicizing agent, precipitation, spherical agglomeration technique, heavy metal evaluation index.

Resumen

Se analizaron muestras de agua subterránea de una comunidad minera en el norte de México. Se encontró una concentración de metales por encima de la concentración máxima permitida por la regulación mexicana. Se utilizó la técnica de aglomeración esférica (TAE) para eliminar metales (Pb, Cu, Cr, Ni, Zn, Mn, Cd). Se probaron dos agentes precipitantes: NaOH y Ca(OH)$_2$. Además, se utilizó el extracto de *Agave tequilana* Weber como agente hidrofobizante para impedir la redisolución de metales. Los altos niveles de remoción de metales demostraron la efectividad en la aplicación de TAE bajo un pH entre 9-11 y dosis de extracto desde 0.3 g de extracto/g de contaminante. Se alcanzaron mejores porcentajes de eliminación al utilizar Ca(OH)$_2$ como precipitante. La eficiencia de eliminación reportada en este caso, arrojó porcentajes de eliminación tan altos como el 99% de remoción de Pb bajo los tres pH utilizados. El índice de evaluación de metales pesados antes del tratamiento fue de 2354.91, pero disminuyó a niveles tan bajos como 4.7 después de la aplicación de la TAE.

Palabras clave: redisolución de metales, agente hidrofobizante, precipitación, técnica de aglomeración esférica, índice de evaluación de metales pesados.

1 Introduction

In recent years, there has been a considerable increase in heavy metal contamination of groundwater due to industrialization (Muya *et al*., 2016). The extraction of minerals and heavy metals has resulted in extensive contamination by heavy metals in ground and surface water (Gu, 2018). This type of contamination in a mining community in Mexico has been reported by Corral-Bermúdez *et al*. (2014). Mining produces large quantities of wastes, which contain heavy metals that are deposited inside mine tailings and exposed to the climatic and oxidizing conditions that lead to acid drainage. This in turn mobilizes heavy metals, which then penetrate the soils and sources of drinking water (Kobielska *et al*., 2018).

Heavy metal pollution is currently a major environmental problem because metal ions persist in the environment due to their non-degradable nature, their toxicity and tendency to bioaccumulate in the environment is a serious threat to the health of organisms (García-González *et al*., 2016; Ayangbenro and Babalola, 2017; Villabona-Ortiz *et al*., 2019), due to this, they must be removed from the water. Some techniques, such as chemical precipitation, membrane filtration and adsorption, etc., have been used to remove heavy metal ions from aqueous systems (Luo *et al*., 2015; Cruz *et al*., 2018; Hernández-Botello *et al*., 2020). However, these techniques have disadvantages such as high sludge volume, high costs for the use of reagents or membranes and extended treatment times (Carolin *et al*., 2017).

Water treatment by means of the Spherical Agglomeration Technique (SAT) is an alternative because it reduces the aforementioned disadvantages, since it offers high levels of heavy metal removal and the formation of an insoluble phase in the liquid medium thanks to an hydrophobicizing agent, for example, saponins (Bailón-Salas *et al*., 2018). The SAT is a process of formation of aggregates, which are held together by liquid bridges, in which discrete particles agglomerate to form large granules (Krishna and Chandra, 2018). It consists of four stages: 1) heavy metals precipitation (15 min), performed by a precipitant agent in a specific pH range; 2) hydrophobyzation step (30 min), that changes the nature of the surface of the metal hydroxide from hydrophilic to hydrophobic using a surfactant (saponins); 3) wetting stage (15 min), in this stage, the hydrophobic chains of the surfactant are unified trough a bridging liquid (n-heptane in this case); and 4) the agglomeration step (90 min), where an initiator (Ca^{2+}) allows the free ions react with the surfactant in the solution, forming the agglomerates, thanks to the change and redistribution of the charges. Under appropriate physicochemical conditions (such as temperature, pH magnitude and stirring speed), the particles to be obtained can be selectively agglomerated and removed from the sludge (Rosseti *et al*., 2003; Gonzalez Valdez *et al*., 2013; Alcazar-Medina *et al*., 2014).

