Relationship of biodiversity with heavy metal tolerance and sorption capacity: A meta-analysis approach

Running title: ***Biodiversity and heavy metal Meta-analyses***

Isis E. Mejias Carpio [a], *Ali Ansari* [a], *and Debora F. Rodrigues* [a,*]

[a] Department of Civil and Environmental Engineering. University of Houston, Houston, TX-77004, USA

E-mail: dfrigirodrigues@uh.edu

*Corresponding Author: E-mail: dfrigirodrigues@uh.edu; Tel: 713-743-1495; Fax: 713-743-4260

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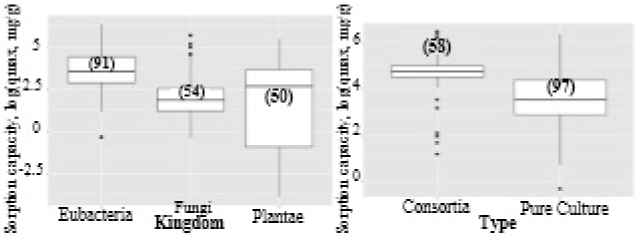

Abstract

Microbial remediation of metals can alleviate the concerns of metal pollution in the environment. The microbial remediation, however, can be a complex process since microbial metal resistance and biodiversity can play a direct role in the bioremediation process. This study aims to understand the relationships among microbial metal resistance, biodiversity, and metal sorption capacity. Meta-analyses based on 735 literature data points of Minimum Inhibitory Concentrations (MIC) of plantae, bacteria, and fungi exposed to As, Cd, Cr Cu, Ni, Pb, and Zn – showed that metal resistance depends on the microbial Kingdom and the type of heavy metal, and that consortia are significantly more resistant to heavy metals than pure cultures. A similar meta-analysis comparing 517 MIC values from different bacterial genera (*Bacillus*, *Cupriavidus*, *Klebsiella*, *Ochrobactrum*, *Paenibacillus*, *Pseudomonas*, and *Ralstonia*) confirmed that metal tolerance depends on the type of genus. Another meta-analysis with 195 studies showed that the maximum sorption capacity is influenced by microbial Kingdoms, the type of Biosorbent (whether consortia or pure cultures), and the type of metal. This study also suggests that bioremediation using microbial consortia is a valid option to reduce environmental metal contaminations.

Keywords: biosorption, heavy metals, microbial remediation, diversity, consortia

1. Introduction

In the past decade, multiple studies indicated that microorganisms can serve as potential alternatives for the sustainable remediation of heavy metals in the environment.[1, 2] These investigations showed that microorganisms belonging to different Kingdoms, i.e. Fungi, Plantae, Eubacteria, are very promising for metal remediation.[3] It is still unclear, however, whether the microorganisms from these Kingdoms have similar metal tolerance and sorption capacities.

Most of the metal sorption studies in the literature use pure cultures, and only recently, researchers have been focusing on microbial communities.[4, 5] The value of investigating pure cultures for heavy metal remediation is the discovery of the mechanisms of metal resistance and sorption capacity. Yet, pure culture studies are not practical for large-scale processes or realistic for *in situ* bioremediation, due to the difficulty in maintaining pure cultures and guaranteeing their optimum metal sorption capacity under different environmental conditions. Alternatively, microbial communities could be a more realistic approach for *in situ* remediation.

The investigation of complex microbial communities for the sorption of heavy metals from the environment is promising, yet an unclear solution. Diverse microbial communities exist in the environment and may hold different sorption capacities or unknown mechanisms of metal resistance. But to date, very few studies have focused on understanding how microbial community diversity affects metal sorption.[1, 6] In this context, we highlight the value of studying complex microbial communities to understand how biodiversity affects metal sorption processes in large-scale applications.

In addition to research in biodiversity, studies in microbial metal tolerance up to date do not explain its effect on heavy metal sorption processes. The metal Minimum Inhibitory Concentration (MIC) of microbial cells is typically used as a first approach to determine the microbial metal resistance. MIC is commonly defined as the lowest metal concentration inhibiting microbial growth.[7] Numerous studies have examined the heavy metal tolerance through the MIC of microorganisms isolated from different habitats, and grown under different conditions. But, to date, that large amount of data is still scattered, with no connection between metal resistance and microbial Kingdoms that could serve for a more effective bioremediation process.

In this study, we collected 930 values from other literature studies that comprise common cells used for metal sorption and metal tolerance, with aims to: i) correlate microbial metal tolerance, in terms of MIC, the type of metal, the microbial Kingdom, and the bacterial genus ; ii) determine if the growth medium has an influence in the MIC; iii) link microbial metal sorption capacity with the types of metal and the microbial Kingdom; iv) associate the type of metal and the microbial Kingdom with the maximum sorption capacity (q_{max}); and v) determine if biodiversity has a significant effect on q_{max} by considering values of consortia and pure cultures. This approach will allow us to gain a better understanding of the role of microbial diversity, metal resistance, and metal sorption in bioremediation processes.

2. Materials and Methods

Parameters investigated

The meta-analyses presented in this study were performed with data collected from the literature. **Table 1** summarizes the categorical and dependent parameters applied. The subsequent sections describe the analyses performed with these parameters.

Table 1- Number of literature results utilized for each analysis

Categorical Parameter	Dependent Parameters	
	Metal Tolerance, MIC	q_{max}
Microbial Kingdom (Eubacteria, Fungi, Plantae)	735	195
Types of Metal (As, Cd, Cr, Cu, Ni, Pb, Zn)	735	155
Bacterial Genus (*Bacillus, Cupriavidus, Klebsiella, Ochrobactrum, Paenibacillus, Pseudomonas, Ralstonia*)	517	N/A
Growth Medium (Minimum and Rich)	735	N/A
Biosorbent Type (Pure Cultures and Consortia)	735	155

N/A: data not available

Analyses of Microbial Metal Tolerance relationships to Kingdoms, Bacterial Genus, Types of Metals, and Biosorbent type

In this analysis, we collected 735 MIC results from the literature, as presented in Table 1. These MIC values belong to microorganisms from different Kingdoms under aerobic growth conditions. The data collected were analyzed using the ANOVA statistical analysis with Rstudio (see supporting information). In all analyses in this manuscript, the prokaryotic Kingdom of bacteria was treated as separate from Archaea, as stated by Woese and Fox.[8]

The first analysis involved sorting the 735 MIC values into seven heavy metal groups (As, Cd, Cr, Cu, Ni, Pb, and Zn). The 'type of metal' was used as a categorical parameter and the MIC values included prokaryotes, eukaryotes, and consortia with both eukaryotic and prokaryotic microorganisms. A natural logarithm transformation of MIC values was done to obtain a normal distribution of the data. The ANOVA statistical analysis was done to determine if there was a statistically significant difference between the MIC values of all metal groups. In addition, the post hoc Tukey's test was done to find out which metals had the highest and lowest values (see supporting information).

