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Clean-up of bodies of water contaminated by heavy metals using dead and live hyperaccumulators (flowering plants and algae)

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Abstract
The pervasive issue of water contamination by heavy metals poses significant environmental and public health challenges, requiring innovative and effective remediation strategies. This study aims to compare the use of dead and live hyperaccumulators that could be implemented as an environmentally friendly, practical and economically viable method for the removal of heavy metals from contaminated bodies of water; in order to do so, *Helianthus annuus, Cynodon dactylon, Jacaranda mimosifolia* (dead) and *Spirulina* sp. (alive) were selected and employed due to their phytoremediation properties. The results demonstrate a significant reduction in Cd, Cu, Pb, and Zn levels in the water samples, further demonstrating the potentials of kinds of hyperaccumulators as a sustainable solution for cleaning heavy metal contaminated bodies of water.

Keywords
Hyperaccumulators, heavy metals, absorption, dead, live, water

Introduction

Usually, the cleaning of bodies of water consists only of the removal of plastics from them; in some more isolated cases, oils are also cleaned from their surfaces, but this only creates an illusion of cleanliness; while not to be underestimated, and ultimately better done than not, these efforts often forget that while the surface of the water is clean, the water per se is still contaminated. Contamination can be both biological and chemical, however between the two there are, in most cases the chemical is the one that is hardest to treat, being heavy metals some of the more serious contaminants due to their harmful effects in low quantities, their hardness to remove, the exponential increase of their presence in the ambient among others. Therefore and in other to contribute to the solution of this problem, we found inspiration in phytoremediation, which consists of "taking advantage of the capacity of certain plants to absorb, accumulate, metabolise, volatilise or stabilise pollutants present in the soil, air, water or sediments..." (Delgadillo-López et al., 2011); we believe it has been misunderstood, usually only using one agent at a time, when in reality several could be used simultaneously, thus achieving greater efficiency in the method. In this way, it is possible the recovery of bodies of water for both human and endemic species use, while at the same time avoiding problems such as the creation of chemical sludge (precipitation and mixing of metals with the bodies of water sediment when trying to decontaminate them), or the use of other chemical agents that can be harmful. Yet, it was hard to decide which hyperaccumulators to use because, and as we consider, there are two main types; dead hyperaccumulators, acting more like an sponge by absorbing the

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heavy metal ions, and the live ones, which ingest the ions and overtime start decomposing, using or just absorbing them; in order to know their advantages and disadvantages contrasted to the other, we decided compare them side to side in a fairly identical scenario. This does not change our view in the joint utilizations of hyperaccumulators and consequently, we believe that by placing the selected hyperaccumulators in the heavy metal contaminated water, the heavy metal ions will be attached to the structures of the dead hyper-accumulators and consumed by the live one, making it possible to remove the heavy metals from the water in which they are found and measure said removal, determining which performs the best taking into consideration the circumstances in which they could be used.

Limitations of the study

It is important to bear in mind that when non-native species are introduced into the environment, in this case the spirulina sp. which must be used alive, they must be contained or highly monitored to prevent them from becoming invasive species or disrupting the ecosystem, thus generating more harm than good; factor that has been considered in the planning and research. Due to the limited budget available for this research, measurements could only be made for groups of hyperaccumulators and not each individual one, however there have been previous studies regarding the capabilities of each one; also, only four metals were measured, which were selected because they, as we consider, are not only the most common but also some of the most harmful to the environment and people.

Materials and methods

In the present study the heavy metals studied were Cd, Cu, Pb and Zn, whereas the hyperaccumulators used were *Helianthus annuus*, *Cynodon dactylon*, *Jacaranda mimosifolia's* flowers (all the mentioned before dead and excluding the roots; for *Helianthus annuus* the leaves were also excluded) and *Spirulina* sp. (alive). The totality of materials used is listed in Table 1.

Table 1
Full list of materials used

Organic matter	Inorganic matter
100 g of *Spirulina* sp. (obtained through private cultivation)	100 cm of cotton thread
200 g of *Helianthus annuus* (obtained through private cultivation)	5 coffee filters
100 g of *Cynodon dactylon* (obtained from Colonia Santín, Toluca)	2004 ml of distilled water
100 g of flowers of *Jacaranda mimosifolia* (obtained from trees in the Ciudad Universitaria, Mexico City and Colonia Santín, Toluca).	7 mg of Cd
100 g of flowers of *Jacaranda mimosifolia* (obtained from trees in the Ciudad Universitaria, Mexico City and Colonia Santín, Toluca).	13 mg of Cu
	7 mg of Pb
	25 mg of Zn
	4 ml of HNO_3 (nitric acid)