The *Agave tequilana* Weber is perhaps the best known of the more than 140 different species of Agave due to its use in the production of tequila, however after the rest of the plant is harvested, the leaves of *A. tequilana* are not used for any industrial process, and are routinely left in the field (Close *et al*., 2017). In 2016, 273.3 million liters of tequila were produced in Mexico, from 941.8 metric tons of raw material (Trejo *et al*., 2018). Around 54% of the agave plant is the agave head that serves as a raw material for the production of tequila, while the rest of the plant is discarded (Iñiguez-Covarruvias *et al*., 2001). The content of saponins in organic extracts from the plant is appreciable (Herbert-Doctor *et al*., 2016; Velázquez *et al*., 2019).

The objective of this work was to investigate the removal of heavy metals from groundwater in a mining community, through SAT, using extracts from *Agave tequilana* Weber leaves as hydrophobicizing agent. In such way, those results will allow us to develop a possible solution for the water pollution problem caused by mining, while giving a second use to biological waste from tequila production industry.

2 Materials and methods

2.1 *A. tequilana Weber extract preparation*

2.1.1 *Foliar material collection*

Leaves of *A. tequilana* Weber were selected from standing plants of the municipality of Arandas, Jalisco (20°42'39" N, 102°23'54" W), where the species is abundant and plantations are easily accessed. Leaves were deposited in plastic containers and stored at low temperature (4 ºC) until processing. The foliar plant material was positively identified as *Agave tequilana* Weber in the herbarium of CIIDIR IPN Durango.

2.1.2 *Extraction and identification of compounds*

After removing the thorns, leaves were washed and cut into pieces that were subsequently dried at 40 °C. The ethanolic extraction was carried out according to the methodology established by Hernández *et al*. (2005). The presence of saponins in the extract was checked by the foam formation test according to Tadhani and Subhash (2006): a volume of 5 ml of ethanolic extract was mixed with 10 ml of water in a test tube. The appearance of foam on the surface of the liquid after vigorously stirring the mixture, denotes the presence of saponins in the extract.

For the identification of saponins, ethanolic extracts from leaves were analyzed in an Agilent 1100 series HPLC/DAD equipment. Volumes of 20 μL of extract were taken to determine the saponin profile, using a gradient method of acetonitrile-water (0% B, 0.5 min; 9% B, 12 min; 13% B, 20 min; 32% B,

40 min; 33% B, 42 min; 43% B, 60 min; 0% B, 65 min), with solvent B being acetonitrile, using a C18 Brownlee Analytical column (4.6×250 mm, 5 μm). The chromatograms were recorded at 260 nm, also, in order to be able to compare the retention times of the peaks in the chromatograms, the spectral data of the sapogenin glycoside standard in the HPLC/DAD equipment were established under a range of 220-400 nm, according to the methodology used by Gonzalez-Valdez *et al.* (2013). Retention times of the compounds in the extract were compared against the peaks observed in the chromatogram of the sapogenin glycoside standard of Quillaja saponaria (Hycel de México, SA de CV, Cat. 6700, CAS No. 74499-23-3, Mexico) (Oleszek, 2002). The extract solution used in the second stage of the SAT was obtained by dissolving 10 g of dry extract in 1 L of distilled water.

2.2 Collection and characterization of groundwater samples

Groundwater samples used in the study were extracted from a well in the San José de Avino community in the Pánuco de Coronado municipality, Durango (well coordinates 24°31'26.1" N and 104°17'54.2" W) (Fig. 1). Sampling point was chosen based on previous studies indicating poor water quality and high metal concentration in the area (Corral-Bermúdez *et al.*, 2014).

Sampling was performed according to the specifications of NOM-014-SSA1-1993, pH and electrical conductivity (EC) were determined with a portable HQ40d device (HACH). Samples were preserved in acidic medium and refrigerated (4 °C).
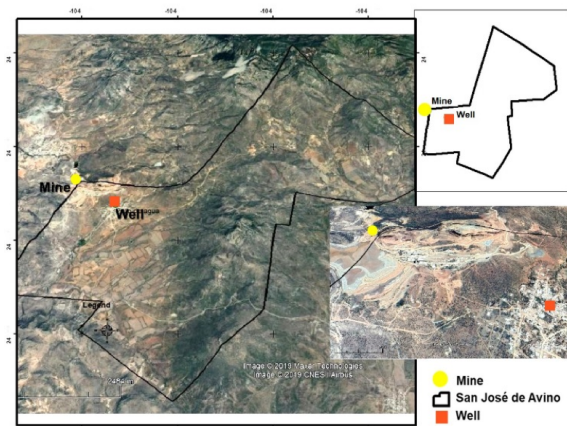

Fig. 1. Well and mine location. The black line polygon signals San José de Avino town land.

