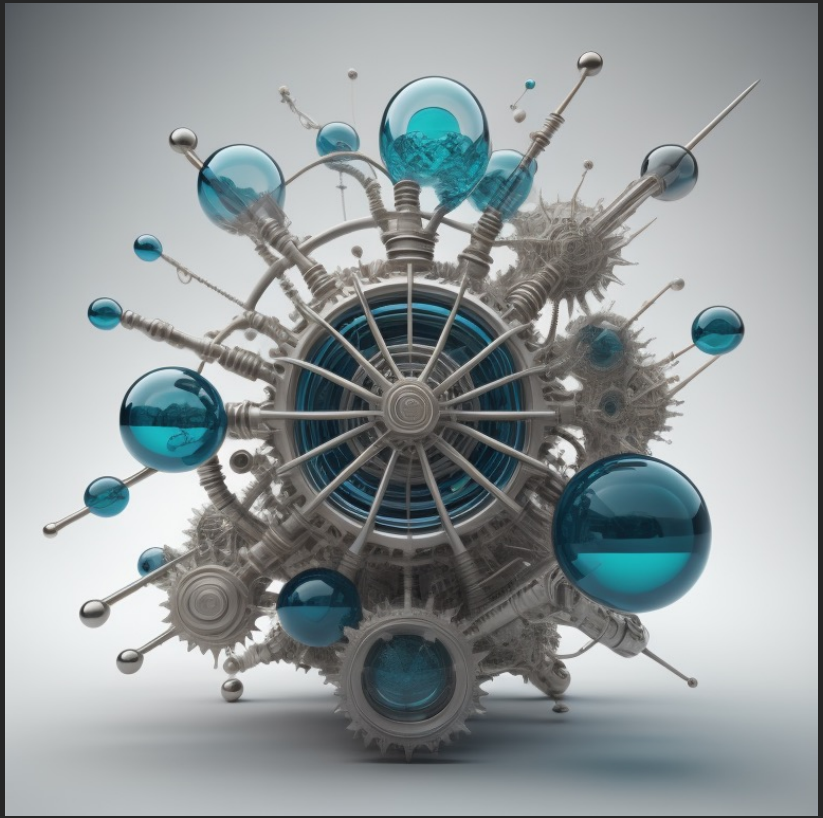
Module 2: Protein engineering for bioremediation



Overview of Module 2 goals

Research:

Genetically engineer a cell surface display peptide to capture cadmium in a model for bioremediation

Communication:

Journal article presentation

Research article

Technical:

Protein engineering:

Site-Directed Mutagenesis cloning

Flow cytometry

Functional assays:

Elemental analysis of metal uptake

Measure metal fluroescence

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 microbes and plants
  - To humans
- How can we mitigate heavy metal contamination?
  - Reusing metals for manufacturing

