The sociality of bioremediation

Hijacking the social lives of microbial populations to clean up heavy metal contamination

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Heavy metals, such as cadmium, copper, mercury, and arsenic, are ubiquitous components of the Earth's crust. Most of these metals are toxic to varying degrees, and life has accordingly evolved ways to deal with them: either by developing mechanisms to detoxify them, or using them for biological functions. As humans began unearthing metals to be turned into consumer products, large amounts of heavy metal-laced trash ended up in landfills or were discarded into the environment. Cadmium from batteries, mercury from incandescent lamps, and copper, gold, and silver from electronics, seeped into soil and waterways with deleterious effects on terrestrial and aquatic biota, as well as human health.

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Some contaminated areas, such as landfills, toxic waste dumps, disused quarries, and mine tailings dumps, have such high concentrations of heavy metals that they pose a risk for human health and the environment, both locally and more widely, as seepage water and wind can distribute contamination over larger areas. Moreover, former industrial sites within cities are not fit for housing or businesses owing to the concentration of heavy metals in the soil. Remediation of these areas is a substantial economic factor for rezoning former industrial areas.

The standard method to clean up soil is removal of the contaminated layer—which can be several meters thick—and chemical conversion and/or removal of metal ions in special treatment facilities. It is a cumbersome and costly process and not particularly suited to deal with large water bodies or large areas of contaminated soil.

Bioremediation is therefore a much cheaper alternative, as it only requires seeding the contaminated soil—or the actual site—with a particular blend of microbes capable of turning a specific toxic metal species into a non-toxic form. For instance, mercury-detoxifying strains of *Pseudomonas* sp. immobilized inside a bioreactor successfully reduced toxic Hg(II) concentrations in chemical wastewater from 3–10 mg/l to under 10 µg/l (in combination with an activated carbon filter) by passing the wastewater continually through the bioreactor [1]. Moreover, several patents now exist on microbial cocktails that are efficient at detoxifying heavy metals (the full mechanism/composition of which has not been released). “Mercrobes”, developed in Germany, have promised to reduce toxic mercury to its elemental form. Non-independently run tests have reported these microbial concoctions are capable of removing 95–99% of mercury contaminants. Alpha Omega bioremediation, based in Ohio, USA, uses another patented blend of microbes that have been successful in detoxifying heavy metals from acid-mine drainage, allowing many mining operations to finally close throughout the USA and Canada.

A wide variety of organisms have evolved mechanisms to deal with harmful concentrations of heavy metals, and microbes are particularly good at it; some even use these materials as a food source. One study showed that 62% of bacterial strains isolated from a

copper-contaminated water system exhibited substantial resistance to copper via a combination of intracellular compartmentalization of metal ions and copper efflux pumps to excrete superfluous copper from the cell [2]. Such resistance can evolve very rapidly: Bacterial communities isolated from sludge before and 97 days after artificial copper contamination exhibited a highly enriched *Cus* efflux system, multi-copper oxidases, and several potentially novel copper resistance genes, compared to their ancestor 97 days before [3].

“...we suggest another reason why remediation may fail [is] sociality-induced costs that are associated with heavy metal resistance.”

Crucially, several bacterial resistance mechanisms (Table 1) reduce the toxicity or the concentration of heavy metals in the environment, making these particular microbes amenable to exploitation for our own requirements. Some microbes can oxidize or reduce heavy metal ions and transform them into their non-toxic form. Mercury resistance in *Pseudomonas putida* is encoded by the *mer*-resistance operon that reduces Hg²⁺ to non-toxic metallic mercury Hg(0). Other resistant strains sequester metals from the environment and safely store them away in internal compartments. Another strategy used by bacteria to detoxify metal ions is the production of siderophores, which are primarily produced by many bacterial species to acquire insoluble iron. Siderophores have been used for bioremediation, owing to their ability to chelate an array

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Table 1. Effect of bacterial detoxification mechanisms on the extracellular environment and microbial community. Decrease in environmental toxicity suggests bioremediation potential.