The metals and elements of toxicological importance measured in samples were manganese, lead, zinc, copper, cadmium, chromium, and nickel. Analysis was performed by Atomic Absorption Spectrophotometry (AAS) according to NMX-AA-051-SCFI-2001 (SCFI, 2001), through PERKIN-ELMER brand equipment, model AAnalyst 700.

2.3 Removal of heavy metals in groundwater samples

Heavy metal removal experiments in well water samples were carried out using 500 mL flasks with deformed walls containing in a 250 mL sample volume. Constant agitation was provided by 3.5×2.5 cm stainless steel propellers rotating at 600 rpm throughout the experiment. Room temperature (20 ± 2 °C) was also maintained (Alcázar-Medina *et al.*, 2020).

Successful SAT application requires four stages to achieve complete encapsulation and removal of the contaminant without the possibility of redisolution (Proal-Nájera *et al.*, 1997). In the first stage of the SAT, the pH of the groundwater samples was adjusted to one of the three values to be tested (9, 10 and 11). The pH increase was achieved by adding one of the tested precipitating agents: NaOH and $Ca(OH)_2$. After the required pH was reached, agitation was kept for 15 min before proceeding to the next step.

In the second stage of the process (30 min), a dosage of 0.3, 0.5 or 1.0 g extract/g contaminant (g of extract per gram of metal present in water) was added in order to hydrophobicize the system. The dose of hydrophobizing agent (extracts) is the third of the factors considered in the research experimental design.

The wetting and conditioning stage of the hydrophobic solids (third stage of the SAT, 15 min) were carried out by adding n-heptane (C_7H_{16}), at a ratio of 6.3 mL per gram of contaminating metal (Me^+) present in the water sample. Once the wetting agent was added, the experiment was kept under constant stirring for 20 min. Finally, the growth stage of the crystalline nuclei by agglomeration was achieved by adding 10 mL of 1M $CaCl_2$ per gram of metal present in the aqueous solution (Fig. 2) (Alcázar-Medina *et al.*, 2020). The experiment kept going for 90 more min under constant agitation.
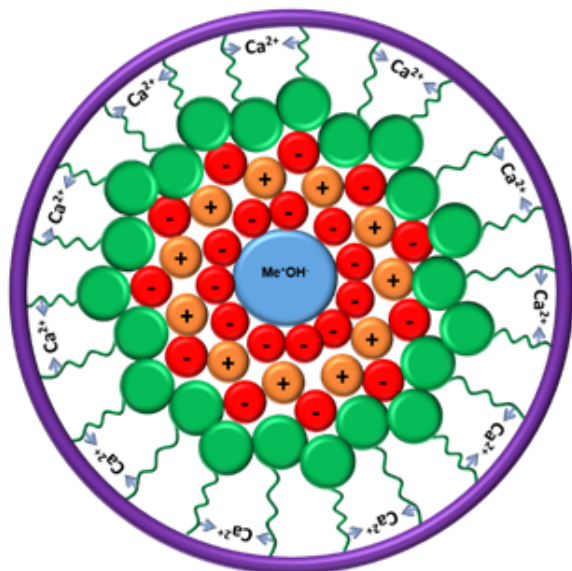
Fig. 2. Charge redistribution at the end of SAT application.

At the end of the described process (150 min), solution was filtered using Whatman #42 filter paper, to remove the formed agglomerates and determine, in the liquid phase, the residual concentration of metals by AAS.

2.3.1 General removal evaluation

To evaluate the scope of heavy metal presence in the sample, the heavy metal evaluation index (HEI) was used; the HEI uses the ratio of concentration of every metal and the concentration limit established by the WHO for drinking water (Edet and Offiong, 2002; Ahmadi *et al*., 2018).