Heavy metals and their uses

Heavy metals


Nickel


Manganese

Cadmium


Mercury


Chromium

Gold

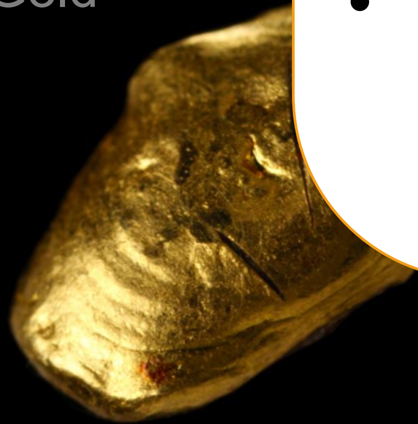
Copper


Cobalt

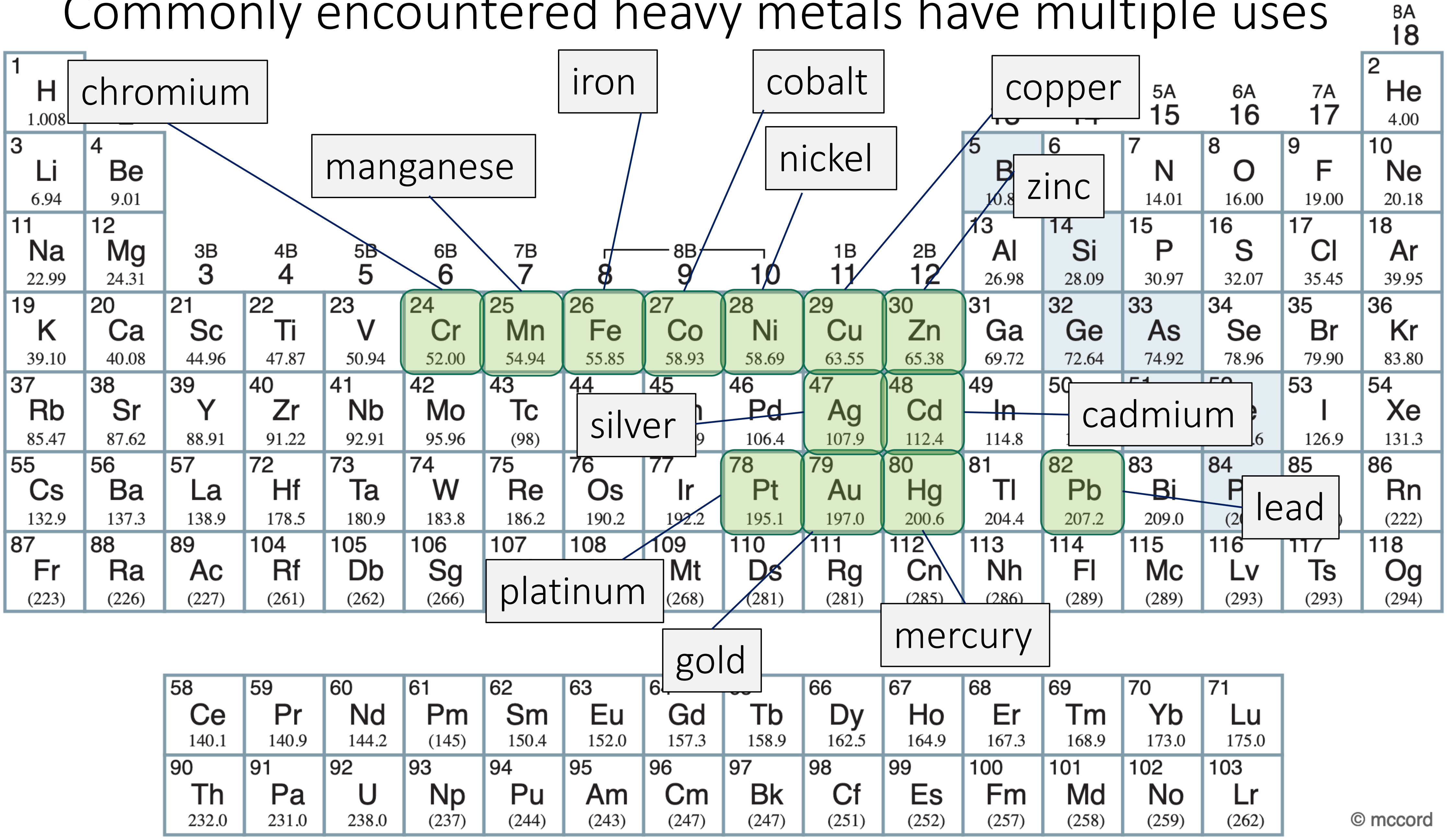
Heavy metals

Heavy metals is poorly defined as a term

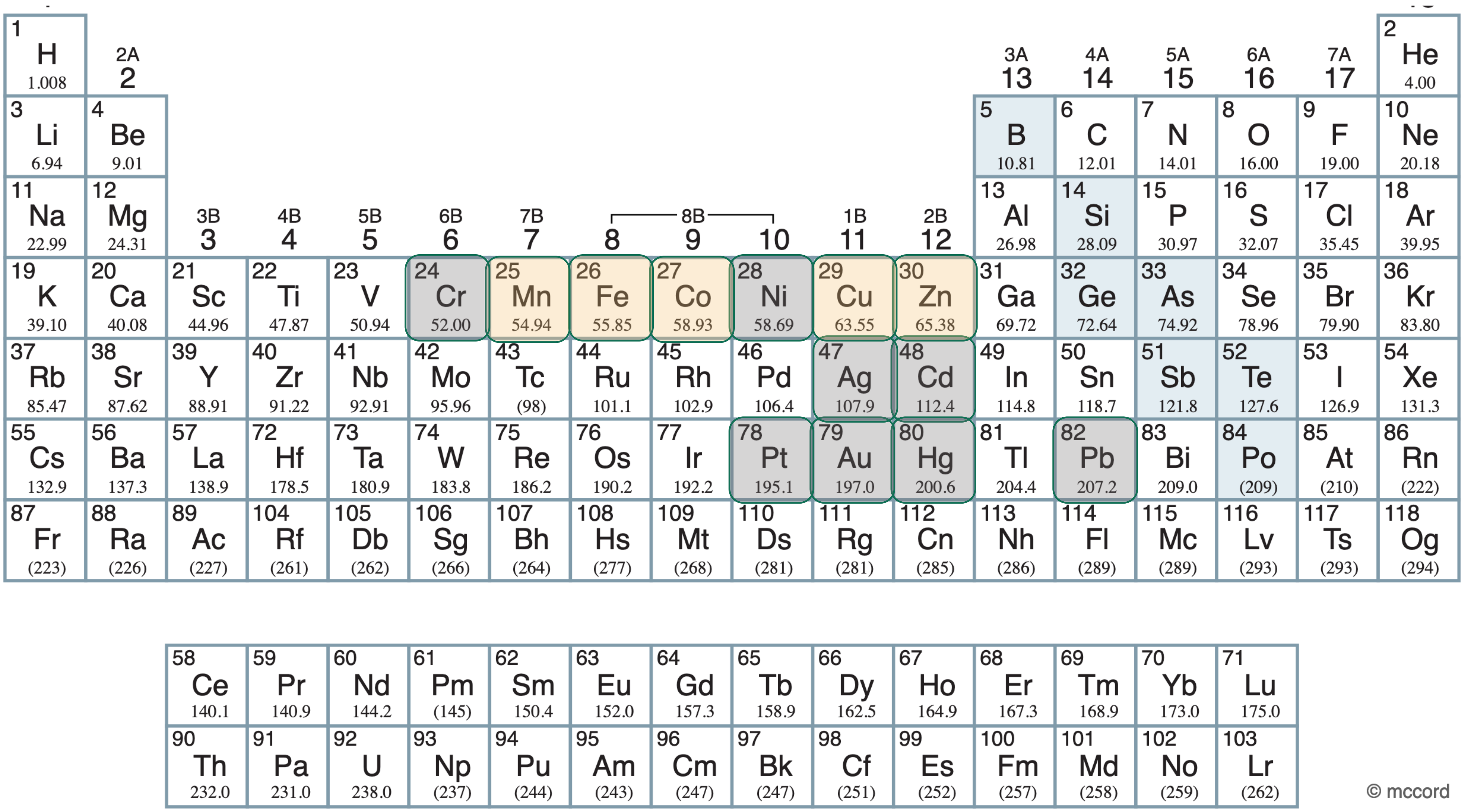
- Relatively high atomic density (greater than 5 g/cm^3)