Resistance	Mechanism (e.g.)	Example	Effect on environmental toxicity	Effect on community
Decrease cell permeability	Downregulation of cell-surface porins	<i>E. coli</i> Ag ⁺ resistance: loss of non-specific OmpC/F transport channels	+	—
Extracellular sequestration	Siderophores, methylation, biosorption, EPS production, biofilm formation	<i>P. aeruginosa</i> Cu ²⁺ resistance: siderophore-Cu ²⁺ chelation	—	+
Intracellular sequestration	Intracellular compartmentalization/bioaccumulation	<i>P. syringae</i> Cu ²⁺ resistance: <i>Cop</i> -operon driven accumulation in periplasm	— ^a	+ ^a
Heavy metal reduction/volatilization	Reduction of toxic metals to non-toxic form	<i>P. fluorescens</i> Hg ²⁺ resistance: <i>Mer</i> operon driven reduction of Hg ²⁺ to volatile Hg (0)	—	+
		<i>P. aeruginosa</i> Ag ⁺ resistance: pyocyanin-mediated reduction of toxic Ag ⁺ to Ag(0)	—	+/- ^b
Rapid efflux of toxic metal from cell	ATPases/chemosmotic transporters/proton gradients	<i>R. metallidurans</i> Zn ²⁺ and Cd ²⁺ resistance: RND-driven efflux pumps	+	—

^aPositive effect on environment is possibly constrained to the lifetime of the cell.

^bThere is a positive effect of reduction in silver toxicity; however, community diversity is likely to decrease in response to pyocyanin production.

of heavy metal species, so that the free ions cannot diffuse into cells. While the fate of the metal–siderophore complex varies between metal types—some complexes enter the cell membrane and some remain in the extracellular environment—siderophore production tends to promote a reduction in the free metal concentration in the environment. For instance, one study documented that siderophores isolated from *Pseudomonas azotoformans* could remove 92.8% of arsenic from contaminated soil [4].

“... an efficiently bioremediating community of bacteria requires that everyone perform their fair share of detoxifying the environment...”

However, bacterial bioremediation to deal with heavy metal pollution is not a very reliable process and often fails to achieve the desired aims. Failure is ultimately associated with poor growth of the bacteria caused by inadequate nutrient and carbon sources, temperature, pH, salinity, toxins, or poor movement of bacteria through their new substrate. Here, we suggest another reason why remediation may fail to work even under optimal growth conditions: sociality-induced costs that are associated with heavy metal resistance (Fig 1).

As mentioned above, microbes can easily evolve resistance to heavy metals, but this often comes at a cost. Specifically, the growth rate of resistant compared with non-resistant bacteria is reduced in the absence of the heavy metal, analogous to the effect of antibiotic resistance. This may be especially relevant for plasmid-encoded efflux-pump genes, which are among the most widespread mechanisms of bacterial resistance. For instance, the non-conjugative plasmid pACYC184 that confers resistance to chloramphenicol in *Escherichia coli* carries a fitness cost in the absence of the antibiotic, but provides a fitness benefit in its presence. Similarly, siderophore production is also costly for the cell, but this is offset by the benefit of siderophores when iron is scarce or in the presence of high concentrations of toxic heavy metals [5]. Nonetheless, the costs of resistance in and of themselves are not likely to be a significant impediment for bioremediation, as the remediating bacteria live in environments that are contaminated with heavy metals. However, resistance can sometimes incur a positive effect on other bacteria by reducing the concentration of soluble heavy metals in the environment (Table 1). Where this is the case, an efficiently bioremediating community of bacteria requires that everyone perform their fair share of detoxifying the environment, just as a well-functioning human society requires that all individuals contribute to the public good. In general, this is in line with what we want from a bioremediating community, so it is in our best interests to

promote these cooperative resistance mechanisms. However, in such cases, since only some individuals need to pay a cost in order to confer resistance to the entire microbial community, problems for the resistant cells could arise.