HEI can be calculated as follows (Ec. 1):

$$HEI = \sum_{i=1}^{n} \frac{M_i}{S_i} \qquad (1)$$

where S_i represents the maximum allowable concentration (MAC) established by the WHO and M_i the measured metal concentration in the sample (Jahanshahi and Zare, 2015).

2.3.2 Statistical analysis

An AxBxC factorial design was used to determine the effects of each factor and the interaction among them on the metal removal (Anderson and McLean, 2018). The analyzed factors were precipitation pH (A), *A.*

tequilana extract dose (B), and precipitant agent used in the processes (C).

Using the general linear method for the experimental design, generated by Statistica® version 7 software, multiple analyzes of variance (MANOVA) were performed to explain the interactions given. MANOVA was validated by the verification of the Pearson determination coefficient (R^2), as well as the analysis of variance for each of the factors, using Fisher's F. Gauss-Markov assumptions (normality, independence and homogeneity of variance) were verified using, subsequently, the minimum significant difference test to evaluate the comparison of means. Finally, a response surface with a polynomial model was obtained, based on the experimental data.

3 Results and discussion

3.1 A. tequilana Weber extract preparation

The yield obtained with respect to the ethanolic extract of foliar material of *A. tequilana* was 16.63%, which is within the range (12.3 to 29.7%) reported by Gonzalez-Valdez *et al*. (2013) for some agaves. The foam test performed according to Tadhani and Subhash (2006), yielded a positive result for the presence of saponins, since the formed foam remained stable for over 30 min on the liquid surface.

Saponin peaks in the chromatograms were identified by HPLC/DAD, comparing the retention times of those characteristic peaks against those of the standard used. When comparing the chromatographic profile obtained by HPLC/DAD for the reference standard against the chromatographic profile obtained for *A. tequilana* extract, two different saponin compounds were detected. Reproducibility was always observed in the chromatograms obtained for the extracts of *A. tequilana*, since the foliar material used comes from the same sampling site; besides, extraction temperature was the same and kept constant during the extraction, which agrees with Hernández *et al*. (2005). Retention times found in this research (34.33 and 38.29 min) match to the retention times characteristic of saponins reported by other researchers (Gonzalez-Valdez *et al*., 2013; Alcazar-Medina *et al*., 2014).

Table 1. Initial pH, conductivity and heavy metal concentration values found in groundwater samples.

	Initial value	Mexican regulation	WHO
pH	5.1	6.5-8.5	6.5-8.5
Conductivity (μS/cm)	4.51	0.06-0.1	NA
Pb (mg/L)	2.1392	0.01	0.01
Cu (mg/L)	210	2	2
Cr (mg/L)	0.0605	0.05	0.05
Ni (mg/L)	0.322	NA	0.07
Zn (mg/L)	4.78	5	3
Mn (mg/L)	111.25	0.15	0.4
Cd (mg/L)	5.25	0.005	0.003

NA: not available

3.2 Characterization of groundwater samples

Table 1 shows the results obtained from the analysis of the groundwater samples used in the study and the MAC by the Mexican regulation (SSA, 2000) and the established for drinking water by the World Health Organization (WHO, 2011).

As table 1 shows, groundwater samples exceed the permissible limits established by the WHO in all chemical parameters tested. Although the limits established by the Mexican regulation are less strict, the sample exceeds the limits in 6 of the measured elements, some as Pb in more than 100 times and Cd in 1000 times.

3.3 Removal of heavy metals in groundwater samples

3.3.1 NaOH as precipitating agent

Results from the heavy metal removal percentages when using NaOH as precipitating agent by modifying pH value are shown in table 2. Final pH value was always between 6.8 and 7.6, thus complying with the established limit set by the Mexican regulation and the WHO. Conductivity final values were always above the established limit due to the high remaining concentration of cations in the solution.

When using NaOH, Cu removal efficiency was found in a range of 98 - 99.79%, which was similar to that obtained in previous works in aqueous models by Bailón-Salas *et al*. (2018), using an extract of foliar material from *Yucca descipiens* Trel. (99.96%) and by Alcazar-Medina *et al*. (2014), using extract of foliar material from *Agave lechuguilla* Torr. (99.97).