- Atomic number > 20
- Exhibit metal-like properties



Commonly encountered heavy metals have multiple uses

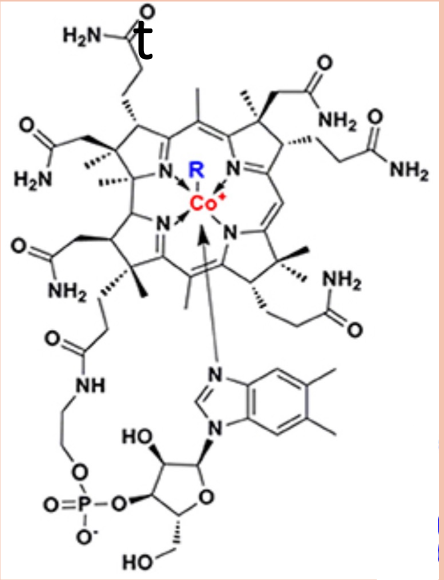


Metals can act as protein co-factors in human biology



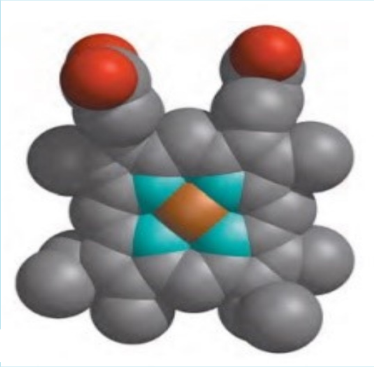
Metals crucial for metabolic activity are also known as essential elements