In the second analysis, the 'MIC' value was used as a dependent parameter and the 'Kingdom' (Eubacteria, Fungi, and Plantae) as a categorical parameter. Within those values, the analysis included pure cultures as well as consortia with either prokaryotes or eukaryotes, and consortia with both eukaryotic and prokaryotic microorganisms. The data included MIC values of the most common metals used in biosorption studies: As, Cd, Cr, Cu, Ni, Pb, and Zn. Most of the MIC studies used were short-term studies (maximum of 2 to 4 days of incubation). Some of the studies included tolerance assays done in one week and two were done in two weeks to a month. A natural logarithm transformation of MIC values was done to obtain a normal distribution of the data, presented in the supporting information. ANOVA statistical analysis was done to determine if there was a statistical difference among the three Kingdoms. In addition, the post hoc Tukey's test was done to find out which group had the highest and lowest values (see supporting information).

The third analysis involved evaluating 517 MIC values of pure cultures of different bacterial genera. This analysis aimed to determine whether specific genera could have different metal resistance. The most common genera of bacterium found to resist high concentrations of heavy metals are *Acidithiobacillus sp.*, *Desulfovibrio sp.*, *E. coli sp.*, *Cupriavidus sp.*, *Ochrobactrum sp.*, *Streptomyces sp.*, *Micrococcus sp.*, *Acinetobacter sp.*, and *Pseudomonas sp.*. However, because not enough data was available for all these genera, we utilized the most commonly studied microbes: *Bacillus sp.*, *Cupriavidus sp.*, *Klebsiella sp.*, *Ochrobactrum sp.*, *Paenibacillus sp.*, *Pseudomonas sp.*, and *Ralstonia sp,* as categorical parameters. A natural logarithm transformation of MIC values was done to obtain a normal distribution of the data. The ANOVA statistical analysis and post hoc Tukey's test were done to determine which group had higher MIC values, as shown in the supporting information. For all analyses, the least square mean graphical representation was plotted with Rstudio.

A fourth analysis involved 735 MIC values, but the MIC values were divided into two groups: minimum and rich media so that the ‘media’ represented a categorical parameter. The ANOVA statistical analysis and post hoc Tukey’s test were done to determine which group had higher MIC values.

A fifth analysis involved the same 735 MIC values, but the MIC values were divided into two groups: pure cultures and consortia so that the ‘biosorbent type’ represented a categorical parameter. The ANOVA statistical analysis and post hoc Tukey’s test were done to determine which group had higher MIC values.

Analysis of Microbial Maximum Sorption Capacity relationship to microbial kingdom and biosorbent type

First, the relationship of the maximum sorption capacity, q_{max}, to the microbial kingdom was investigated with q_{max} values for various heavy metals from 195 published studies. The ‘Kingdom’ (Eubacteria, Fungi, and Plantae) was used as a categorical parameter, whereas q_{max} was used as a dependent parameter.

In a second analysis with 155 studies, the “metal” was used as a categorical parameter, whereas the q_{max} was used as a dependent parameter. The data included MIC values of common metals used in biosorption studies: Cd, Cr, Cu, Ni, Pb, and Zn only for the bacteria Kingdom.

A third analysis included using “biosorbent type” (pure cultures or consortia) as a categorical parameter with the same 155 studies (97 for pure cultures and 58 for consortia from the bacteria

Kingdom). These values were sorted into two main groups: Pure Cultures (PC) and Consortia (C). (Table 1).

All consortia studies utilized in this analysis were a complex mixture of microorganisms obtained from environmental samples (e.g. soil, water or wastewater) grown in the laboratory with minimum media under aerobic conditions. None of the consortia studies were done in the study site. Most of the q_{max} values were obtained from Langmuir isotherm data where excess metals are present. The q_{max} values were calculated by the authors of each study. A few studies had only sorption capacity values, which were used from the highest reported observed values, or the maximum metal concentrations observed to be adsorbed, see Supporting Information. A natural logarithm transformation of qmax values was done to obtain a normal distribution of all of the data. The ANOVA statistical analysis and the post hoc Tukey's test with Rstudio were done for the three analyses to determine which group had higher sorption capacity values, and the least square mean graphical representation was exported from Rstudio.

3. Results and Discussion

3.1 Relationships among microbial kingdoms, bacterial genera, heavy metal tolerance, and bioremediation capability

Microorganisms can differ in metal resistance and in their ability to remove heavy metals. In the scientific literature, diverse microorganisms from the Eubacteria, Fungi, and Plantae Kingdoms have shown evidence of heavy metal resistance. Within such Kingdoms, though, some metal-resistant microorganisms have never been investigated for heavy metal remediation capability. [9,10] Thus, we first compared the MIC of different microbial Kingdoms, without considering their remediation capability, to understand whether a particular Kingdom is more tolerant to heavy metals than others. Additionally, we also investigated the tolerance of different Eubacterial genera to determine whether different genera can have different metal tolerances. We selected Eubacteria as a representative group for genus investigation since it is the most studied Kingdom in the literature. The next step of our evaluation was to compare the remediation capability of these Kingdoms in terms of metal sorption capacity. These results allowed us to determine (i) whether heavy metal tolerance is intrinsic to any particular microbial Kingdom; (ii) more specifically, whether the metal tolerance depends on the type of genus, and (iii) whether microorganisms from a particular Kingdom that are tolerant to metals can play a significant role in metal sorption capacity and hold bioremedation capabilities.

Relationship between microbial metal tolerance and microbial Kingdom

The relationship between microbial metal tolerance and Kingdom is key to determine the types of microorganisms that can survive in environments contaminated with heavy metals and potentially play a role in bioremediation processes. It is important, however, to first understand whether microbial metal tolerance is related to the type of metal since different metals have different redox capabilities, solubilities in water, and toxicity mechanisms under aerobic conditions. The analysis presented in **Figure 1** shows that there is a statistically significant difference between the different metals and the overall microbial tolerance under the same redox conditions (aerobic), with a p-value <0.001. In the results, the metals group together with various levels of toxicity. For instance, Cu and Cd seem to be the most toxic among the metals investigated in this study, since they had the lowest MIC values, as shown in Figure 1. The MIC values of these two metals are not significantly different from each other (Tukey's test $p>0.01$), but are significantly different from As, Ni, Pb and Zn (Tukey's test $p<0.001$). Cr, Ni and Zn, also seem to group together as having moderate toxicity, relative to the other metals. The MIC values of these two metals are not significantly different from each other (Tukey's test $p>0.01$), but are significantly different from As and Pb (Tukey's test p<0.001). In the case of As and Pb, they group together showing the least toxicity, since the MIC values of these two metals are not significantly different from each other (Tukey's test p>0.01), but are significantly different from the rest of the metals (Tukey's test p<0.001). The tolerance to metals was also statistically different for the diverse microbial Kingdoms, as seen in the ANOVA test results in Figure 2 with p < 0.001.

