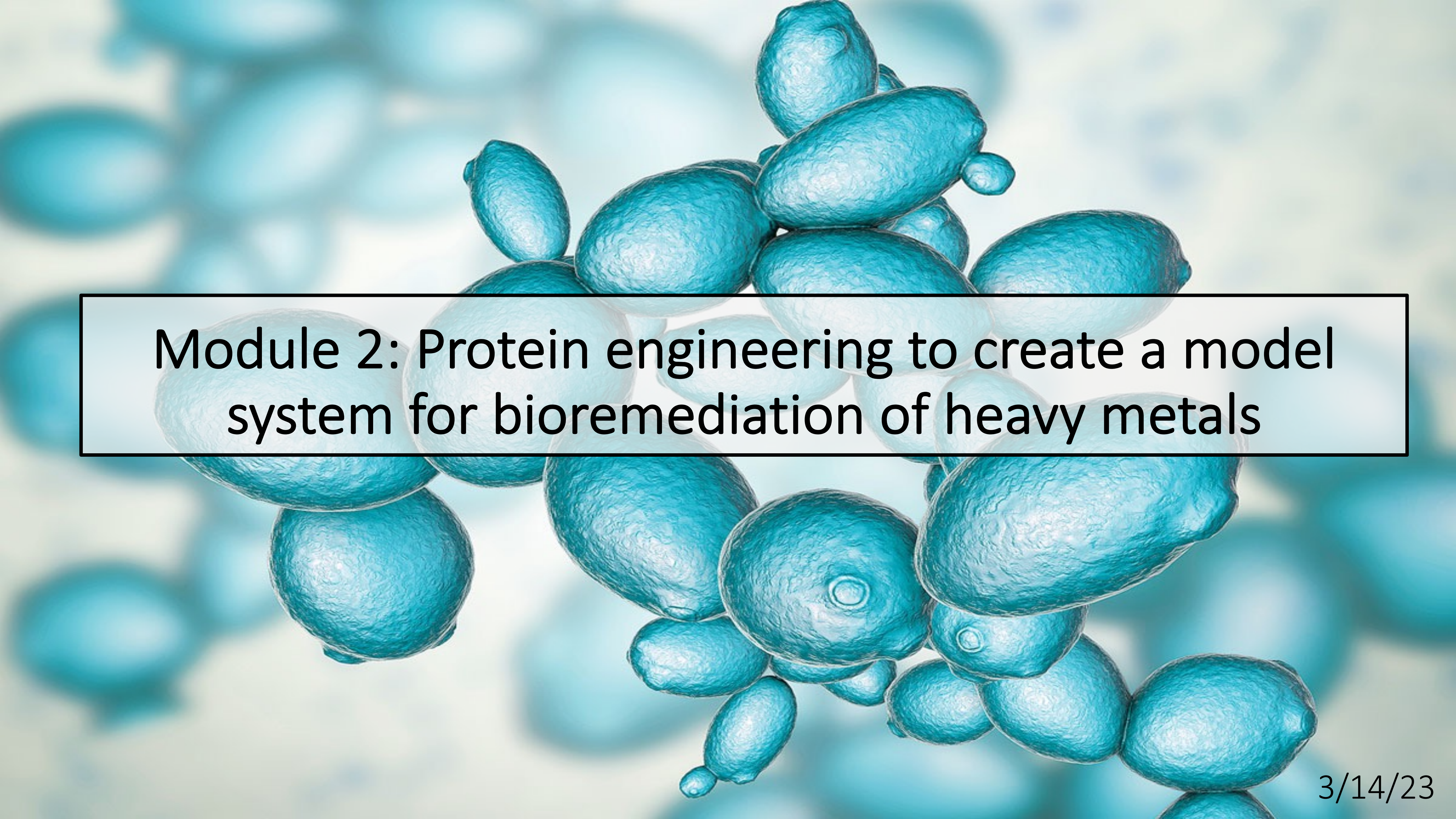


Module 2: Protein engineering to create a model system for bioremediation of heavy metals

3/14/23

Overview of Module 2 goals

Research:

Genetically modify a yeast iron transporter to preferentially take up cadmium as a model for bioremediation

Communication:

Journal article presentation

Research article

Technical:

Protein engineering:

Site-Directed Mutagenesis

Mutant expression

Functional assays:

Elemental analysis of metal uptake

Cell tolerance of metal uptake

Module Outline

- **M2D1**: Environmental heavy metal contamination — Intro
- **M2D2**: Model system – target selection and engineering approach
- **M2D3**: Model system – choosing and modifying a chassis — Design
- **M2D4**: Screening a system—high throughput vs functional screens
- **M2D5**: Analysis of elemental metals – laboratory and field approaches — Test
- **M2D6**: Applying remediation strategies—advantages and pitfalls
- **M2D7**: Engineering a problem-specific bioremediation solution — Apply
- **M2D8**: Comm Lab — Review

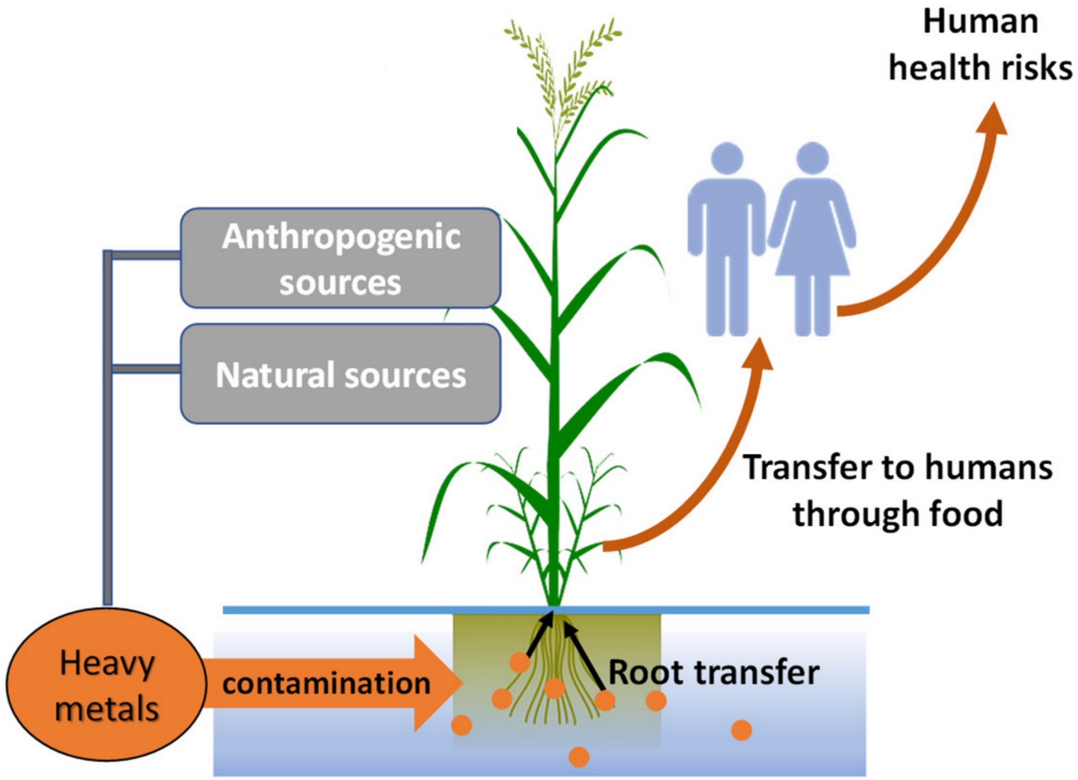
Overview of today's lecture

- Heavy metals
  - What are they?
  - What are their uses?

- How do heavy metals get into environment?
  - Geogenic sources
  - Anthropogenic sources

- What happens after heavy metal exposure
  - To soil
  - To plants
  - To humans

- How can we mitigate heavy metal contamination?



adapted from Uddin et al.