Cobal



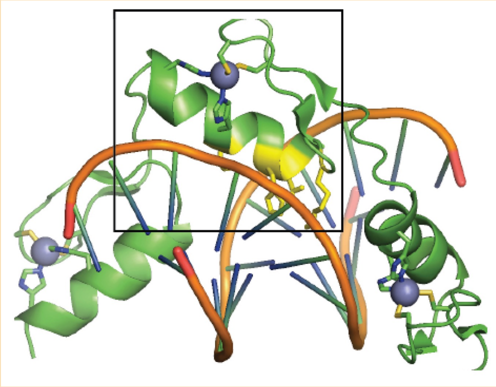
vitamin B12

Iron



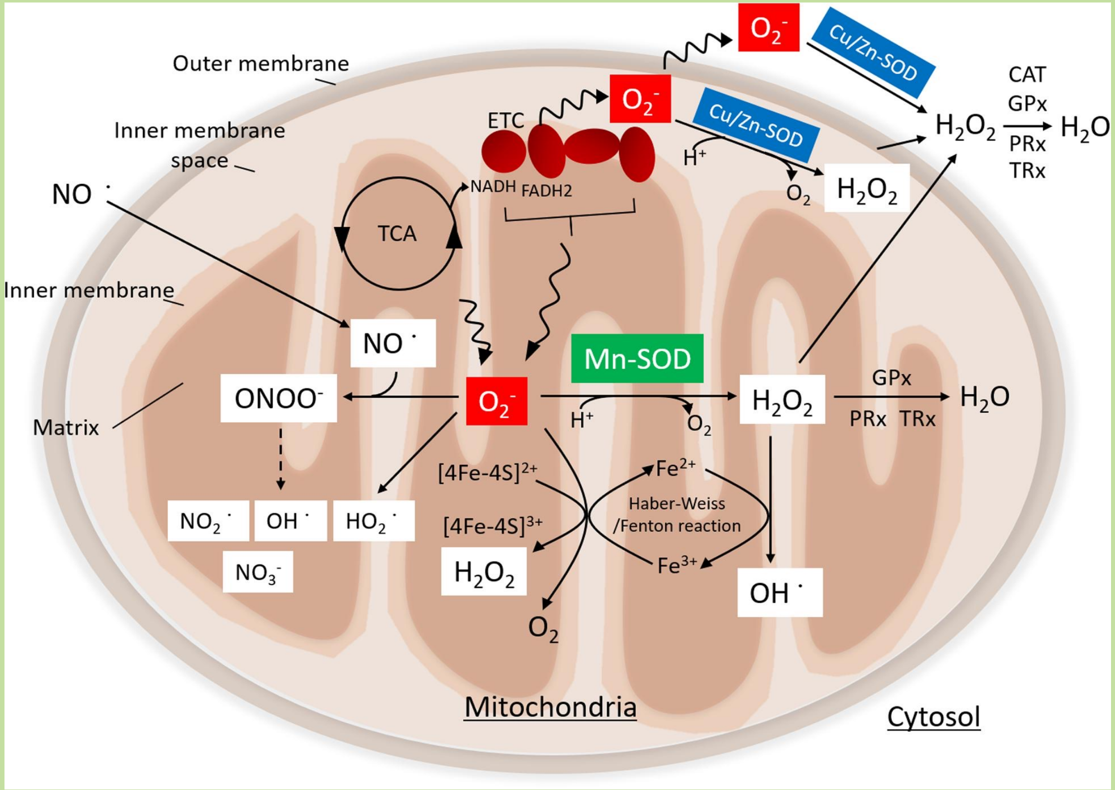
Heme B subunit

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	8	8B 9	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)