“... non-detoxifiers can gain a fitness advantage in the presence of Hg²⁺ reducers, while the detoxifiers suffer a fitness cost in the presence of non-detoxifiers.”

Natural selection is typically more effective at the level of the individual than the group, because individuals replicate faster and can invade other groups. Consequently, there will inevitably be selection for non-detoxifying mutants who pay a reduced or no cost of detoxification but still reap the benefits provided by their detoxifying counterparts. This fitness advantage may facilitate invasion of detoxifying groups, which can impose a large growth cost on the group as a whole, in a microbial “Tragedy of the Commons” scenario (see Sidebar A). The net result is reduced bioremediation efficiency.

The problem of common, vital, and limited resources has been extensively modeled in a wide range of scenarios from car traffic to greenhouse gas emissions to show how pursuing individual benefits

Figure 1. Competition and co-operation between detoxifying and non-detoxifying microbes.

Non-detoxifying microbes (blue) can either benefit or pay a cost, depending on how other microbes (white) in the environment deal with heavy metal toxicity. In (A), the detoxifying microbes take in and store heavy metals (red shapes) internally in their cells. As a result, non-detoxifying microbes experience a benefit because they do not pay the cost of taking in the heavy metals and the environment becomes less toxic. In (B), the detoxifying microbes pump out heavy metals from their cells, resulting in the environment becoming more toxic. Hence, non-detoxifying microbes pay a fitness cost because they cannot deal with the increase in toxicity (i.e., they cannot likewise pump out the metals). In (C), the detoxifying microbes produce metal-chelating molecules called siderophores (green shapes). These molecules bind to heavy metals and prevent heavy metals from diffusing into cells. The concentration of free-floating heavy metals in the environment is therefore reduced. Similar to (A), the non-detoxifying microbes experience a benefit because they do not pay the cost of producing the siderophores, but the environment becomes less toxic.

contrary to the best interests of the group leads to the collapse of societal function. A few studies have recently addressed the Tragedy of the Commons scenario in the specific context of bioremediation. Richard Ellis and coworkers from Imperial College London demonstrated that the reduction of toxic Hg^{2+} to non-toxic, volatile $\text{Hg}(0)$ is a social trait; non-detoxifiers can gain a fitness advantage in the presence of Hg^{2+} reducers,

while the detoxifiers suffer a fitness cost in the presence of non-detoxifiers [6]. We also recently demonstrated that populations of copper-chelating siderophore producers are vulnerable to invasion by non-producing cheats and that copper toxicity can select for rapid *de novo* evolution of cheats from clonal populations of producers [7].

While these single-species studies are vital for understanding the social nature of

these traits, they tell us little about whether social selection actually matters in real life. In reality, the Tragedy of the Commons may be even harder to overcome because the beneficiaries of bioremediation are not just mutant conspecifics but the whole microbial community. In a spatially structured environment, microbes often exist in close physical contact with other species, such as in multi-species biofilms, and so, benefits of bioremediation are less likely to accrue to clone mates alone. More generally, when multiple species rely on the same resource, individual species may benefit by losing an essential biosynthetic “leaky” function, such as a vital public good, provided the function is retained by at least one other species in the community—this is the gist of the Black Queen Hypothesis that assumes an evolutionary advantage in losing gene functions within a microbial community (see Sidebar A). Thus, any detoxifying trait that incurred a community benefit could be sequentially lost from the community, even when diversity is high. This is likely to be initiated by the most numerically dominant species, and perhaps a key constituent of the bioremediating community.

In short, costly mechanisms of bioremediation can be exploited when they accrue benefits to others, meaning that such “cooperative” mechanisms of resistance are more likely to be lost when surrounded by the microbial community. Selection should instead favor selfish mechanisms of resistance, such as reducing cell permeability or developing efflux pumps. Interestingly, P_{1B} -type ATPases, which can function as efflux pumps, are among the most widespread resistance mechanisms in microbes [8], although it is of course not clear if this is influenced by social selection, other associated benefits of efflux pumps such as antibiotic resistance, or simply that they confer the greatest resistance at the least cost. We are currently carrying out experiments to investigate the importance of social interactions for the evolution of heavy metal resistance strategies.