Heavy metals and their uses

Heavy metals

Nickel

Manganese

Cadmium

Mercury

Chromium

Gold

Copper

Cobalt

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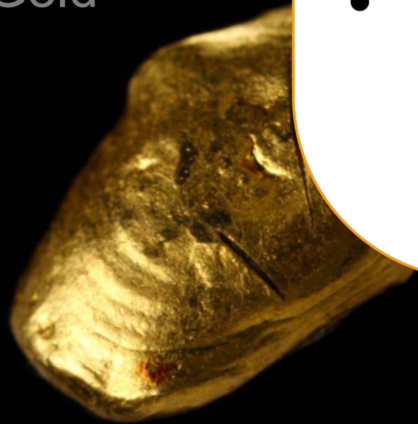
Heavy metals





<u>Heavy metals</u> is poorly defined as a term

- Relatively high atomic density (greater than 5 g/cm^3)
- Atomic number > 20
- Exhibit metal-like properties

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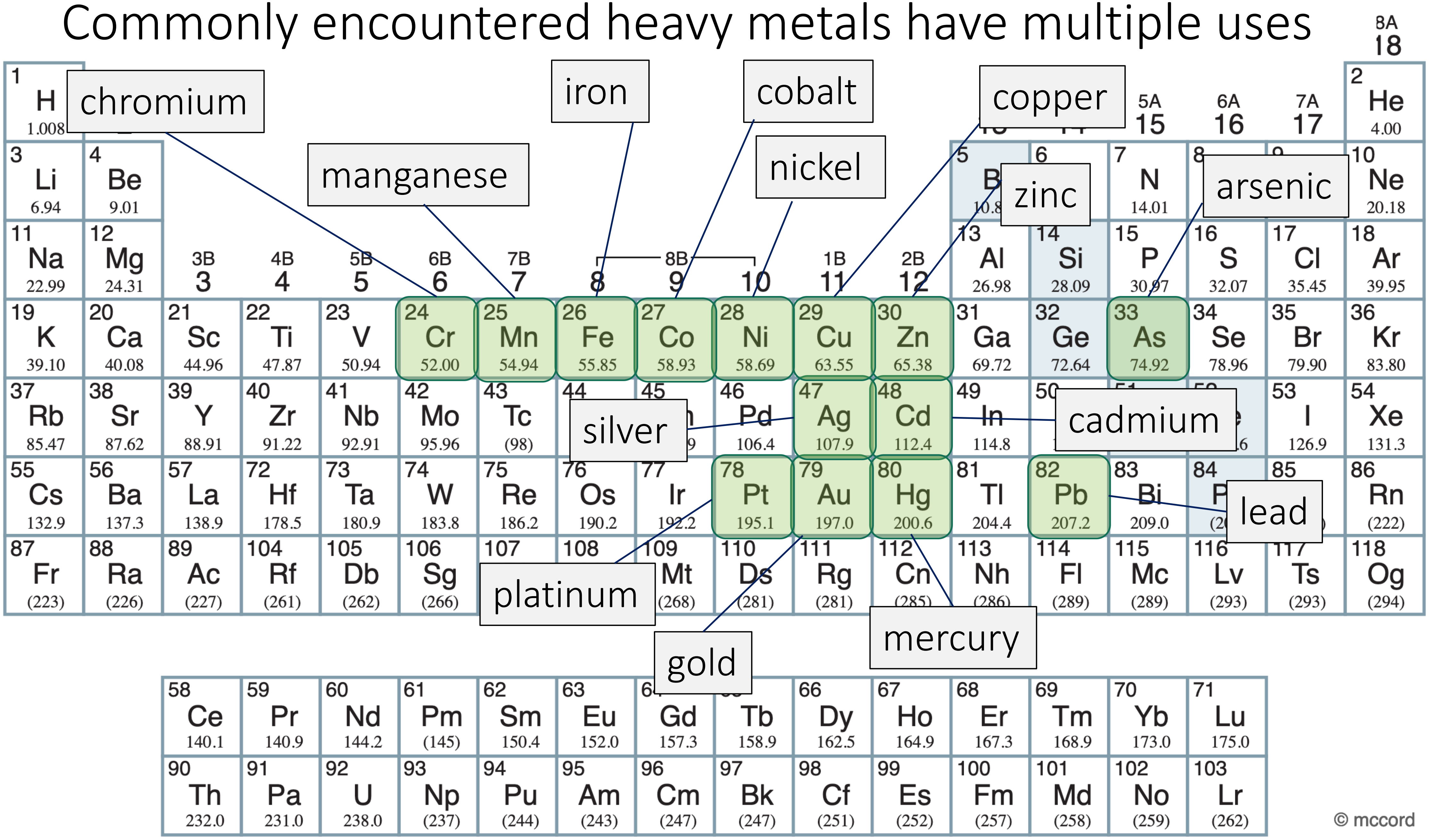
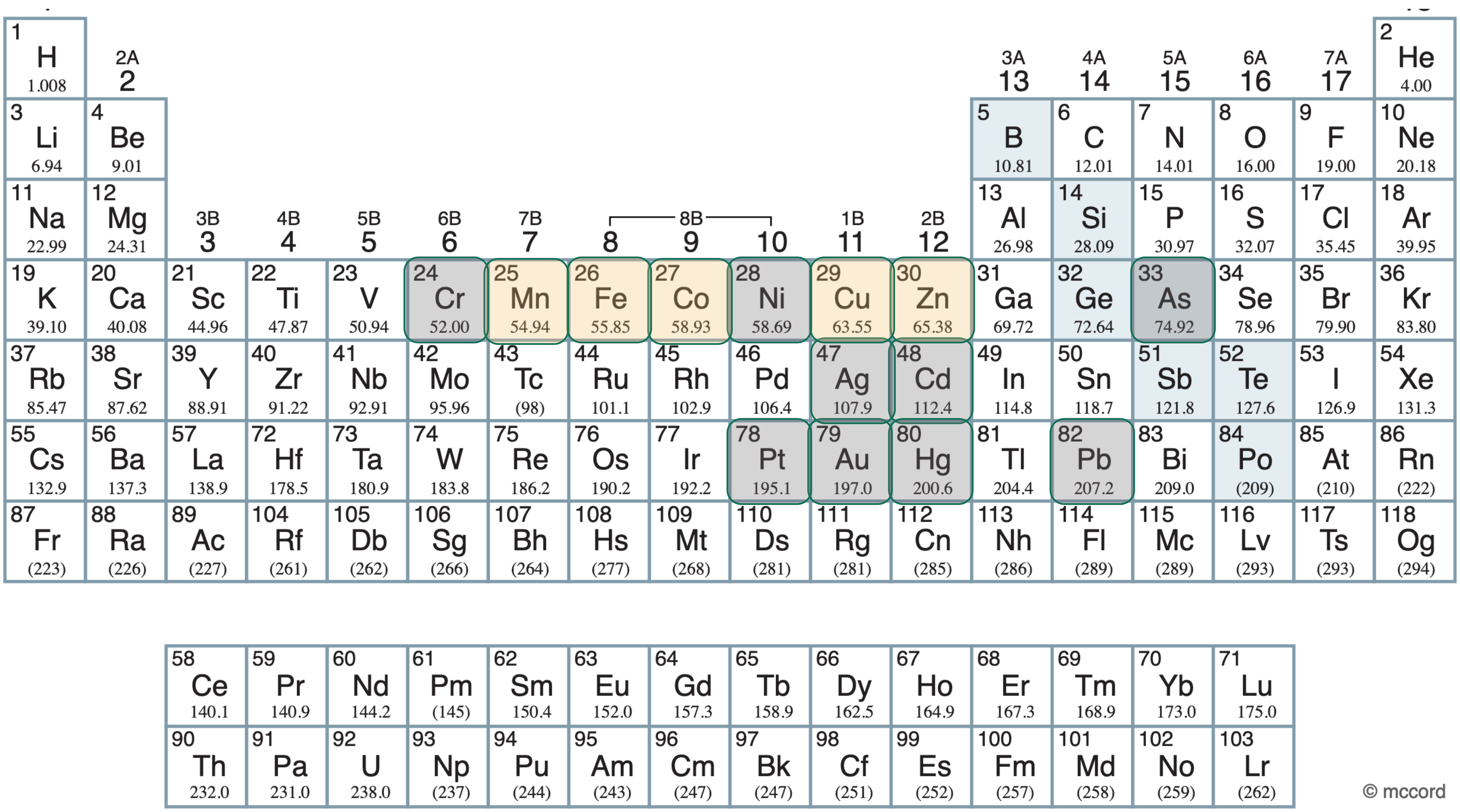
Commonly encountered heavy metals have multiple uses