Experimental design

Two beakers were first treated with nitric acid, dissolved at a 1:1 ratio with distilled water, to prevent the metal ions from adhering to their walls, then 1000 ml of distilled water were added to each. Subsequently 5 g of Cd, 10 g of Cu, 5 g of Pb and 20 g of Zn were mi-xed into one beaker (water sample #1) and 2 g of Cd, 3 g of Cu, 2 g of Pb and 5 g of Zn were mixed into the other one (water sample #2); it was chosen to mix different amounts of heavy metals into the samples due to the negative effects it could have in the spirulina sp. and considering the difference in mass of the hyperaccumulators; next the pH of both water samples was measured to see if the proposed

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method has any influence in it. Afterwards, 200 g of the dead hyperaccumulators (100 g *Helianthus annuus*, 50 g *Cynodon dactylon* and 50 g *Jacaranda mimosifolia*) were added into each of the two bags, each made of two coffee filters and sewn together with thread to avoid the spreading of the hyperaccumulators facilitating their extraction, as shown in Figure 1; another bag was also made, this time with a single coffee filter in which 100 g of *Spirulina* sp. were placed, being the bag of the live hyperaccumulator. Subsequently, the first bag of dead hyperaccumulators was submerged, in its entirety, in the water sample #1 for a total of 120 seconds, after the time has passed it was extracted and pressed in order to remove all the water it could have absorbed, then the pH of the solution was measured. For the second bag of dead hyperaccumulators the above steps were repeated and it was also submerged in water sample #1. At the end of the second pH measurement of the solution, an aliquot was separated and dissolved, due to measurement requirements, to a 1:3 aliquot-distilled water ratio and later marked as treatment sample #1. Afterwards, the bag with the spirulina sp. was introduced into the water sample #2, in which was kept submerged for 5 minutes; once this time had elapsed, the bag was removed, the pH of the solution was measured, an aliquot was separated and dissolved to a 13:37 aliquot-distilled water ratio, then labelled as treatment sample #2. Next, the treatment samples were sent to a laboratory certified by the EMA to measure the heavy metals concentrations; This was done by Atomic Absorption Spectrometry according to the standards of Mexico's Ministry of Economy. It is worth mentioning that all the bags were properly discarded after their use.

Figure 1. *The two dead hyperaccumulators and the smaller livehyperaccumulator bags.*

Results

Measurements

To obtain the correct data, and because the samples had to be dissolved to make the measurements, we calculated the real concentrations following Eq. [1]:

$$a = \frac{b + c}{d} \qquad [1]$$

where a is the actual metal concentration in mg, b is the ml of water of which the concentration to be known, c is the mg of metals in the aliquot dissolved and d is the ml of water of the aliquot.

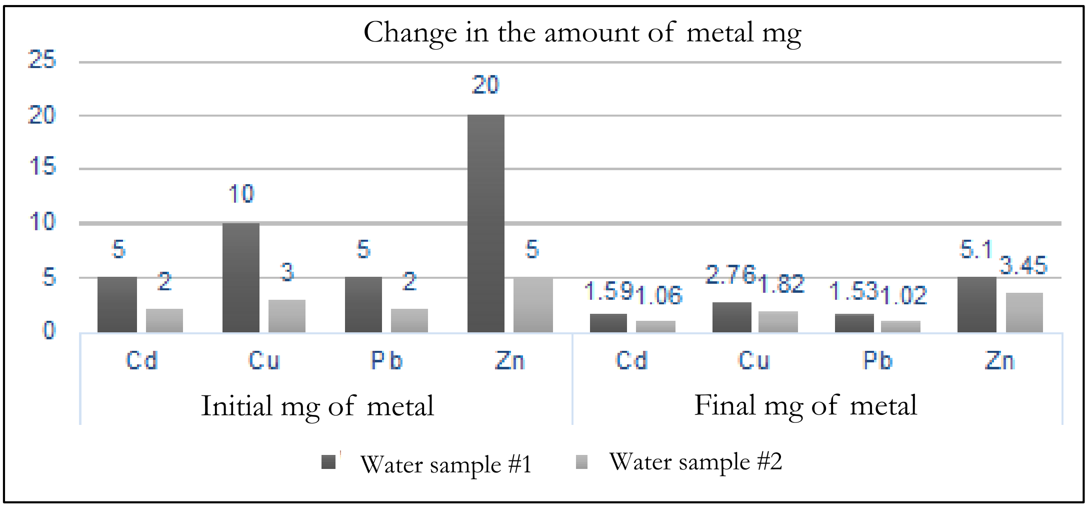

Figure 2 *Initial and final concentration of heavy metals per Liter of water in the two water samples*

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Changes in the samples

The total metal concentration in the treatment sample #1 decreased by 72.5% on average respect to the original one; similarly, the total metal concentration in the treatment sample #2 decreased by 38.7% on average with respect to the original one. The individual decrease per metal is shown in Figure 2 for each treatment sample and the overall for dead and live hyperaccumulators is show in Table 2.

For the water sample #1, the colouring of the solution changed from transparent at the beginning to a light orange shade after adding the first bag of dead hyperaccumulators, it changed again when the second bag was added to a darker shade of orange; It did not change when the bag with the live hyperaccumulator was added. The original pH of both water samples marked a value of 1, which did not change throughout the measu-rements done.