Likewise, removal efficiencies were similar to 99.5% removal reported by Li *et al*. (2017), by using zero-valent nanoscale iron. According to Cuppett *et al*. (2006), the preferential form at low pH levels (typically below pH 6) is the cupric ion (Cu^{2+}). Thus, at the beginning of the experiment and according to sample pH, Cu^{2+} was present in groundwater sample. At pH levels 6.5-12, metal precipitates most frequently as copper hydroxide (Cuppet *et al*., 2006); after adding precipitating agent and reaching higher pH, Cu precipitated as copper hydroxide, thus yielding the high Cu removal percentages showed in the study.

Pb removals of 99.98% were achieved. These removal values were similar to Pb adsorption on sepiolite-supported nanoscale zero-valent iron, with an efficiency of 99.9% (Fu *et al*., 2015); but superior to those obtained by adsorption chitosan-coated montmorillonite beads in single- and multi-metal system (94.08%) (Tsai *et al*., 2016).

The only metal that shows a remarkably better removal in pH 9 is nickel, coinciding with Balladares *et al*. (2018), who reported maximum Ni removal under the same pH. Average heavy metal removal from the 3 extract concentrations presented the next patterns when adding NaOH as precipitating agent under pH 9: Mn<Cd<Ni<Zn<Cr<Cu<Pb, pH 10: Ni<Zn<Cd<Mn<Cr<Cu<Pb and pH 11: Ni<Zn<Cr<Cu<Cd<Mn<Pb.

3.3.2 Ca(OH)$_2$ as precipitating agent

Ca(OH)$_2$ is a strong base which dissociates in water according to the next reaction (Chen *et al*., 2009):

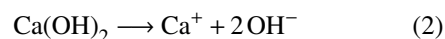$$Ca(OH)_2 \longrightarrow Ca^+ + 2\,OH^- \qquad (2)$$

Table 2. Removal % for every extract dose and pH with NaOH as precipitating agent, in experiments carried out at room temperature and a constant agitation of 600 rpm.

Extract Dose	pH	Pb (%)	Cu (%)	Cr (%)	Ni (%)	Zn (%)	Mn (%)	Cd (%)
0.3	11	99.79±0.10	99.79±0.02	97.19±0.24	64.05±9.77	95.72±0.24	99.59±0.42	99.16±0.68
0.5	11	99.98±0.02	99.68±0.05	99.00±0.70	47.77±5.64	95.75±0.19	99.98±0.02	99.49±0.83
1	11	99.88±0.06	98.93±0.02	98.85±1.10	41.26±5.64	95.88±0.14	99.94±0.01	99.98±0.00
0.3	10	99.99±0.01	99.77±0.03	98.44±0.14	57.54±14.92	95.91±0.24	98.18±0.23	98.44±0.60
0.5	10	99.95±0.03	99.59±0.02	97.38±2.08	41.26±5.64	95.66±0.16	97.85±1.32	97.53±1.23
1	10	99.91±0.04	98.90±0.03	98.93±0.13	86.84±5.64	95.25±0.29	96.02±1.43	95.90±1.22
0.3	9	99.91±0.02	99.72±0.03	98.29±0.23	77.08±5.64	91.57±2.08	70.97±11.29	88.56±9.36
0.5	9	99.87±0.00	99.44±0.03	98.52±0.10	83.59±0.00	89.06±2.29	65.85±5.67	76.17±4.41
1	9	99.89±0.02	98.59±0.05	98.86±0.32	80.33±5.64	89.09±1.62	68.57±5.96	78.77±3.22

Then, metal hydroxides can form through a series of interactions among hydroxyl ions and divalent or trivalent cations (Balladares *et al.*, 2018):

$$Me^{2+} + 2OH^- \longrightarrow Me(OH)_2 \qquad (3)$$

$$Me^{3+} + 3OH^- \longrightarrow Me(OH)_3 \qquad (4)$$

So, stoichiometrically, better results were to be expected when using this hydroxide as precipitating agent given that yields formation of 2 OH- instead of one, as obtained when using NaOH. Table 3 shows the general removal results. General removal % when using Ca(OH)$_2$ were remarkably better than the ones from NaOH. Removal percentages for Cr and Ni are not shown as the final values for these metals were lower that the detection limit for the measurement technique employed. Final pH average values were 8.1 in the experiments with initial pH of 9, 8.5 in experiments with pH starting at 10 and 9 in experiments with initial pH of 11, being this last value above the established limit for drinking water. Conductivity was again above the allowed limit due to an elevated number of cations present.