On a more optimistic note, the application of ecology and evolution to the study of microbial resistance provides some novel answers to the question of how we could increase the efficiency and durability of bioremediation. Thus, enhancing direct benefits of cooperative detoxification could reduce the fitness

Sidebar A: Further reading**Mechanisms of metal resistance in microbes**

Various ways in which bacteria deal with excess heavy metals

Ji G, Silver S (1995) Bacterial resistance mechanisms for heavy metals of environmental concern. *J Ind Microbiol* 14: 61–75

Chien CC, Lin BC, Wu CH (2013) Biofilm formation and heavy metal resistance by an environmental *Pseudomonas* sp. *Biochem Eng J* 78: 132–137

Muller M, Merrett ND (2014) Pyocyanin production by *Pseudomonas aeruginosa* confers resistance to ionic silver. *Antimicrob Agents Chemother* 58: 5492–5499

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiol Rev* 27: 313–339

Wolfe-Simon F, Blum JS, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, Weber PK, Davies PCW *et al* (2010) A bacterium that can grow by using arsenic instead of phosphorus. *Science* 332: 1163–1166

Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa* heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 69: 2313–2320

Cowan SW, Garavito RM, Janssonius JN, Jenkins JA, Karlsson R, König N, Pai EF, Pauptit RA, Rizkallah PJ, Rosenbusch JP (1995) The structure of OmpF porin in a tetragonal crystal form. *Structure* 3: 1041–1050

The use of bacteria in bioremediation

Environmental effects, application and mechanisms of bioremediation

Alexander M (1999) *Biodegradation and Bioremediation*. Academic Press: London, UK

The evolution of cooperation

Studies investigating the breakdown and persistence of cooperative traits

Hardin G (1968) The tragedy of the commons. *Science* 162: 1243–1248

Mc Ginty SE, Rankin DJ, Brown SP (2011) Horizontal gene transfer and the evolution of bacterial cooperation. *Evolution* 65: 21–32

Morris JJ, Lenski RE, Zinser ER (2012) The black queen hypothesis: Evolution of dependencies through adaptive gene loss. *MBio* 3: 2 e00036–12

Nogueira T, Rankin DJ, Touchon M, Taddei F, Brown SP, Rocha EPC (2009) Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. *Curr Biol* 19: 1683–1691

Adaptation and diversity

A diverse natural microbial community is essential for rapid and efficient adaptation to toxic environments

Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sørensen SJ, Bååth E, Bloem J, de Ruiter PC *et al* (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *2*: 279–294

advantage for non-detoxifying ‘cheats’ (Fig 1). For instance, although siderophores can confer heavy metal resistance to *P. aeruginosa*, their primary function is iron acquisition: Siderophores chelate unavailable ferric iron (Fe^{3+}) and transport it into the cell to be used for cellular functions as ferrous iron (Fe^{2+}). Since iron is an important constituent of bacterial growth, any mutant that fails to produce siderophores will suffer a fitness cost (particularly in a structured environment). Hence, when iron is unavailable, the direct benefits of siderophore production—iron acquisition and detoxification—are substantial. In practice, the addition of lime-containing materials can stimulate an alkaline-rich and

Fe^{3+} -promoting environment, thus restricting iron availability.

Increasing the spatial structure of the substrate could also increase the cost of cheating, ultimately by keeping producers and their products together. Consequently, freeloaders would pay a fitness cost owing to the toxicity of their local environment—and a further cost of not being able to access iron if iron restriction was also imposed (above). The addition of organic matter such as manure can improve spatial structure in clay and sandy soils and has been reported as enhancing the efficiency of bioremediation [9]. However, this improvement has been attributed to enhanced microbial growth, and not microbial social dynamics.