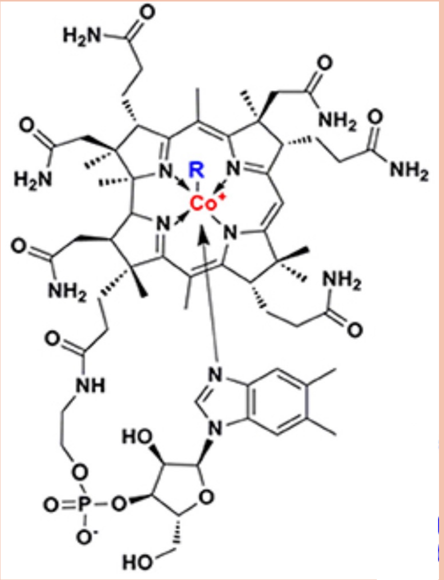


Metals can act as protein co-factors in human biology



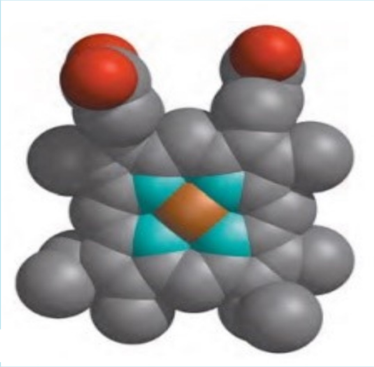
Metals crucial for metabolic activity are also known as essential elements

Cobalt



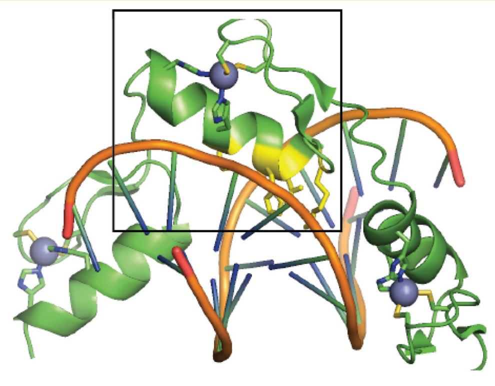
vitamin B12

Iron



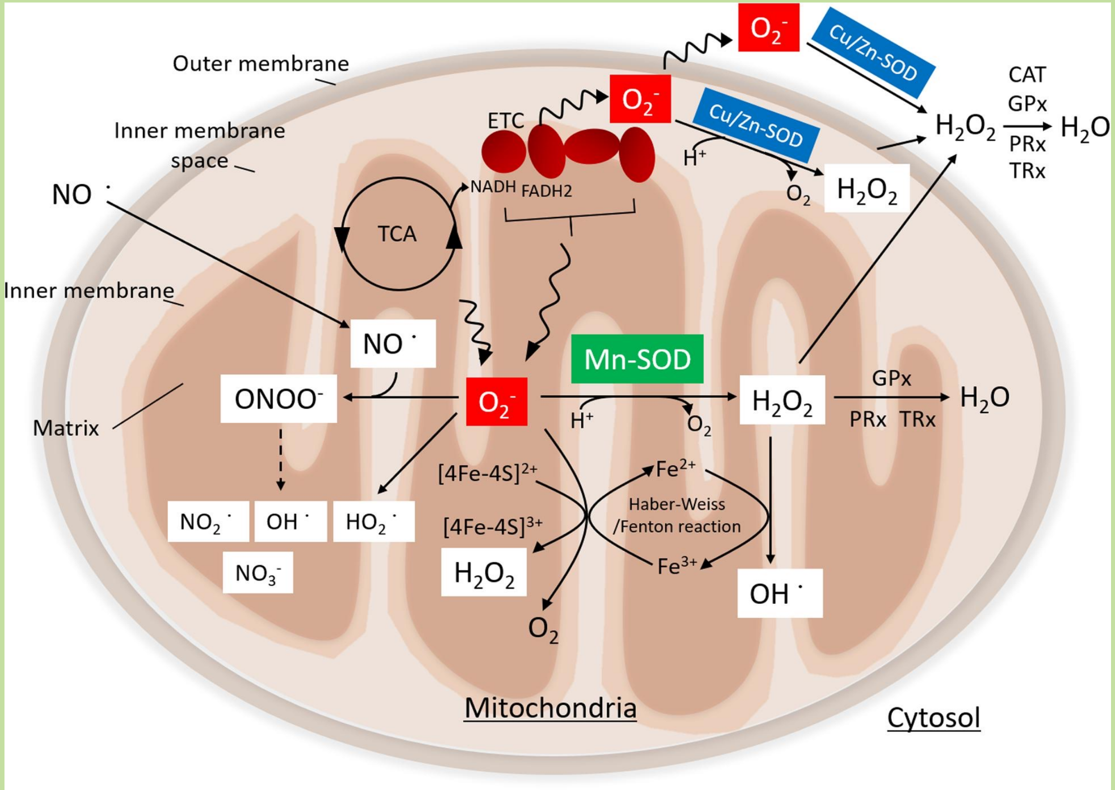
Heme B subunit

Zinc



zinc finger motif

Manganese / Copper



Mitochondrial SOD cofactors

Heavy metals have value as “precious metals”

1A 1	2A 2	3B 3	4B 4	5B 5	6B 6	7B 7	8B 8	8B 9	8B 10	1B 11	2B 12	3A 13	4A 14	5A 15	6A 16	7A 17	8A 18
1 H 1.008																	2 He 4.00
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.07	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.87	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.38	31 Ga 69.72	32 Ge 72.64	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.96	43 Tc (98)	44 Ru 101.1	45 Rh 102.9	46 Pd 106.4	47 Ag 107.9	48 Cd 112.4	49 In 114.8	50 Sn 118.7	51 Sb 121.8	52 Te 127.6	53 I 126.9	54 Xe 131.3
55 Cs 132.9	56 Ba 137.3	57 La 138.9	72 Hf 178.5	73 Ta 180.9	74 W 183.8	75 Re 186.2	76 Os 190.2	77 Ir 192.2	78 Pt 195.1	79 Au 197.0	80 Hg 200.6	81 Tl 204.4	82 Pb 207.2	83 Bi 209.0	84 Po (209)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra (226)	89 Ac (227)	104 Rf (261)	105 Db (262)	106 Sg (266)	107 Bh (264)	108 Hs (277)	109 Mt (268)	110 Ds (281)	111 Rg (281)	112 Cn (285)	113 Nh (286)	114 Fl (289)	115 Mc (289)	116 Lv (293)	117 Ts (293)	118 Og (294)

58 Ce 140.1	59 Pr 140.9	60 Nd 144.2	61 Pm (145)	62 Sm 150.4	63 Eu 152.0	64 Gd 157.3	65 Tb 158.9	66 Dy 162.5	67 Ho 164.9	68 Er 167.3	69 Tm 168.9	70 Yb 173.0	71 Lu 175.0
90 Th 232.0	91 Pa 231.0	92 U 238.0	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)

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Precious metals have economic and cultural value

Silver: $21 / oz

Ag

Gold: $1,858 / oz

Au

Platinum: $979 / oz

Pt

Many heavy metals play a role in manufacturing

1A 1	2A 2	3B 3	4B 4	5B 5	6B 6	7B 7	8B 8	8B 9	8B 10	1B 11	2B 12	3A 13	4A 14	5A 15	6A 16	7A 17	8A 18
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37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.96	43 Tc (98)	44 Ru 101.1	45 Rh 102.9	46 Pd 106.4	47 Ag 107.9	48 Cd 112.4	49 In 114.8	50 Sn 118.7	51 Sb 121.8	52 Te 127.6	53 I 126.9	54 Xe 131.3
55 Cs 132.9	56 Ba 137.3	57 La 138.9	72 Hf 178.5	73 Ta 180.9	74 W 183.8	75 Re 186.2	76 Os 190.2	77 Ir 192.2	78 Pt 195.1	79 Au 197.0	80 Hg 200.6	81 Tl 204.4	82 Pb 207.2	83 Bi 209.0	84 Po (209)	85 At (210)	86 Rn (222)
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90 Th 232.0	91 Pa 231.0	92 U 238.0	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)