High chromium removal was achieved. Final concentrations of the element were in all cases below the quantification limit of the method used in the measurement, which was 0.2 ppb. These values were

higher than those obtained with entrapped nanoscale zero-valent iron in chitosan beads (98.4%) (Liu *et al.*, 2010), through adsorption with Zero valence iron nanoscale supported by sepiolite; and an efficiency superior to that reported by Kaya *et al.* (2016), using the polymer inclusion membrane (97.69%).

The application of the SAT was also effective for zinc removal with the use of calcium hydroxide, yielding removal levels slightly higher than 97.70%. When chitosan-coated montmorillonite beads were used in a multi-metal system, 96% removal was reached in batch experiments (Tsai *et al.*, 2016); when using hydrogel modified biochar, at pH 8 and an adsorbent dose of 10 g/L, a 95.86% zinc removal was achieved (Sanyang *et al.*, 2014). Removal values in this research are also higher than those reported by Iconaru *et al.* (2018) when hydroxyapatite was used in aqueous media (40.77%).

Cu concentration at the end of treatment with an extract dose of 0.3 g extract/g pollutant was low enough to comply with regulations, decreasing treatments costs. When starting the process in pH 11, final pH above 8.5 results, but in lower pH, Mn and Cd removals decreased as well.

Table 3. Removal % for every extract dose and pH with Ca(OH)$_2$ as precipitating agent, in experiments carried out at room temperature and a constant agitation of 600 rpm.

		Pb (%)	Cu (%)	Cr (%)	Ni (%)	Zn (%)	Mn (%)	Cd (%)
Extract Dose	pH							
0.3	11	99.78±0.15	99.31±0.08	>99.95	>99.95	97.70±1.98	99.8±0.08	>99.95
0.5	11	99.35±0.17	99.05±0.12	>99.95	>99.95	88.18±5.57	99.8±0.05	>99.95
1	11	98.43±0.20	97.13±0.07	>99.95	>99.95	74.21±6.32	98.4±1.15	>99.95
0.3	10	99.54±0.34	99.40±0.05	>99.95	>99.95	87.12±4.67	95.1±1.05	>99.95
0.5	10	99.17±0.11	98.71±0.04	>99.95	>99.95	85.91±2.78	95.0±0.10	>99.95
1	10	95.84±0.29	96.80±0.04	>99.95	>99.95	87.86±4.22	96.5±0.85	>99.95
0.3	9	99.76±0.08	99.30±0.01	>99.95	>99.95	72.63±3.00	81.5±3.80	99.43±0.14
0.5	9	98.58±0.08	98.50±0.07	>99.95	>99.95	57.79±6.32	77.9±0.89	99.37±0.04
1	9	97.71±1.24	96.65±0.24	>99.95	>99.95	46.13±2.62	75.7±3.20	99.40±0.15

Removal efficiencies followed the next pattern: pH 9: Mn<Cd<Zn<Cu<Pb<Cr<Ni, pH 10: Mn<Cu<Cd<Zn<Pb and pH 11: Cu<Zn<Mn<Cd<Pb. Cr and Ni were not considered as their concentration were below the quantification limit of the analytical method.

3.3.3 General removal comparison

General heavy metal removal results, when using different precipitating agents, are shown on fig. 3. Even though stoichiometrically better results were presumed when using Ca(OH)$_2$ and considering individual removal percentages, general results showed that this assumption is true when working with initial pH of 10 and 11, but not in lower initial pH, when better results were obtained with NaOH.