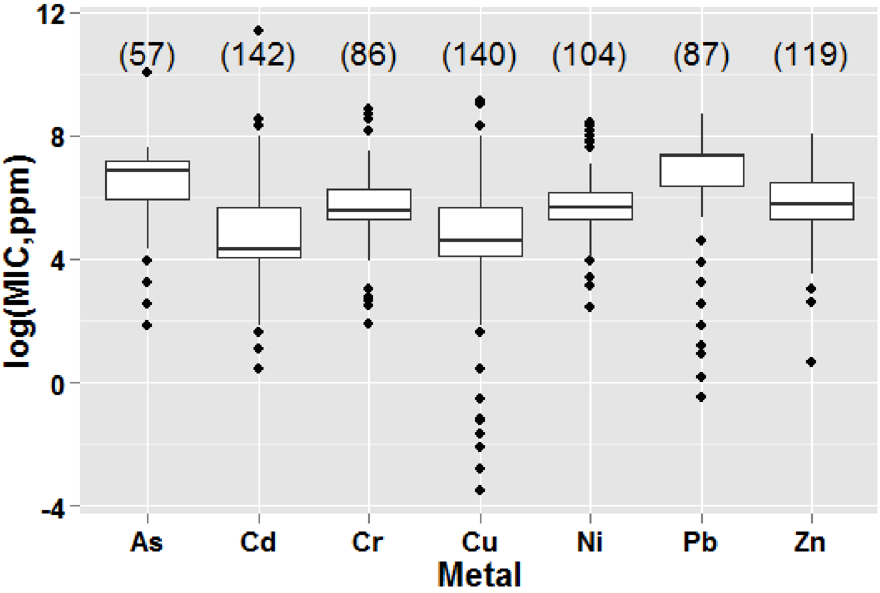

Figure 1- Microbial metal tolerance, expressed as the Minimum Inhibitory Concentration, as a function of metal type analyzed with ANOVA. The data presents results from 735 literature studies with bacteria, fungi, and plantae. Current effect: F(6,724)=39.486, p < 0.001

The results of the relationship between metal tolerance and Kingdom show that Fungi have the highest tolerance to heavy metals, in terms of MIC, followed by Eubacteria, and Plantae (Figure 2). The Fungi group presented statistically significant higher MIC values than the other Kingdoms, with a p < 0.001 confidence level (**Table S4**). Even though the cells from these studies were grown under different conditions, i.e. composition of nutrients in the culture media, incubation temperatures, and rotational speed, the confidence level of the analysis suggests that, in average, Fungi have higher metal resistance than other Kingdoms. These results are not surprising since Fungi carry diverse mechanisms of metal detoxification found both in prokaryotes (metal efflux pumps) and in eukaryotes (intracellular sequestration), which makes them more resistant to diverse heavy metals. [11,12] For instance, *Candida albicans* and *Candida tropicalis* are human pathogens known to resist high concentrations of diverse metal ions, such as Cu^{2+}, Zn^{2+}, Cr^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, As^{3+}, and Se^{6+} via different mechanisms.[13,14] Among the different mechanisms of tolerance, intracellular sequestration mechanisms are typically seen in Fungi.[12]

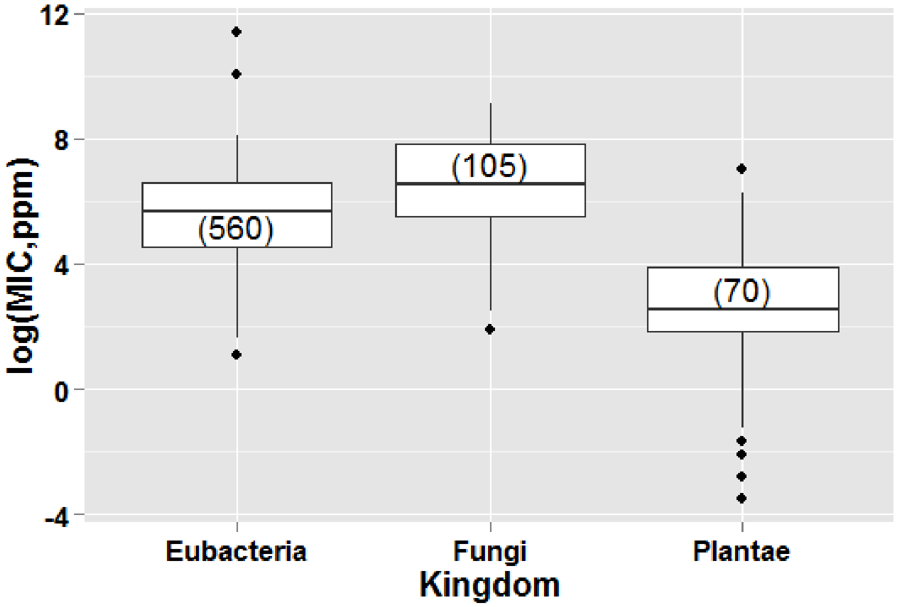

Figure 2- Microbial metal tolerance, expressed as the logarithm of the MIC, as a function of microbial Kingdom analyzed with ANOVA. The data presents results from 735 literature studies. Current effect: F(2,724)=250.7619, $p < 0.001$.

The intracellular sequestration in Fungi can involve accumulation by binding metals to low molecular weight, cysteine-rich proteins present in the cytoplasm, called metallothioneins (MT).[15] Even though MT can be found in prokaryotes, they are more typically seen in eukaryotes. MT are well-known contributors to the high metal resistance in Fungi. The gene sequence that encodes the metal-binding MT in the yeast *Saccharomyces cerevisiae* was first revealed in 1984.[16] Since then, important findings with MT in genetic engineering have revealed that the more stable eukaryotic MT can be inserted in prokaryotic cells to enhance metal tolerance and accumulation.[17,18,19,20]

Fungal intracellular sequestration of metals can also occur through phytochelatins (PCs), which are peptides, oligomers of glutathione that are enzymatically synthesized in the cytosol and can form PC-metal complexes.[21] The first vacuolar PC transporter was first revealed in the yeast *Schizosaccharomyces pombe.* [22,23,24] With the well-recognized role of PCs in metal sequestration in Fungi, new studies have genetically engineered PCs in existing bacterial species to increase their resistance against metal ions. For example, a recent study cloned and expressed PCs from *S. pombe* into *Pseudomonas putida KT2440* to increase the mRNA expression of *SpPCS*, which resulted in enhanced bacterial Cd accumulation [25].