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Heavy metals are used to manufacture common materials

Dyes and Pigments

Prussian Blue

Pb | Fe | Co

As | Ni

Cu | Zn

Fe | Cr | Ni

Stainless Steel

Heavy metals are frequently used in coating and electroplating for everything from automotive to aerospace machinery

Zn

Ni Cu

Au Pt

Cd Mn

Ag Cr

Chrome plating

Batteries utilize heavy metals

Alkaline batteries

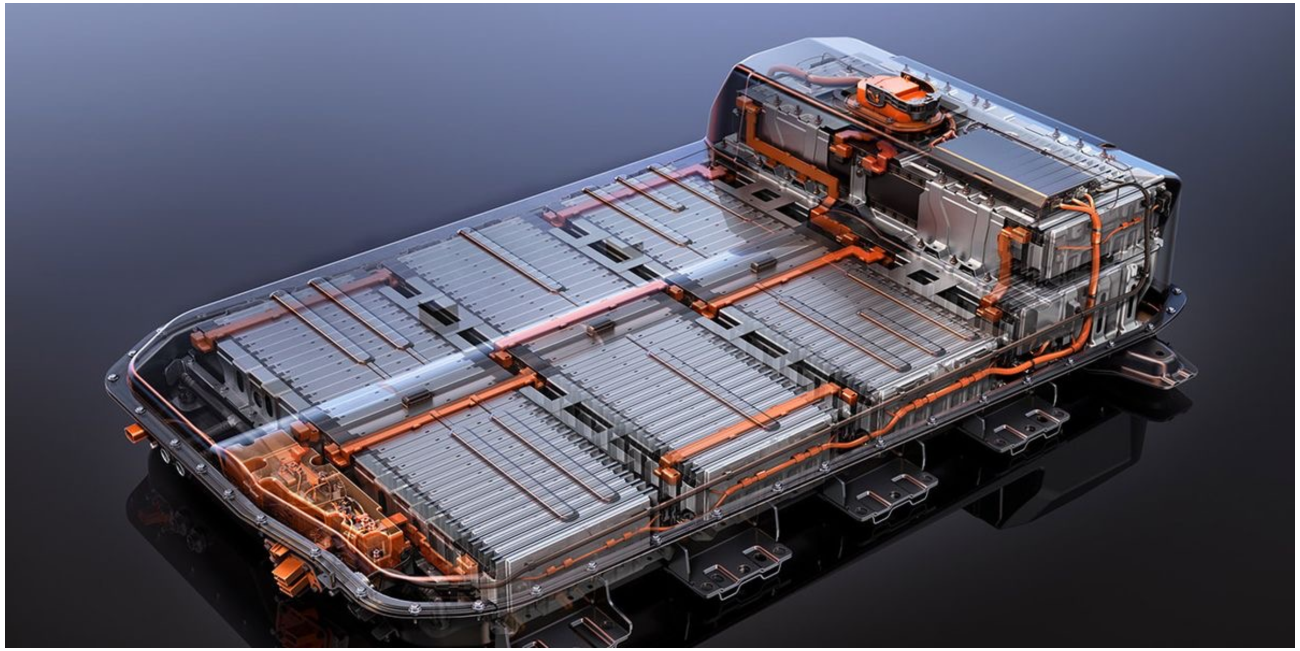
Cd Ni

Mn Zn

Lead-Acid battery

Pt Au Pb

EV Battery

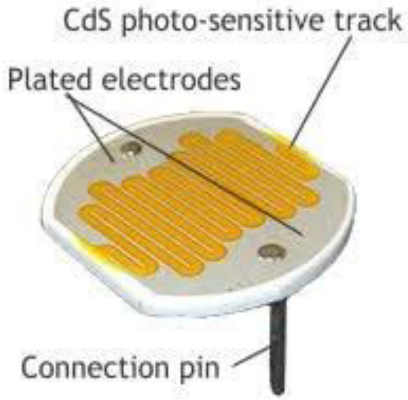


Cu
Ni
Mn
Co

Photovoltaic cells, photoresistors, infrared detectors all use heavy metals

Infrared detectors & Photoresistors

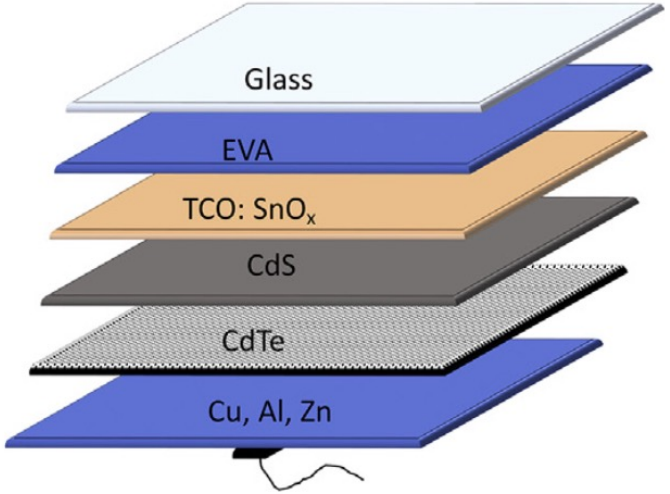
Cd Pb Cu Hg



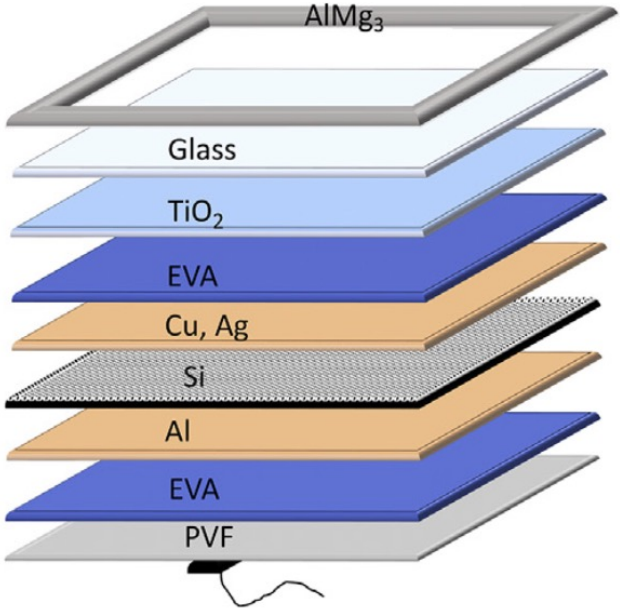
Solar photovoltaic cells

Cu
Cd
Ag
Zn
Pt

Cadmium-Telluride



crystalline-Silicon



Some heavy metals are highly toxic at low exposure levels

1A 1	2	3B 3	4B 4	5B 5	6B 6	7B 7	8B 8	9	10	1B 11	2B 12	13	14	15	16	A 17	8A 18
1 H 1.008																	2 He 4.00
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Environmental contamination and its consequences