A similar story is told by the results from the HEI calculation (table 4). The higher the pH, the lower the HEI from the treated sample. Before SAT application, HEI from samples reached a value of 2354.91; after treatment, a remarkable decrease in HEI was observed. As was expected, lower HEI values were reached under the application of Ca(OH)$_2$, except for initial pH 9 and a dose of 0.3 g extract/g pollutant, where NaOH performed better and achieved higher removal percentage and lower HEI than Ca(OH)$_2$; and pH 11

with a dose of 1 g extract/g pollutant, where again, NaOH yielded lower HEI, but removal percentage remained higher for Ca(OH)$_2$ application.

As removal percentage calculation does not consider the MAC, such parameter gives an accurate idea of the general SAT efficiency, whereas HEI reflects the ratio among concentration measured and concentration accepted by the regulation. Consider, for example, a heavy metal with a very high initial concentration, hundreds of times above the MAC that is removed to around 99% and still, its final concentration is several times above MAC.
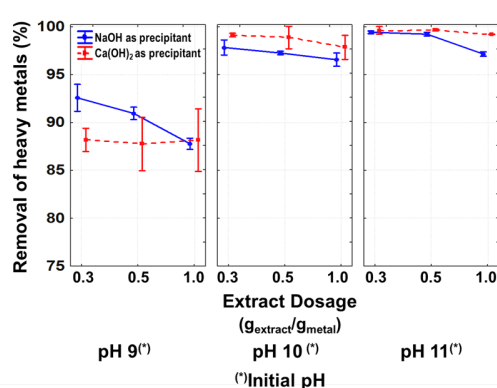

Fig. 3. General removal percentages under different SAT conditions.

Table 4. HEI under different SAT conditions*.

pH	Extract dose	HEI	
		NaOH	Ca(OH)$_2$
9	0.3	282.4	290.5
9	0.5	513.4	326.1
9	1	460.2	320.5
10	0.3	34.1	22.1
10	0.5	52.7	32.8
10	1	86.4	27.4
11	0.3	16.9	5.4
11	0.5	12.9	5.7
11	1	4.7	16.3
Mean value		162.6	116.3

*HEI from initial sample: 2354.91.

At the same time, an element with a low initial concentration not that far from MAC, when poorly removed, will affect greatly the value for removal percentage but its contribution for HEI value will be negligible.

In the past, HEI has been used to establish a criteria to classify water samples according to their level of heavy metal contamination. Limit values for low, medium and high contamination level are usually set by considering the mean HEI value. Edet and Offiong (2002), developed the HEI to classify surface water samples and set the limits to be HEI<400 for low risk, between 400 and 800 for medium risk and above 800 for high risk. Bhuiyan *et al*. (2010) studied groundwater from the vicinity of a coal mine and set HEI<40, between 40 and 80, and above 80, for low, medium and high risks, respectively. In 2015, Singaraja *et al*. worked with groundwater from coastal aquifers and got a mean HEI of 124, thus setting the HEI under 100 for low, between 100 and 200 for medium, and above 200 for high risks.

In the present study, limits were set as follows: for NaOH as precipitating agent, HEI under 160

is cataloged as low risk, between 160 and 320 for medium and above 320 for high; for Ca(OH)$_2$, values were set as below 120 for low risk, between 120 and 240 for medium and above 240 for high. Comparing our results with the limits set by Bhuiyan *et al*. (2010), samples treated with initial pH of 10 or 11, and doses of extract of 0.3 and 0.5 g extract/g contaminant, fall in the low or medium risk category. HEI from original groundwater sample is cataloged as being high risk according to all consulted literature.

Considering HEI and removal percentages, authors from present study have considered the best SAT conditions for these experiments to be initial pH 11 and application of 0.3 g extract/g pollutant, when Ca(OH)$_2$ is used as precipitating agent.