Eubacteria are the second most tolerant Kingdom to heavy metals and the most studied. Diverse–mechanisms of resistance have been suggested for bacterial cells, such metal efflux pumps, metal reduction, cell wall binding, EPS sequestration, metal volatilization, and intracellular sequestration. Several bacteria are able to change the oxidation state of metals, such as iron, chromium, uranium, and arsenic, through various metabolic processes.[26] A variety of microbes can also produce hydrogen sulfide to aid in the immobilization of metals, and the majority falls within the phylum Proteobacteria.[27] The efflux of chromate, Cr^{6+}, out of the cytoplasm has been extensively studied, particularly for the strain *C. metallidurans.*[28,29] Metal sequestration via transport or efflux pumps is commonly seen among bacteria, as described previously.[29,30] Volatilization of mercury has been known to remove significant amounts of mercury from contaminated surface waters, wastewaters, and soils.[31] The metal volatilization mechanism has been well studied, particularly for plasmid-borne genes that provide mercury resistance in several bacterial species.[32,33,34] Lastly, the cell wall binding is another mechanism of resistance among bacteria that gives this Kingdom a high level of metal tolerance. In Gram-positive, the peptidoglycan may contain teichoic acids, polymers of glycerol or ribitol joined by phosphate groups, which have proven to participate in the metal binding. The binding in Gram-positive bacteria occurs by the phosphoryl and/or by the carboxyl groups of the peptide chains. The lipopolysaccharide and phospholipids provide the primary sites for metal interaction in Gram-negative bacteria.[36] Some of the mechanisms of resistance that make this Kingdom tolerant is further discussed in the next section.

The least tolerant Kingdom belong to the Plantae Kingdom. The microorganisms from this Kingdom, mainly algae, are the least resistant to heavy metals. Although the metal MICs of different algal species are relatively low (**Figure 2**) when compared to bacterial, and fungal species, living and non-living algal cells are still used for biosorption of different metal ions.[37, 38] Although the metal MICs of different algal species are relatively low when compared to bacterial and fungal species, non-living algal cells are typically used for biosorption of different metal ions.[37,38] Outer membrane adsorption or cell wall binding have been shown as an important mechanism in algal metal biosorption because of their large surface area-to-volume ratio available for contact with the metal-rich environment.[39] Many organisms in the plantae Kingdom contain cellulose as their main cell wall component, which have shown to sorb various metals in *Ulva lactuca*,[40] a Chlorophyta. Among the groups of plantae, Phaeophyta, Chlorophyta, and Rhodophyta are the most investigated for bioremediation. These different types of plantae have important differences in their cell wall structure and some internal organelles. For instance, they all have different and complex polysaccharide contents in the cell wall.[41] Thus, the cell wall chemistry of the different plantae species plays a key role in the number of electrostatic interactions and complexation with metal ions available for bioremediation. But living plantae species are not resistant to high metal concentrations. For example, previous research indicated that zinc concentrations above 20 mg/L inhibited the algal growth of *C. pyrenoidosa.* [42]

The results of this analysis suggest that the overall microbial tolerance to metals is different for microorganisms from different Kingdoms, with Fungi being the most tolerant to heavy metals.

Relationship between microbial metal tolerance and microbial genus

Besides evaluating the effect of the Kingdom on metal tolerance, we evaluated the effect of the bacterial genera on the MIC. Since the Eubacteria Kingdom has the most data available, the MIC values of this Kingdom were further parsed out in order to evaluate the genus with the highest tolerance to heavy metals (Figure 3).

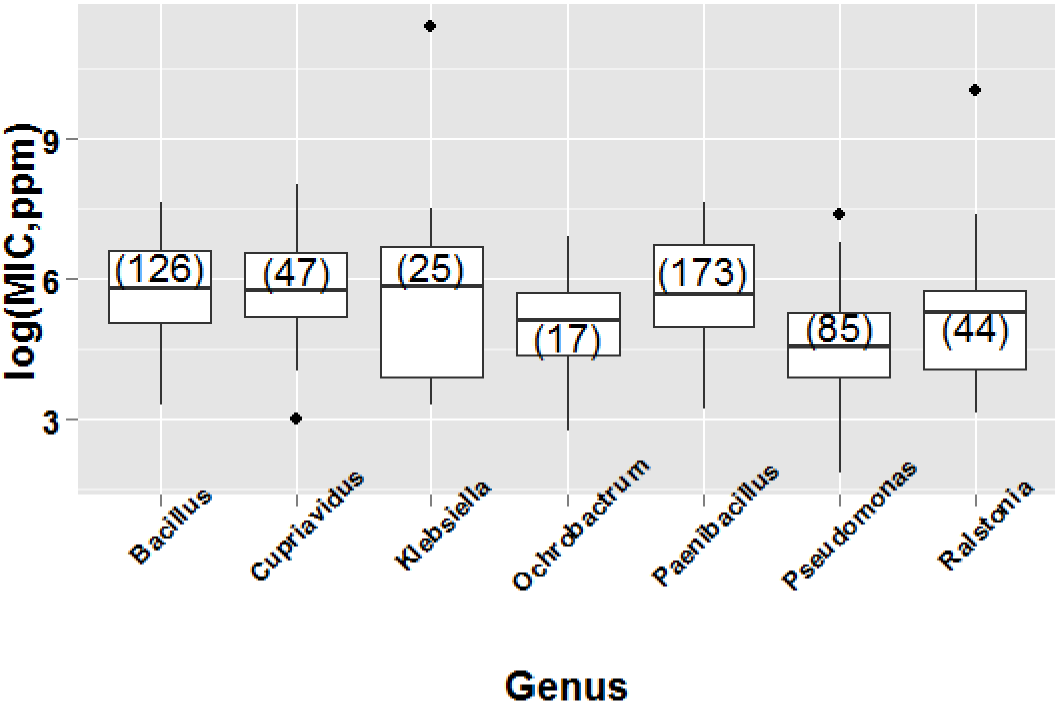

Figure 3- Microbial metal tolerance, expressed as the logarithm of the MIC, as a function of bacterial genus (analyzed with ANOVA. The data presents results from 517 literature studies. Current effect: F(6,504)=23.941, p < 0.001

The results in Figure 3 show that there is a statistical difference between the metal tolerances of different genera. Through the Tukey's post hoc test (see supporting information), it was found that *Bacillus*, *Curpiavidus*, *Klebsiella*, and *Paenibacillus* are the most resistant, whereas *Ochrobactrum*, *Pseudomonas*, and *Ralstonia* are the least resistant.