Another approach would aim to discourage “selfish” resistance mechanisms, such as efflux pumps and reducing cell-surface permeability, which ultimately result in an increase in extracellular toxicity and hence can have a negative effect on others. Clearly, this is not consistent with what we want from a bioremediating community. Increasing the cost associated with selfish resistance and the benefit of cooperative resistance might encourage the evolution of bioremediation-friendly adaptations to heavy metal toxicity. For instance, the cost associated with overexpressing the MexEF-OprN multidrug efflux pump increases under anaerobic conditions as enhanced H^+ influx increases cytoplasmic acidification [10]. Moreover, the loss of non-specific transport channels, such as OmpF mutations conferring resistance to Ag^+ in *E. coli*, is likely to reduce access to water, ions, glucose, and other nutrients. Limiting nutrient access could thus reduce the benefit associated with porin-based forms of resistance.

While diverse communities may exacerbate the Tragedy of the Commons to some extent, there are other general benefits of diversity, including better community-wide resource use, reduced susceptibility to invasion from both pathogens and novel competitors, and enhanced resilience to environmental change. In the context of detoxification, not only are diverse communities more likely to contain intrinsically resistant species, but their standing genetic diversity increases the likelihood and speed with which novel resistance traits, such as low-cost remediation, evolve.

Despite the initial advantage diversity has on heavy metal resistance, heavy metal contamination is most commonly associated with a reduction in microbial diversity and consequently ecosystem functioning, as some species are inevitably intrinsically resistant. Keeping community diversity high is likely to be of great importance for productivity, and lessons can be learned from the recent success of using fecal transplants to treat *Clostridium difficile* infections in humans. This involves replacing the *C. difficile*-dominated gut microbiota of an ill patient with the highly diverse gut microbiota of a healthy individual. Could it similarly be possible to transfer a “healthy” soil microbiota that is effective at bioremediation to an environment with a poorly diverse microbial community? Moreover, are such “healthy” microbial communities more likely to be characterized by a few

well-adapted species that are sufficient to perform efficient bioremediation, or are the sparse, less common species playing a role too? These are questions that are crucial for understanding how microbial communities shape ecosystems, in the context of both medicine and the environment.

“... enhancing direct benefits of cooperative detoxification could reduce the fitness advantage for non-detoxifying cheats.”

Finally, horizontal genetic elements (HGEs) are another factor that promote cooperation and thereby facilitate bioremediation. Detoxification genes located on plasmids, transposable elements, or carried by temperate phages are potentially less vulnerable to non-detoxifiers, as mutant cells are continuously re-infected with the cooperating element. Consequently, cooperative genes tend to be over-represented on mobile elements. The introduction of either synthesized or natural conjugative plasmids or temperate phages to a bacterial community containing susceptible species could impede invasion by freeloaders. However, in an unstructured environment, another plasmid that does not code for cooperation can similarly spread through the population, and so the social dilemma repeats itself. Hence, increasing spatial structure as mentioned above in combination with the addition of HGE-encoded cooperative traits could be a convincing strategy to encourage more efficient bioremediation.

Bioremediation is a low cost and environmentally friendly alternative to chemical processing of contaminated soils or water, but it suffers from a lack of reliability. This could well change with increasing knowledge about microbial societies: During the past decade, microbes have been viewed less as isolated entities and more as socially interacting communities, which has yielded important medical advances such as the treatment of *C. difficile* infections described above. We have discussed how social interactions may matter for bioremediation, but this is equally applicable for other environmental processes such as biomining, methane production, and plastic degradation. Bringing together the fields of microbial bioremediation and social evolution could ultimately enhance the efficiency of using microbial populations to clean up our mess.

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Conflict of interest

The authors declare that they have no conflict of interest.

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copper-enriched activated sludge revealed by metagenomic analysis. *Appl Microbiol Biotechnol* 98: 10255–10266

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