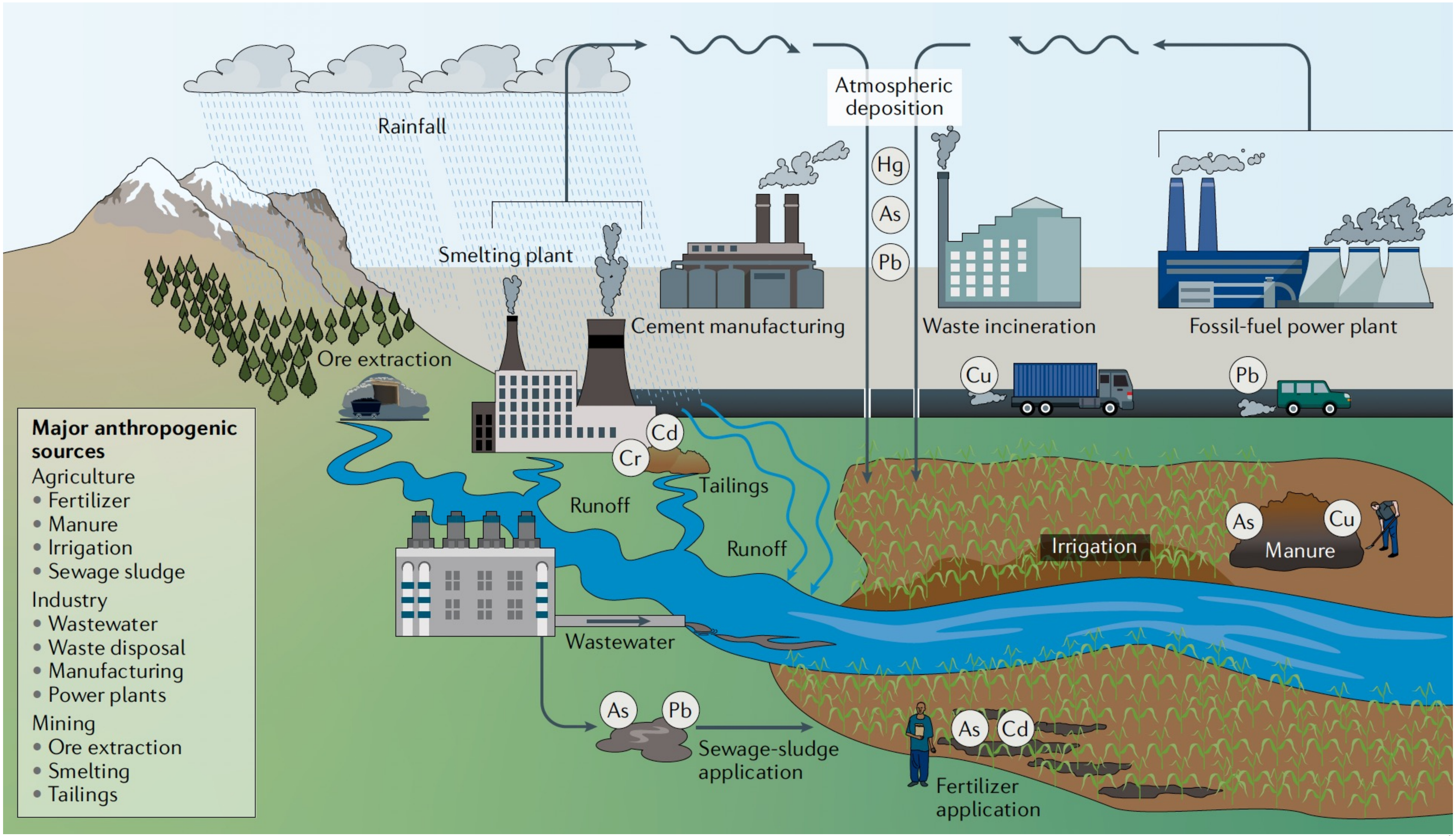
There are 2 main routes of heavy metal release into the environment

Geogenic sources

- Weathering of rock
- Volcanoe

Anthropogenic sources

- Agrochemicals
- Industrial activity
- Smelting and mining activity
- Sewage and waste disposal



Hou et al. 2021

Agrochemicals release heavy metals into the soil

Fertilizers

- Sewage sludge fertilizer contains heavy metals
- Fly ash from coal plants
- Inorganic phosphate-based fertilizers increase cadmium in the soil
  - Some disagreement if the fertilizers release cadmium or increase bioavailability

Pesticides and fungicides

- Can contain heavy metals as contaminants

Industrial activity contributes to heavy metal contamination

- Coal-fired power stations release:
  - Cu, Zn, Cd, Ni

- Chemical processing which involves heavy metals is required to produce common goods
  - Plastics
  - textiles
  - electronics
  - wood preservatives
  - automotive components

- The waste generated in manufacturing can leach into the environment

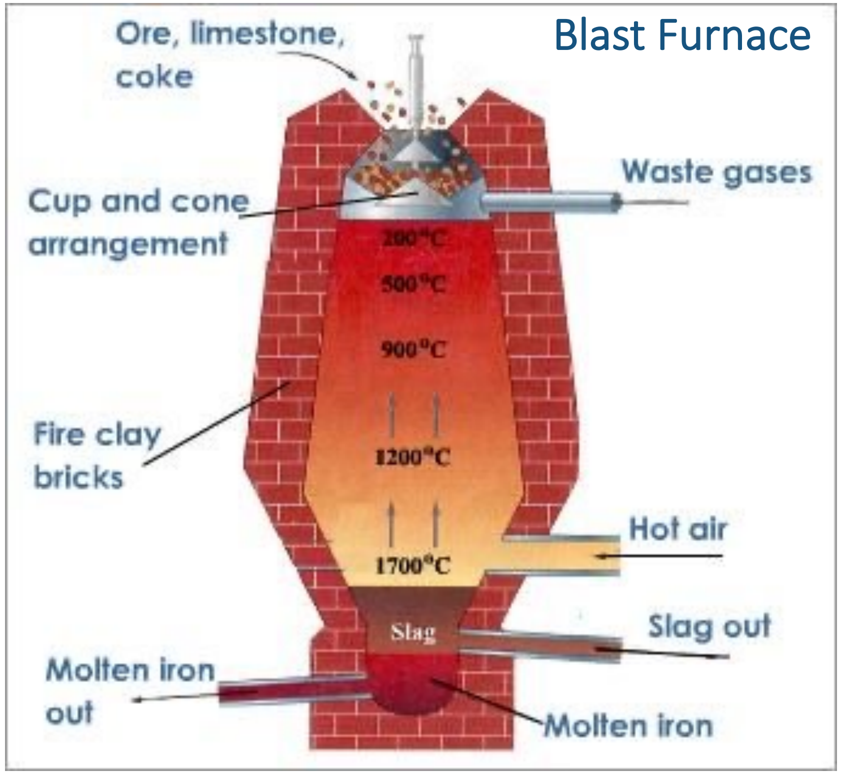
Smelting and mining activity produce metal contaminants

Mining

- Disruption of sedimentary layers can release embedded heavy metals
- Waste runoff from mining sites contaminates water