3.3.4 Statistical analysis

Residuals analysis of the 3x3x2 random block model fulfilled assumptions of normality, independence and homogeneity of variance. Coefficient of variance for the experimental series performed was greater than 0.090, thus indicating a high level of confidence in these experiments.
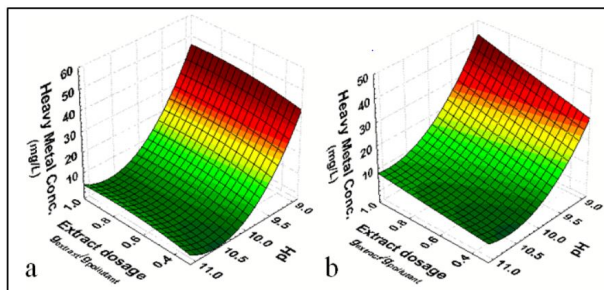

Fig. 4. Response surface plots for SAT application using *A. tequilana* Weber extracts and NaOH (a) and Ca(OH)$_2$ (b) as precipitating agents under different pH and extract addition.

Fig. 4 shows the response graph for the SAT application when using NaOH (Fig. 4a) and $Ca(OH)_2$ (Fig. 4b) as precipitating agents in the removal of heavy metals from groundwater.

As can be seen from figure 4, lower heavy metal concentration are expected in all pH and extract dose combination when using $Ca(OH)_2$ as precipitating agent, thus this chemical is recommended by the authors in this kind of process. Heavy metal final concentration depends upon the factors tested according to equations (5) (when NaOH was used) and (6) (when precipitating agent is $Ca(OH)_2$):

$$
\begin{aligned}
FC_{NaOH} = &\ 1698.6876 + 43.34 * d - 323.31 * pH \\
&- 14.8458 * d^2 - 1.86 * d * pH + 15.3087 pH^2
\end{aligned}
\tag{5}
$$

$$
\begin{aligned}
FC_{Ca(OH)_2} = &\ 1022.4267 + 76.9648 * d - 194.2343 * pH \\
&+ 0.9153 * d^2 - 6.4322 * d * pH + 9.2125 * pH^2
\end{aligned}
\tag{6}
$$

where FC represents the final concentration of the metals measured, d stands for extract dose and pH is the initial pH of the experiment.

The MANOVA showed statistically significant differences among the values of the 3 factors tested, as well as its double and triple interactions, which indicates that statistically different removal percentages will be reached when applying different values for these parameters.

3.4 Environmental implications

According to Chen *et al.* (2009), hydroxyl ions generated through NaOH and $Ca(OH)_2$ dissociation in water react with metals to form metal hydroxides. Amphoteric metals like Cr, Zn and Pb would redissolve under pH changes outside their pH precipitation values. Under SAT, not such precipitation occurs given that the metal hydroxide is encapsulated by the hydrophobicizing agent, tequilana extract in this research, and agglomerate charges neutralized by later SAT stages. This way, it was possible to efficiently use a residue from the tequila production in the removal of heavy metals from groundwater.

Conclusions

SAT application proved to be efficient in the removal of heavy metals present in real water samples at different pH levels and under a considerable dosage range of surfactant (0.3-1.0 g of extract/g of metal).

The effect of the precipitating agent, either NaOH or $Ca(OH)_2$, on the removal of heavy metals, directly affects the final concentration of each of the metals present in the system. However, the application of $Ca(OH)_2$ as a precipitating agent showed, in general, better results and yields than the NaOH agent, since lower concentration of the former is used to reach the optimum precipitation pH, reaching a lower residual concentration of metals. pH is the most important parameter in heavy metal removal by SAT, effect attributed to metal precipitation. Nevertheless, in order to avoid metal redisolution, all four SAT stages are necessary.

HEI can be lowered in order to achieve levels of medium or low risk in certain conditions. According to MANOVA, the three factors tested as part of the experimental design and the interactions among them, have a significant effect over the heavy metal removal. Best SAT conditions for these experiments are initial pH 11 and a dose of 0.3 g extract/g pollutant, when $Ca(OH)_2$ is used as precipitating agent.

Nomenclature

AAS	Atomic absorption spectrophotometer
d	Extract dose
EC	Electrical conductivity
FC	Final metal concentration
HEI	Heavy metal evaluation index
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
MAC	Maximum allowable concentration
MANOVA	Multiple analyzes of variance
Mi	Measured metal concentration in the sample
Me^{2+}	Divalent metal ions
Me^{3+}	Trivalent metal ions
R^2	Pearson determination coefficient
S_i	Maximum allowable concentration
WHO	World Health Organization

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