Cupriavidus and *Ochrobactrum* are suitable for metal remediation due to their reduction mechanisms. Bacteria have played major roles in the reduction of chromate ions, Cr^{6+}, to insoluble trivalent chromium, Cr^{3+}. Three reduction mechanisms have been suggested for the reduction of Cr^{6+}: 1) in aerobic conditions, chromate reduction happens through soluble chromate reductases that use NADH or NADPH as cofactors, (ii) in anaerobic conditions, Cr^{6+} acts as an electron acceptor in the electron transport chain, and (iii) through chemical reactions with microbially generated compounds such as amino acids, nucleotides, sugars, vitamins, organic acids or glutathione that can effectively reduce chromium.[29]

Klebisella was shown to use the metal precipitation mechanism for metal tolerance.[43] The metal oxidation/reduction mechanism often overlaps with the metal precipitation mechanism since some metals are reduced to an insoluble state where they precipitate. Yet, not all microorganisms capable of reducing metals induce their precipitation.[44] Biological precipitation of metals has been widely considered for metal bioremediation in water, [45, 46,9] and the precipitation may be mediated by the production of hydrogen sulfide or by cellular respiration. In a previous study, *Klebsiella aerogenes* S45 appeared to use the formation of insoluble cadmium sulfide as the primary detoxification mechanism.[43]

Bacillus and *Paenibacillus* typically use the EPS sequestration mechanisms for metal resistance.[47,48] Metal ions can bind to the EPS by ionic interactions with the negatively charged functional groups, or, for example, by forming salt bridges with carboxylate groups from two different polysaccharide chains in the EPS matrix.[47] The study by Perez et al., (2008) revealed the chemical composition of the EPS from the Gram-positive *Paenibacillus jamilae* with 28% of the weight being uronic acids, [47] while a similar uronic acid content (38%) was seen in the EPS from the Gram-positive *Bacillus firmus*.[48] The EPS from both of these strains played a key role in the heavy metal sequestration within the matrix.

Pseudomonas also uses the reduction mechanism to increase its metal tolerance. The first aerobic bacterium found to be resistant to chromate was *P. fluorescens* LB300, and its resistance was attributed to *chr* genes in the plasmid pLHB1.[49] The enzyme ChrR from the CHR protein superfamily was found to be responsible for the effective reduction of Cr^{6+} in *P. putida*,[50,51] and the enzyme's crystal structure was recently published by Eswaramoorthy et al.[52] Other bacteria able to reduce chromate by the presence of *chr* in different plasmids include *O. anthropi,*[53] *E. coli,* [54] and *C. metallidurans.*[28] These bacteria are among the species with the highest chromium resistance.

For some aerobic species, such as *Ralstonia* and *Pseudomonas* the metal resistance is owed to a combination of various mechanisms. *Ralstonia* has shown tolerance to lead by a combination of mechanisms of uptake, sequestration, and efflux. In *Ralstonia metallidurans* CH4, for example, the efflux ATPase pump PbrA, which contains heavy metal-associated metal binding domains, can expel Pb^{2+} out of the cell.[55] The *Pseudomonas* genus has also shown various metal resistance mechanisms. The chromate resistance of this genus is owed to a combination of chromate reduction and chromate efflux. [49] The efflux of Cd^{2+} and Cu^{2+} is also mediated by ATPase pumps. Proteomic analysis of *P. putida* exposed to copper and cadmium revealed that the P-type ATPase CadA2 was associated with Cd^{2+} efflux, whereas the Cu^{2+} resistance was linked to the activation of the gene encoding the P-type ATPase transporter (*pac*S) and genes for metal-binding proteins in the outer membrane, the periplasmic and the cytosol (*por*D, *cop*Z or *pac*Z, *cop*A1, *cop*A1, and *cop*B1).[56] Other reviews are available to detail the microbial chromium resistance of various species. [29,57,58]

Although the mechanisms of resistance are known to protect the microbial cells against heavy metal toxicity and to carry out cellular functions, other questions remain: are the microorganisms with the highest metal resistance able to sorb heavy metals more efficiently from the environment? And can they sorb certain metals better than others? Would the water chemistry affect their tolerance? In the latter one, even though there are no studies *in situ* investigating the relationship between water chemistry and metal tolerance of microorganisms, some studies have used different growth media to simulate different water chemistries and determined metal tolerance.

Relationship between microbial metal tolerance and growth medium

Many studies with these microorganisms suggested that the composition of the growth media, *i.e.* rich versus minimum media, would influence the microbial metal tolerance due to the higher chance of forming metal complexes with biomolecules present in the rich medium. The studies using minimum media typically aimed to better correlate or simulate the aquatic chemistry of the environment since the minimum media is typically composed by salts only and one carbon source; whereas rich media can contain salts, different sugars and other biomolecules from cell extracts (e.g. polysaccharides, proteins, lipids). The effect of the growth medium on the MIC was not evaluated by the authors of the respective studies presented here. In order to determine whether the growth media can influence the metal availability and consequently the level of metal tolerance in the different Kingdoms, the media (minimum or rich) was also included in the ANOVA statistical analysis (Figure 4).

In this study, it was found that the growth medium does not influence significantly the metal tolerance level (p=0.6716). The results of this analysis may be due to the fact that microorganisms can secrete complex biomolecules, called exopolymeric substances (EPS), during growth in any type of media, including minimum media. These biomolecules can effectively complex with metals and compete with cells for metals.[114] These secreted EPS could have made the minimum media more complex and similar to the biomolecules also found in rich media, which would explain the similar responses observed between rich and minimum media in this analysis.

Note that both minimum and rich media are synthetic solutions made in the laboratory and do not represent the exact chemistry conditions of the environment. Although the analysis of the composition of specific media is outside of the scope of this study, it is important to highlight that the water chemistry will have an effect on the heavy metal bioavailability and hence will affect toxicity towards microbial cells.

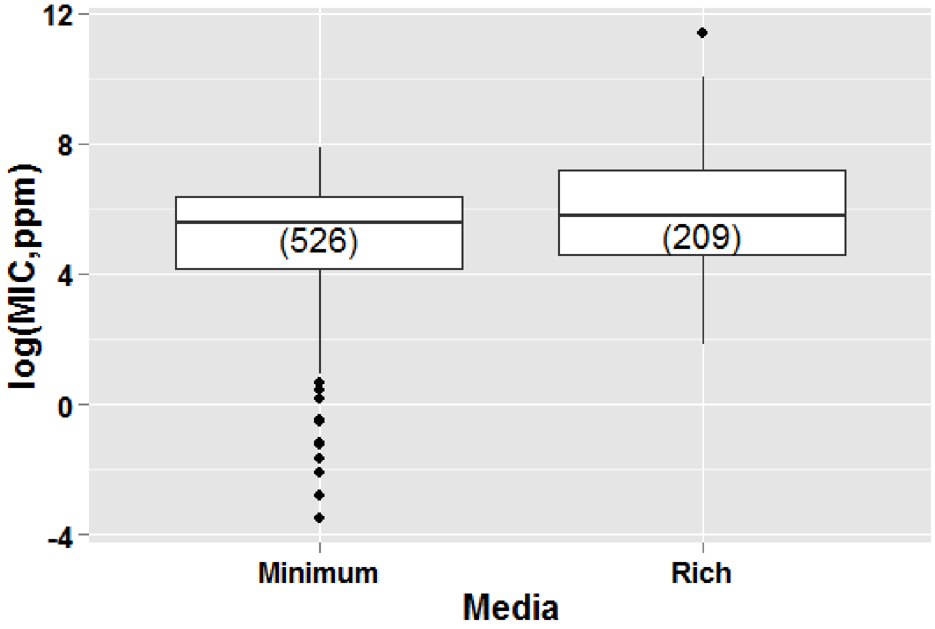

Figure 4- Microbial metal tolerance, expressed as the logarithm of the MIC, as a function of growth medium (analyzed with ANOVA. The data presents results from 735 literature studies (see supporting information for the references). Current effect: F(1,724)=0.1799, p =0.6716