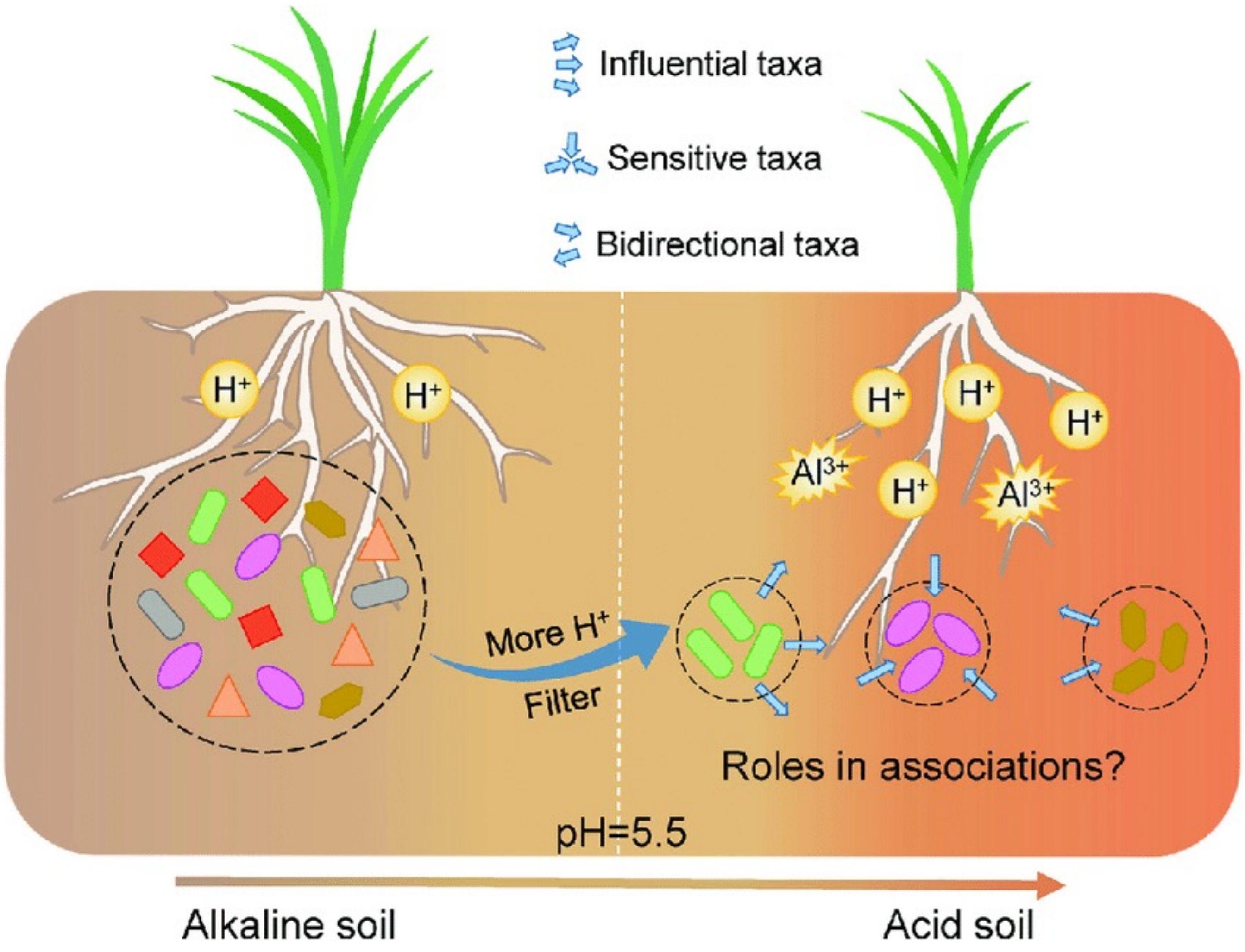
Smelting

- Slag generated from refinement of metal can contain contaminants
  - Smelting zinc produces slag containing lead and cadmium
- Heavy metal particulates are also released



Heavy metals fundamentally change soil microbial richness

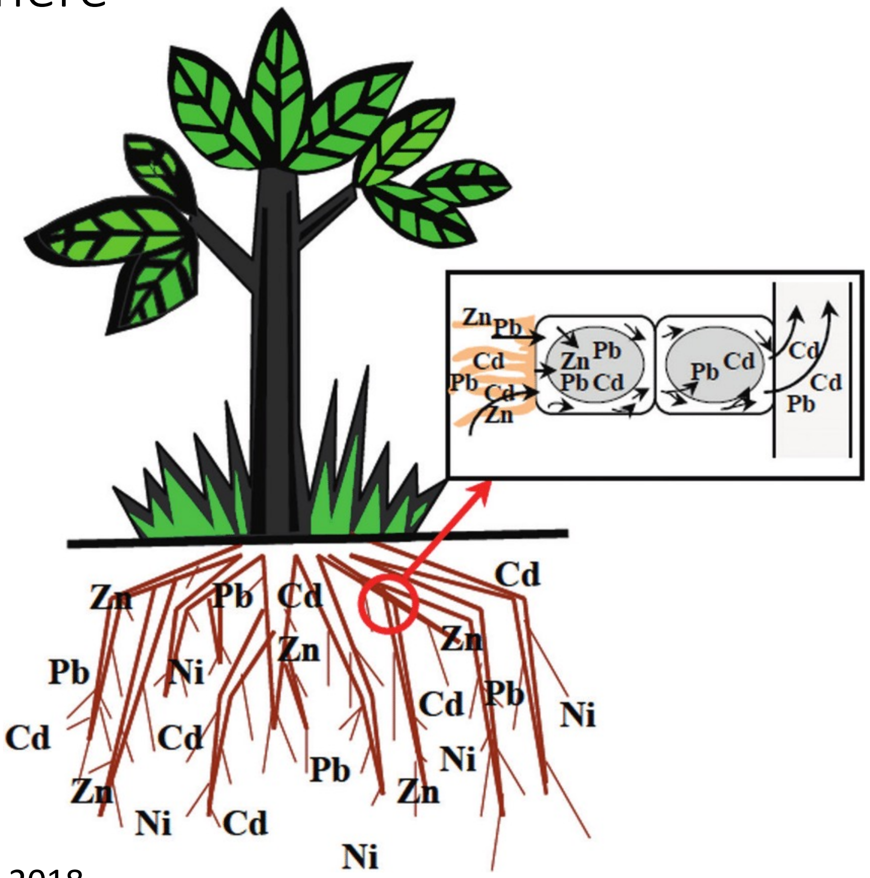
- Decrease in **soil viability**
  - lower microbial biomass
  - less biodiversity
- Reduced **nitrogen fixing**
- Reduced microbial **metabolism**
  - reduced essential enzyme activities
  - reduced litter breakdown
- Altered microbial communication
- Changes in soil ecosystem



Zhang et al. 2022

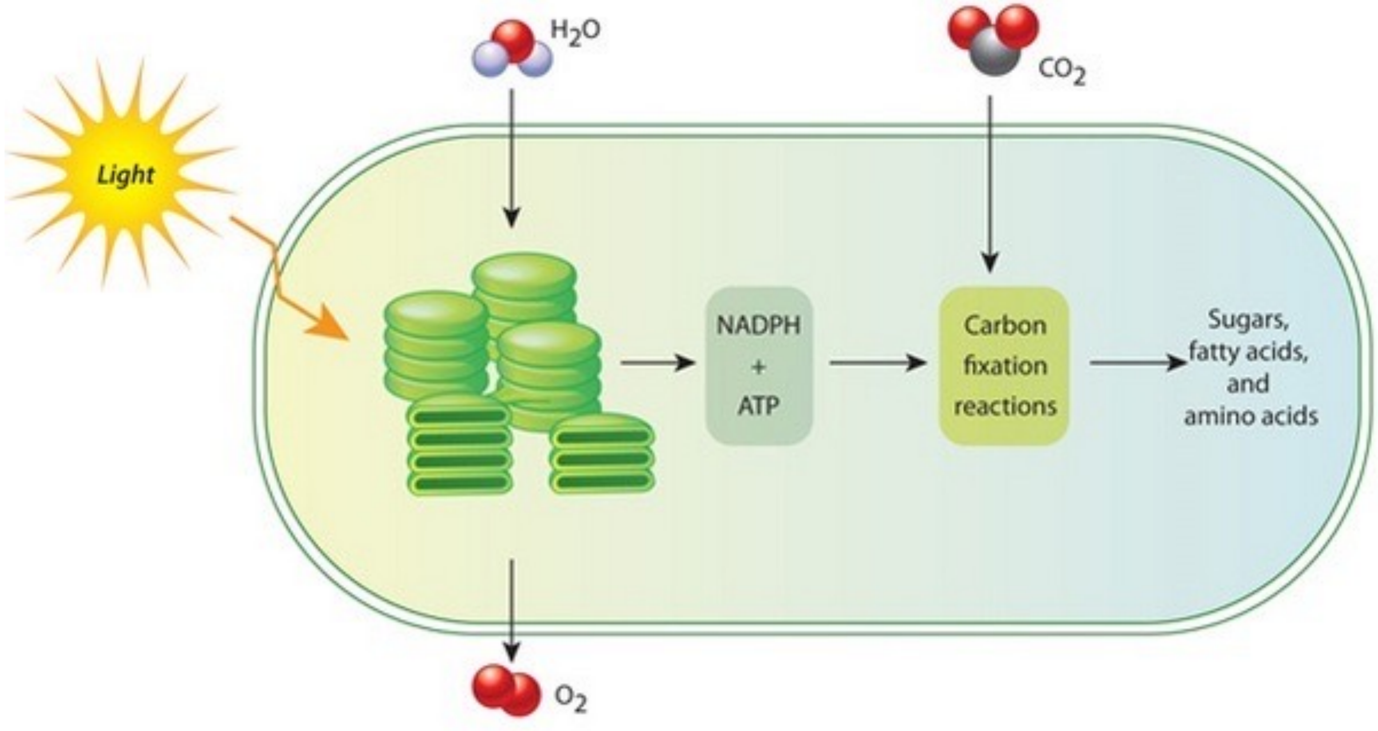
Heavy metal accumulates in plants and disrupts essential biology