Table 2. *Performance of each hyperaccumulator group*.

Hyperaccumulator group	Total mass of the hyperaccumulators	Initial total massof metals	Total massof metal absorbed	mg of metal absorbed per g of hyperaccumulator mass
Live	100 g	12 mg	4.65 mg	$4.65 * 10^{-2}$
Dead	400 g	40 mg	29.02 mg	$7.255 * 10^{-2}$

Discussion

Although there is and important difference between the capabilities to absorb the heavy metals between dead and live hyperaccumulators, being the dead ones the group that overperformed compared to the live one, it is fair to remark the fact that the live hyperaccumulators have the cappability to continuing absorbing and digesting the heavy metals as long as they are given time, while the dead hyperaccu-mulators have a fixed amount of heavy metals they can absorbe and once reached, the absorbance will irremediably finish. Yet this doesn't mean they necessarily have to be used nor understood separately, if the joint absorption of heavy metals is measured it shows an average reduction of 64.7%. If analyzed as so, the results would corroborate the effectiveness of a joint treatment by theoretically managing to de-

Table 3. *SEMARNAT water quality standards (SEMARNAT, Normas Oficiales Mexicanas, 2013)*

MAXIMUM PERMISSIBLE LIMITS FOR HEAVY METALS AND CYANIDES																				
PARAMETERS (*)	RIVERS						NATURAL AND ARTIFICIAL RESERVOIRS				COASTAL WATERS						SOIL			
	Use in agricultural irrigation (A)		Urban public use (B)		Protection of aquatic life (C)		Use in agricultural irrigation (B)		Urban public use (C)		Fishing, navigation, and other uses (A)		Recreation (B)		Estuaries (B)		Use in agricultural irrigation (A)		Natural wetlands (B)	
mg/l	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.	D.A.	M.A.
As	0.2	0.4	0.1	0.2	0.1	0.2	0.2	0.4	0.1	0.2	0.1	0.2	0.2	0.4	0.1	0.2	0.2	0.4	0.1	0.2
Cd	0.2	0.4	0.1	0.2	0.1	0.2	0.2	0.4	0.1	0.2	0.1	0.2	0.2	0.4	0.1	0.2	0.05	0.1	0.1	0.2
Cn	1.0	3.0	1.0	2.0	1.0	2.0	2.0	3.0	1.0	2.0	1.0	1.0	1.0	3.0	1.0	2.0	2.0	3.0	1.0	2.0
Cu	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0
Cr	1.0	1.5	0.5	1.0	0.5	1.0	1.0	1.5	0.5	1.0	0.5	1.0	1.0	1.5	0.5	1.0	0.5	1.0	0.5	1.0
Hg	0.01	0.02	0.005	0.01	0.005	0.01	0.01	0.02	0.005	0.01	0.01	0.02	0.01	0.02	0.01	0.02	0.005	0.01	0.005	0.01
Ni	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0	2.0	4.0
Pb	0.5	1.0	0.2	0.4	0.2	0.4	0.5	1.0	0.2	0.4	0.2	0.4	0.5	1.0	0.2	0.4	5.0	10.0	0.2	0.4
Zn	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0

(*) Measured in total
D.A. = Daily Average – M.A. = Monthly Average
(A), (B), (C) = Type of receiving body according to the Federal Rights Law

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crease the concentration of Zn to one that complies with the SEMARNAT (Mexico's environment ministry) water quality standards, shown on Table 3, in all aspects, the concentration of Cu would have complied to the daily limits, yet it wouldn't for the monthly ones, the concentration of Pb would be 1.55 mg/L short of the monthly limits and 2.05 mg/L short of the daily limits, both for irrigation use, the concentration of Cd would be 2.25 mg/L short of the monthly limits and 2.45 mg/L short of the daily limits, both for irrigation use (all of them in the rivers section). It is worth mentioning that the dead hyperaccumulators bags presented some ruptures when extracted. Emphasizing that the dead and live hyperaccumulators used, in their respective contexts, have shown the capacity to absorb Ag, As, Au, Ba, Cr, Co, Hg, Li, Ni, Sn, U (Aceñolaza et al., 1999; Cuizano and Navarro, 2008) among others in different quantities is important because it allows to estimate that they are even more effective in the phytoremediation process than has been demonstrated in the present research.

Conclusions

As previously stated, we do not want to generate division between which kind of hyperaccumulators is better than the other, because it really depends on each independent situation to determine the ones that are best suited for the needs presented as well as taking into consideration the ones available; We rather intend to give more information regarding the phytoremediation topic, along with proposing another way to extract heavy metals from bodies of water than the ones currently being used that can be harmful in the long term, expensive or complex to implement in more marginalized communities, which are the ones that tend to be more affected by the contamination. Also, we want to acknowledge the possibility to repeat the study by now taking into consideration different variables in order to be able to give a more complete picture regarding the different capabilities of the hyperaccumulators.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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