Generally, the ionic form of a metal is more toxic, because it can form toxic compounds with other ions. Electron transfer reactions that are connected with oxygen can lead to the production of toxic oxyradicals, a toxicity mechanism now known to be of considerable importance in both animals and plants.[60] Some oxyradicals, such as superoxide anion (O_2^-) and the hydroxyl radical (OH^-), can cause serious cellular damage.[60] The water chemistry is essential in the bioavailability of heavy metals. Some of the parameters that affect the water chemistry are pH, sulfate and nitrate ions, total hardness, redox potentials, electrical conductivity/salinity, solids, and organic matter.

The pH can affect metals' bioavailability and mobility. When the pH in water becomes more acidic, metal solubility increases and the metal particles become more mobile. For instance, water coming from draining mining areas is often very acidic and contains high concentrations of dissolved metals with little aquatic life [61]. In water with higher pHs, the heavy metals tend to precipitate as hydroxides, or as sulfides and carbonates depending on the sulfate concentration and hardness [62].

In the presence of sulfates or high total hardness in the water system, metal ions tend to precipitate typically as sulfides, sometimes sulfates, or carbonates. Toxicity studies with different heavy metals in water with different hardness demonstrated that the metals were less available to biological systems.[62,63] Therefore, heavy metals were shown to be less toxic in soft waters. Additionally, changes from reducing to oxidizing conditions in aquatic systems, which involve the transformation of sulfides and a shift to more acidic conditions have been shown to increase the mobility of heavy metals, such as Hg, Zn, Pb, Cu, and Cd. On the other hand, the mobility was shown to be reduced for Mn and Fe under oxidizing conditions.[62]

In the case of salinity or ions in water, the effect of ligands on heavy metals' mobility is usually determined from their respective stability constants.[59] In this respect, the influence of salinity will depend on the difference between the different stability constants.[59] Another parameter that affects heavy metal bioavailability is the presence of solids or particles, such as silt and clay, in the water. These particles tend to complex with metals, and in some cases precipitate in the sediment, which would make the heavy metal less available to the biota.[64]

Finally, the organic matter present in the water is another important factor that controls the accumulation, mobility, and bioavailability of heavy metals. Naturally dissolved organic matter, such as humic material or amino acids present in both fresh and salt water, has shown to affect very little the mobility of Cd, Ni, U, and Zn.[65] But, As, Cr, Cu, Pb, and V species, on the other hand, was markedly affected by natural organic chelators. The study indicated that the observed effects were associated with the formation of metal–NOM complexes.[65] It has, however, been suggested that upon biodegradation of the humic acid these metals can become again available. Based on these previous studies and our analsyis of the growth media, clearly the evaluation of water chemistry needs to be further investigated in relation to microorganisms, as present growth media and conditions seem to not really represent well the diverse water chemistries found in the environmental.

Relationship between metal sorption capacity and microbial kingdom

Different literature reviews have pointed out that microorganisms have different metal sorption mechanisms.[66] In this study, we examined whether the sorption capacity is sensitive to the microbial Kingdom. **Figure 5** and **Table 3** indicate that there is a statistically significant difference between metal sorption capacity and Kingdom, with a $p < 0.001$ confidence level.

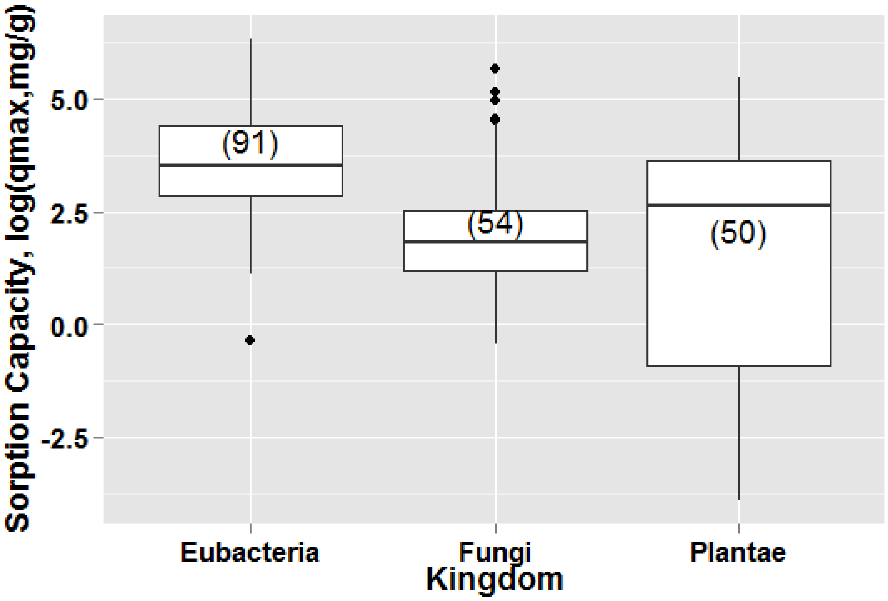

Figure 5- Microbial Metal Sorption Capacity as a function of the three different kingdoms analyzed with ANOVA. The data presents results from literature studies. Current effect: F(2, 192)=25.546, p<0.001.

Table 2- ANOVA statistical analysis of qmax for different microbial kingdoms

	Effect	DF	F	p
ANOVA Analysis 1				
	Microbial Kingdom	2	25.546	<0.001
	Residuals	192		

The results showed that microorganisms from the Eubacteria Kingdom present statistically significant higher sorption capacity values than the rest of the groups (**Figure 5**). This result clearly shows that tolerance is not directly related to the bioremediation ability of a group of microorganisms. Yet, the confidence level of the statistical analysis suggests that on average, Eubacteria have historically revealed higher metal sorption capacities than the other Kingdoms. It is possible that the mechanisms of metal sorption or the biodiversity of this Kingdom may have caused its higher metal sorption. They are the most widely studied Kingdom for bioremediation processes. Eubacteria are frequently investigated as metal biosorbents and biotransformants because of their genomic versatility, short generation times, and metal tolerance. These traits facilitate rapid microbial adaptation to a wide range of environmental conditions.