- Most heavy metal enters the plant through the roots and accumulates there



Adiloglu, 2018

- General stress response
  - obstruct chloroplast structure
  - disrupt electron transport



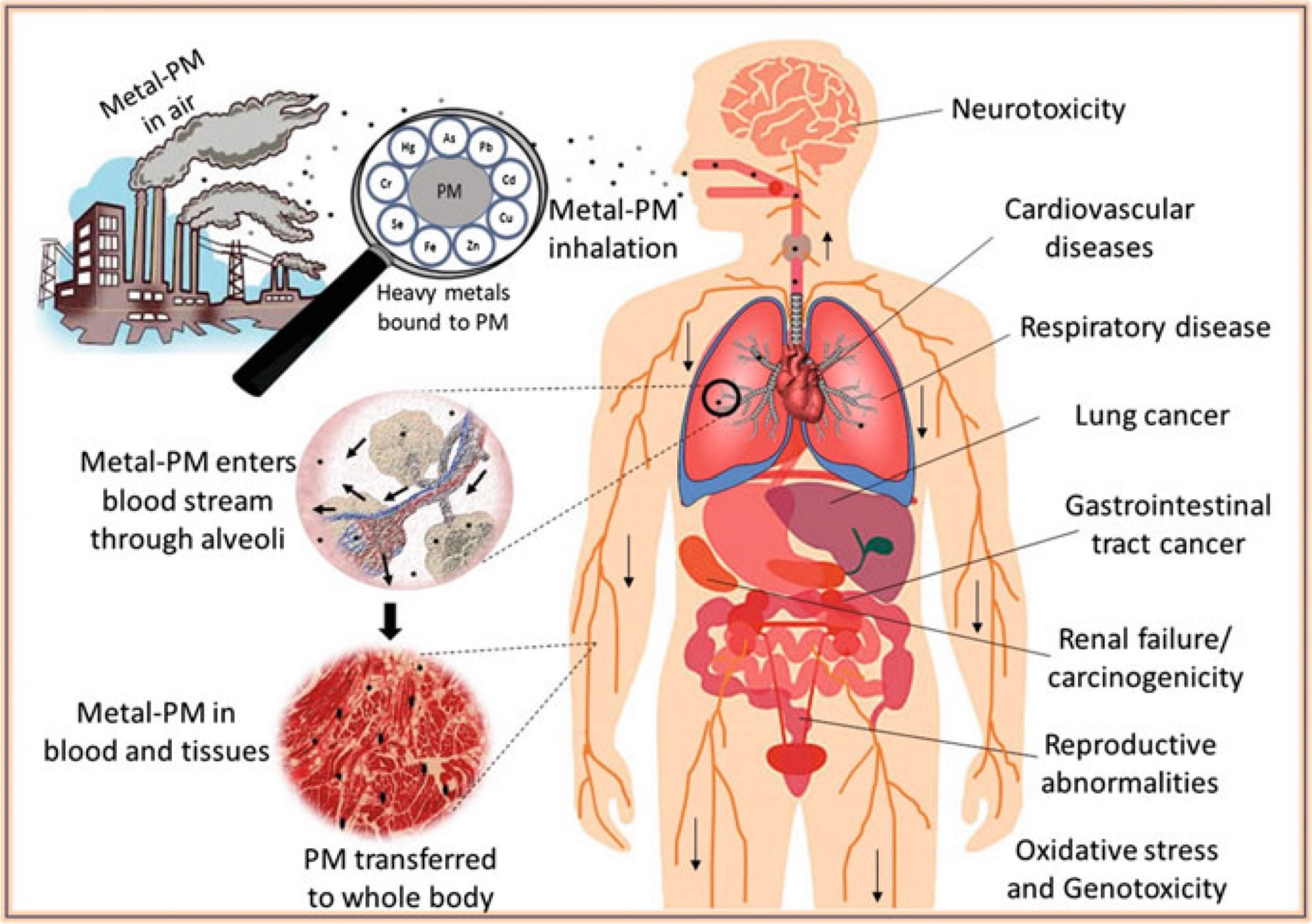
Heavy metal exposure has wide ranging effects on human health

Routes of exposure

- Inhalation
- Ingestion
- Dermal

Health effects

- Systemic toxicity
- Damage of multiple organs



Reviews of Environmental Contamination and Toxicology. Volume 253

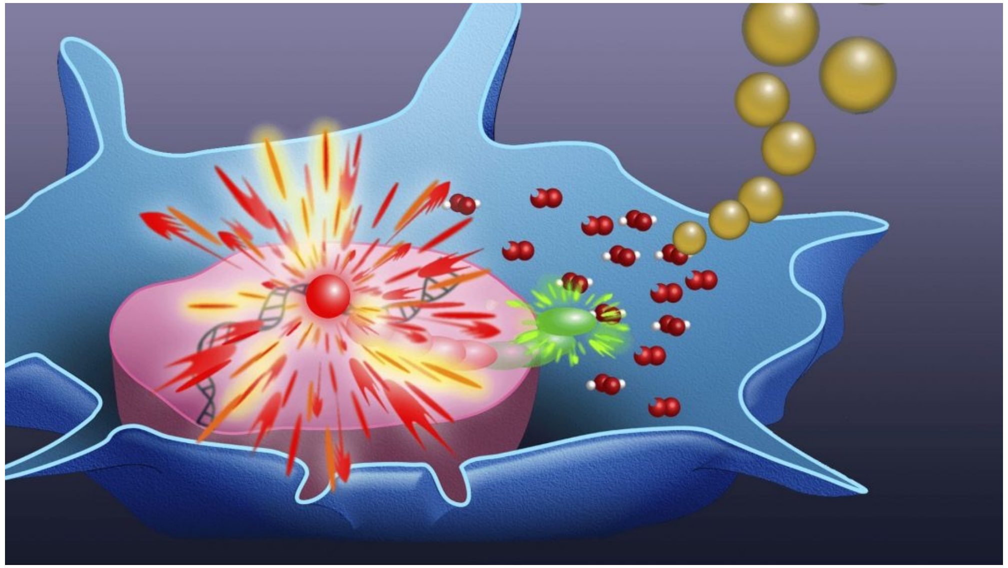
There are multiple proposed mechanisms for metal toxicity

Protein disruption

- Inhibit enzymes through thiol, sulfhydryl, amide group binding
  - Broad enzyme inhibition
- Inhibits enzymes involved in DNA damage repair
  - Many heavy metals are known or putative carcinogens
- Replace essential metal cations and cofactors