Plantae and fungi did not have a statistically significant difference in sorption capacity (Tukey test, p=0.443, see supporting information). The principal mechanisms of metal sorption by Plantae are through biosorption and intracellular sequestration.[67] Biosorption is mainly attributed to the cell wall properties, where both electrostatic attraction and complexation can play a role. Within the Plantae Kingdom, algae have been extensively studied for their metal sorption. Chlorophyta (or green algae), for instance, have xylan and mannan polysaccharides in the embedding matrix, which contain hydroxyl and carboxyl functional groups. Although the MIC for certain algal species of this group are quite low, they can still sorb metal ions effectively. [42] In a previous study, the green algae *Chlorella pyrenoidosa* was seen to sorb up to 95.6% of 20 mg/L Zn^{2+} ions, and the metal binding occurred in the algal cell wall.[42] Therefore, the cell wall chemistry of green algae enables the effective adsorption of metals from water, even though the metal MIC is relatively low.

Fungi also have lower sorption capacity values than bacteria. This may be attributed to the higher surface area for metal sorption per milligram of cell mass when compared to fungi.[113] Literature shows that both living and dead cells of fungi can uptake toxic and precious metals, which make them suitable for heavy metal bioremediation of water and wastewater. The most widely studied fungus for heavy metal sequestration is yeast. [68, 69] For instance, Volesky et al. (1995) studied the yeast *S. cerevisiae* uptake capacity for various metal ions grown aerobically and anaerobically. The metabolically-active aerobic yeast could uptake metals in this order of preference: Zn > Cu ~ Cd > U; anaerobic yeast in this order: U > Zn > Cd > Cu.[68] The uptake of metals by yeast has been attributed to intracellular sequestration and by the presence of extracellular materials produced by yeasts.[14]

3.2. Role of Biodiversity on Metal Tolerance and Sorption Capacity

All the studies analyzed in the previous sections included only pure cultures. Although pure cultures can have multiple metal resistance mechanisms, consortia can perform complicated functions that individual populations cannot.[70] Consortia can also be more robust to environmental fluctuations, such as metal concentrations.[70] Therefore, complex microbial communities are typically more attractive for large-scale processes and for *in situ* bioremediation. In this study, we aim to establish relationships among biodiversity, metal tolerance, and sorption capacity. For that purpose, we will compare results from studies with consortia, which contain multiple microbial species with various mechanisms of metal resistance, with results from studies with pure cultures. This approach will allow us to determine whether complex microbial communities are better at tolerating and removing heavy metals than pure cultures.

Relationship between diversity and metal tolerance

The best way to determine whether microbial communities are more tolerant to metals than pure cultures is to perform an analysis comparing microbial metal resistance between consortia and pure cultures. Consortia in the present study represent communities of microorganisms found in heavy metal contaminated environments that were grown in the laboratory in synthetic minimum growth media prior to experimentation (e.g. MIC and sorption).

The relationship between microbial metal resistance and biodiversity is critical to understand whether microbial diversity influences the mechanisms of metal tolerance. **Figure 6** indicates that there is a statistically significant difference between the two groups, with a $p < 0.001$ confidence level. The confidence level suggests that, on average, consortia have higher heavy metal resistance.

The higher metal resistance of consortia may be explained by the effective communication between the microbial cells among different species. [91] Through communication between cells, microbial consortia can trigger changes in gene expression in response to high levels of metals. [71] The response to metal concentrations may happen by metabolic variations in several community members, shifting the concentration and fate of dissolved metabolites, to increase the tolerance for metals. [72]

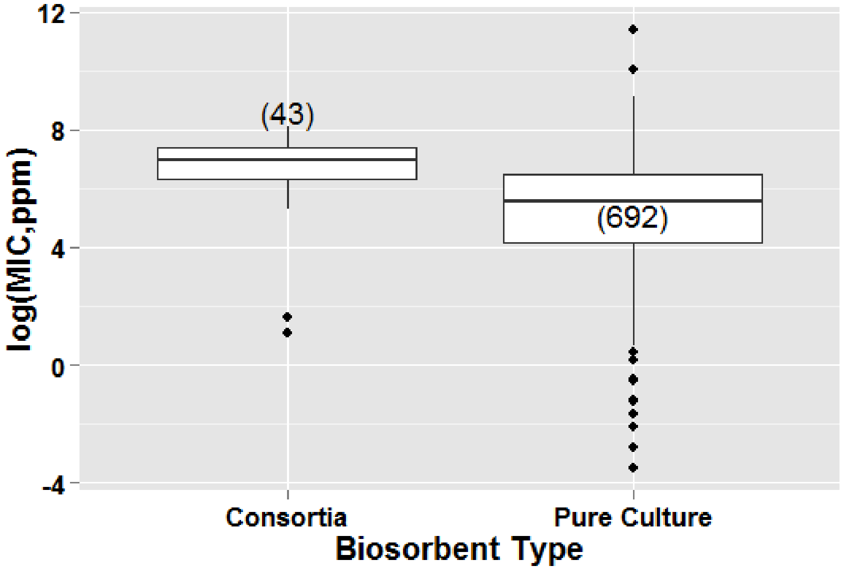

Figure 6- Microbial metal resistance, expressed as the minimum inhibitory concentration, as a function of the biosorbent type. ANOVA results from 735 literature studies. Current effect: F(1,724)= 20.108, p<0.001.

Various metal tolerance mechanisms utilize Quorum Sensing (QS) to increase microbial metal resistance. Some metals, such as copper, have shown to induce production of Reactive Oxygen Species (ROS) through auto-oxidation or Fenton-like reactions.[73] The production of ROS leads to activation of oxidative stress pathways by QS, which increases the tolerance of microorganisms for metals.[71] A resistance mechanism, shown in the Gram-negative *P. aeruginosa*, is facilitated through QS of an acyl-HSL signal named 3OC$_{12}$-HSL. This signal is produced by the enzyme Las-

I and is recognized by the Las-R regulator, which directly binds to the *cueR* to trigger its expression for copper export.[71]

Another tolerance mechanism occurring in Gram-positive bacteria, through oligopeptide signaling, wherein the oligopeptide is sensed by a transmembrane receptor component of a two-component signal transduction module (TCS). The TCS then activates an intracellular response mechanism.[74, 75] The QS plays an important role in providing bacteria with the ability to respond phenotypically to specific metal concentrations.[76, 77]

The effect of microbial diversity on the mechanisms of resistance is still a debated topic. This analysis, however, shows that microbial metal resistance is influenced by biodiversity and that more studies, especially *in situ*, are needed to better understand the resistance mechanisms in microbial communities in real environmental settings.