Oxidative stress

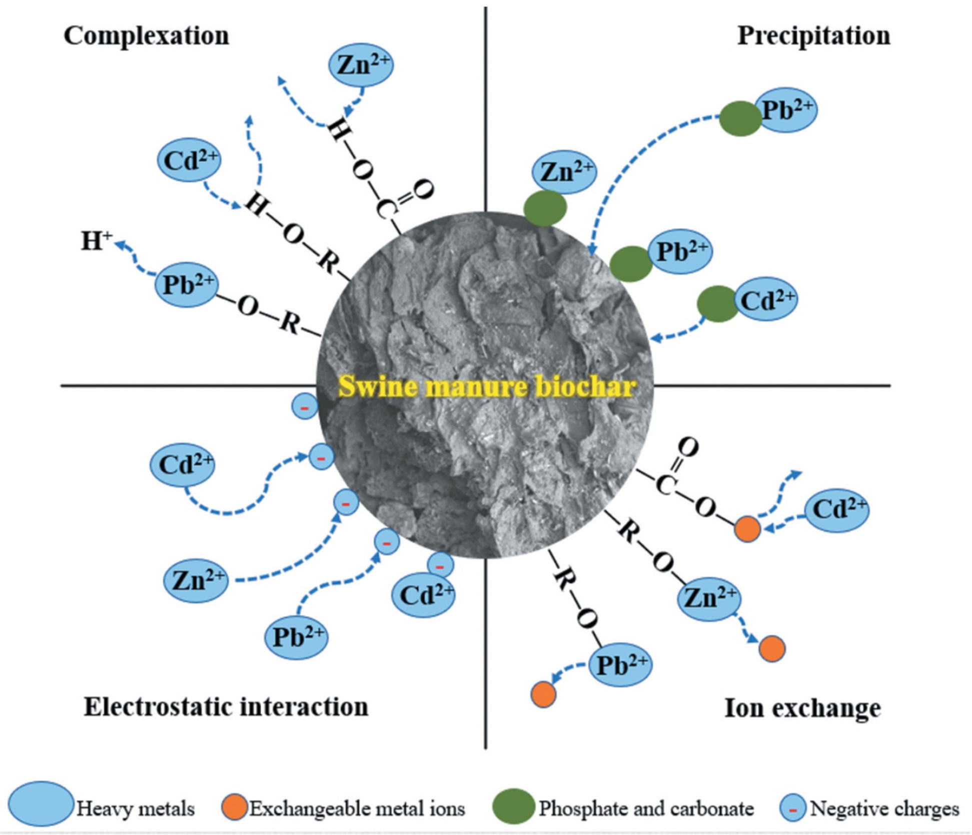
- Disrupt mitochondrial function
- Generate reactive oxygen species



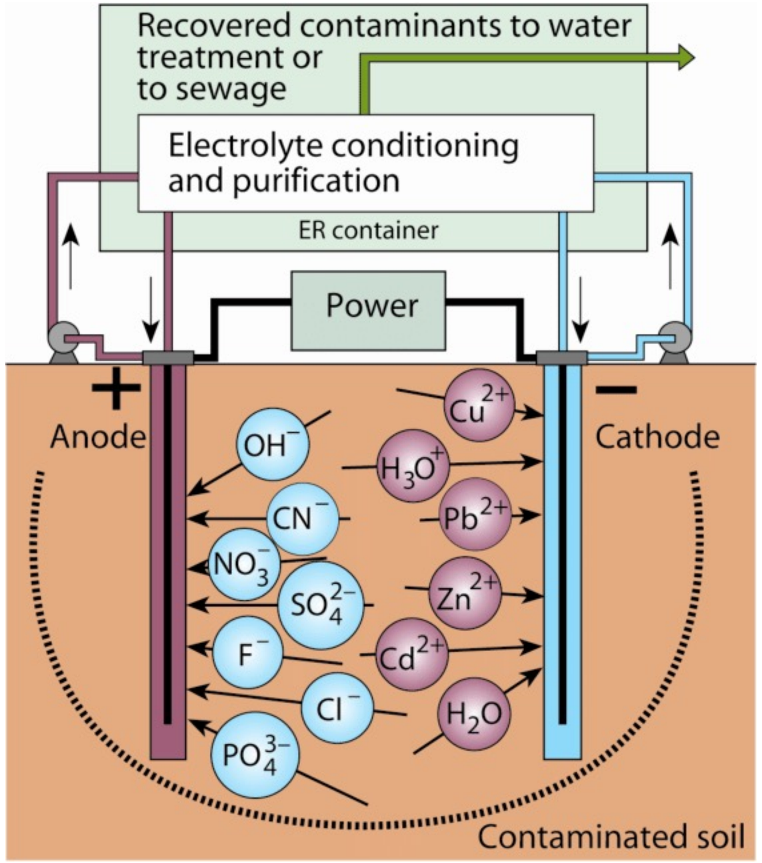
What can we do to mitigate this issue?

Physical and chemical mitigation of heavy metal contamination

Soil Amendment with Biochar



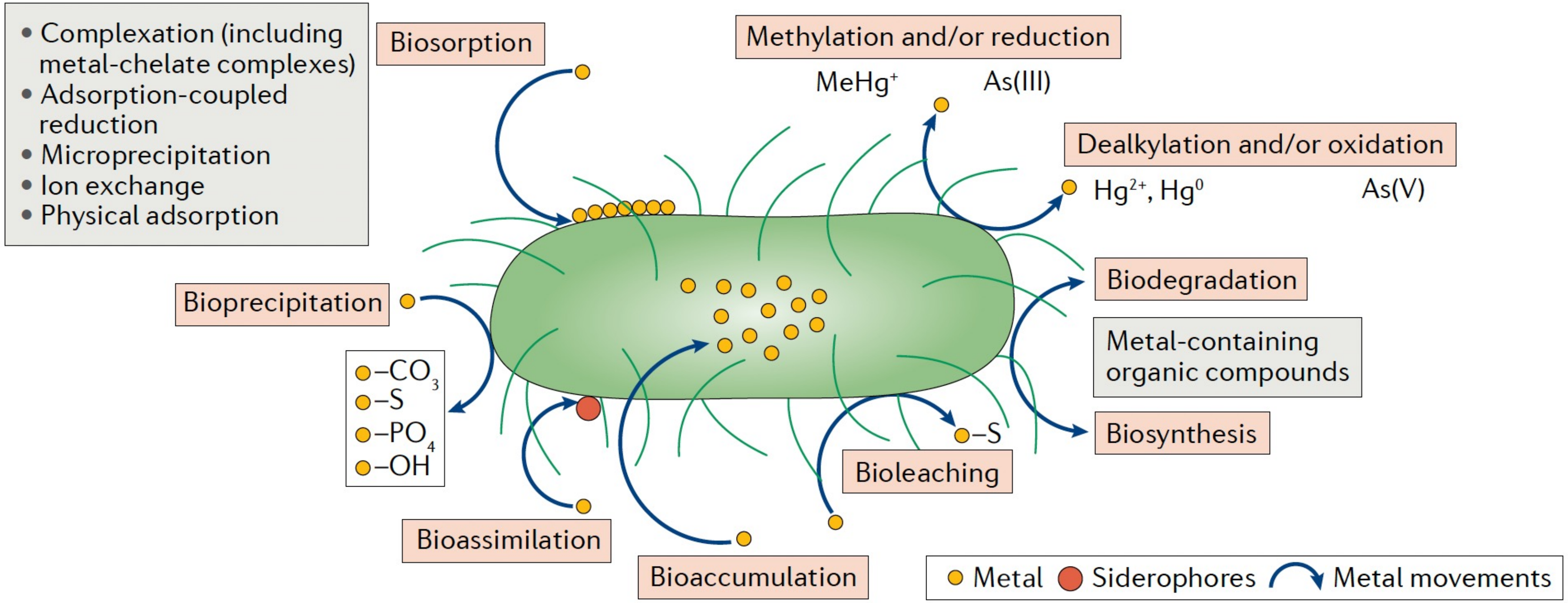
Electrokinetic remediation



- Soil excavation / soil washing
- Chemical precipitation from wastewater

Bioremediation is a useful tool to mitigate heavy metal contamination

- Bacteria, yeast, and plants have natural defenses against heavy metal damage
- These defenses can be engineered to create effective remediation models for pollutants



How does this all relate to your Mod2 project?

- Begin the early stages of the process to create bioremediation model
- Alter a Saccharomyces cerevisiae cell surface protein
  - Fet4
  - Low-affinity iron permease reported to take up other metals
- Use rational design protein engineering to create a mutant form of Fet4
  - Reduce preference of Fet4 for iron and identify mutations that increase preference for cadmium
- Explore mutagenesis and functional screening

In lab today and tomorrow

- Examine secondary and tertiary structure of Fet4 and previous literature to determine mutations that have the potential to alter affinity of the transporter from iron to cadmium

- Design mutagenesis primers to create your designed mutation