The relationship between metal sorption capacity and microbial diversity

Different literature reviews have pointed out that microorganisms have different metal sorption mechanisms. [66] The sorption has shown to depend on the metal redox potential [78] and/or the metal solubility in water.[79] In this study, we examined whether the sorption capacity is sensitive to the type of heavy metals for both pure cultures and consortia. **Figure 7** indicates that there is statistically significant difference between all the metal groups and the sorption capacity, with a $p < 0.001$ confidence level. The confidence level of the analysis suggests that, on average, there is a difference in the sorption capacity between the different types of heavy metals. This effect is similar to the results obtained between metal tolerance and type of metal.

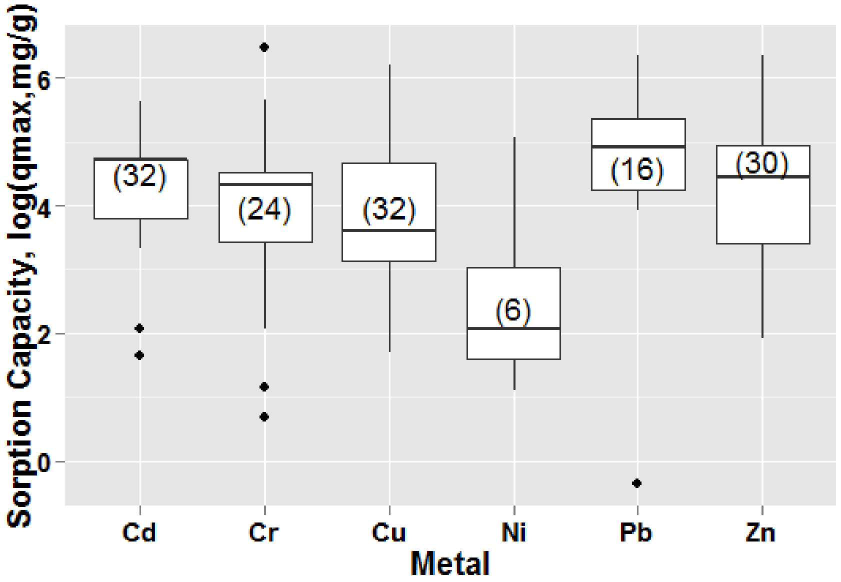

Figure 7- Bacterial Metal Sorption Capacity as a function of different heavy metals analyzed with ANOVA. The data presents results from 155 literature studies. Current effect: F(5, 148)=6.510, p<0.001

The correlation between metal sorption capacity and type of metal is not surprising since the microbial detoxification or sorption of metals in contaminated environments is controlled by oxidative and reductive (redox) transformations of the element or biosorption. [2, 79] [58] The result of this analysis suggests that the sorption capacity is different for different metal ions, which may be explained by the difference in oxidation-reduction potentials(E^0), solubility, ionic radius, valence, and affinity to functional groups for each metal.[110-112] The choice of microbial species for metal remediation is still a debated topic among researchers, because of the variety of mechanisms responsible for metal sorption, the amount of species available, and the different metal sorption capacities of the different species.[82, 83] Most of these studies, so far, have focused on heavy metal treatment by pure cultures.[35, 84-86] The major issue of using pure cultures for heavy metal remediation is that pure cultures cannot be maintained in large-scale treatment processes.

Therefore, understanding metal sorption processes of complex microbial communities is more relevant for real environmental engineering applications.

The maximum metal sorption capacity of pure cultures (PC) and consortia (C) was evaluated in this assessment to understand which group could present higher efficiency as a biosorbent. **Figure 8** shows that the C group presented statistically significant higher values than the PC group, with a $p < 0.001$ confidence level (**Table 5**). Even though all the batch sorption studies from these previous studies were performed under different experimental conditions, i.e., composition of nutrients in the culture media, incubation temperature, and rotational speed, the confidence level of the statistical analysis suggests that in average, the microbial consortia have historically shown higher metal sorption capacities than pure cultures. These results also demonstrate the importance of investigating complex microbial communities for metal sorption capacity applications.

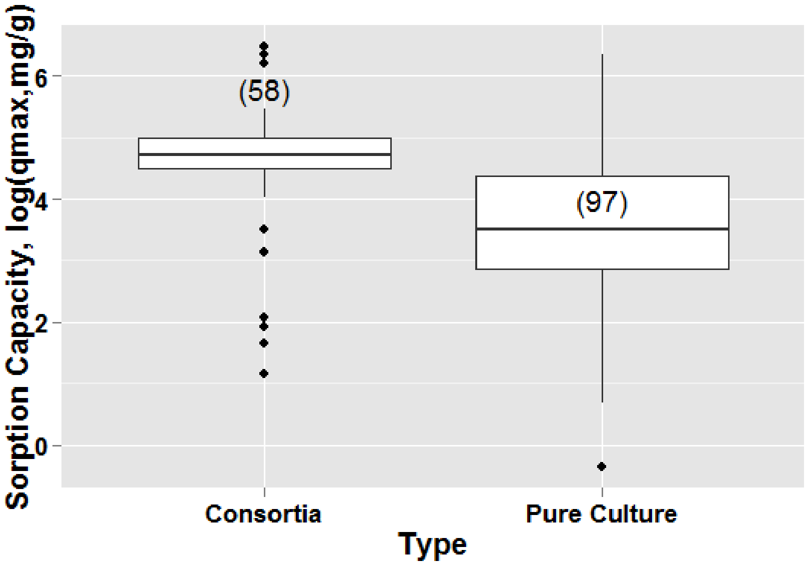

Figure 8- Maximum Heavy Metal Sorption Capacity of Microbial Pure Cultures (PC) and Consortia (C) obtained from 155 research studies.[9, 10, 36, 87-109]**. Current effect: F(1, 148)=34.558, p<0.001**

4. Conclusion

Metal sorption by microorganisms have proven effective and shown great potential for bioremediation applications. Microbes have historically shown tolerance against heavy metals and ability to remove metals from the environment.

In this study, the authors confirmed that the overall microbial metal tolerance and the maximum sorption capacity differ with the type of metals. Fungi have historically shown the highest tolerance to heavy metals, in terms of MIC, followed by Eubacteria, and Plantae. In addition, the Eubacteria Kingdom has shown higher sorption values than Plantae and Fungi. Most importantly, microbial diversity may play an important role in bioremediation as consortia were found significantly more resistant and with a higher maximum sorption capacity than pure cultures. Most of the studies so far, however, have focused on pure cultures. Therefore, the lack of integrated knowledge on the physiology, diversity, and functionality of microbial communities able to remove metals in the environment, hinder their effective application in bioremediation of heavy metals. Based on the current studies, the effective application of microorganisms for metal remediation will depend on the integration of diverse tools from the fields of microbial ecology, biotechnology, and environmental engineering for the development of optimal heavy metal bioremediation techniques.

Supporting information:

References used for meta-analysis, normal distribution figures, Tukey's test results, statistical analysis with RStudio, and model results

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