Precious metals have economic and cultural value

Silver: $24 / oz

Ag

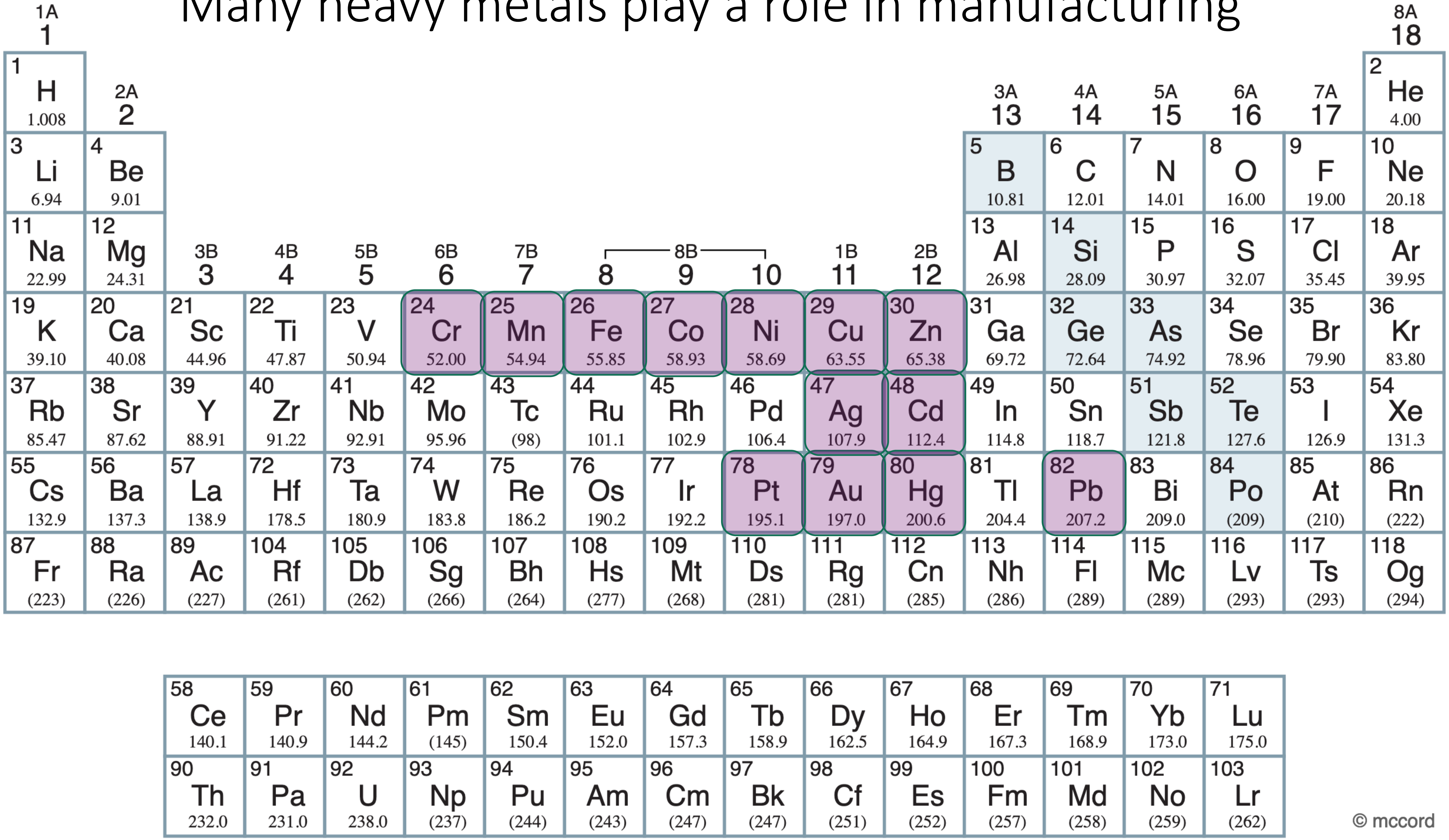
Gold: $2,198 / oz

Au

Platinum: $912 / oz

Pt

Many heavy metals play a role in manufacturing



Heavy metals are used to manufacture common materials

Dyes and Pigments

Prussian Blue

Fe

Co Pb Ni

Cd Cu Zn

Fe Cr Ni

Stainless Steel

Heavy metals are 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

Lead-Acid battery

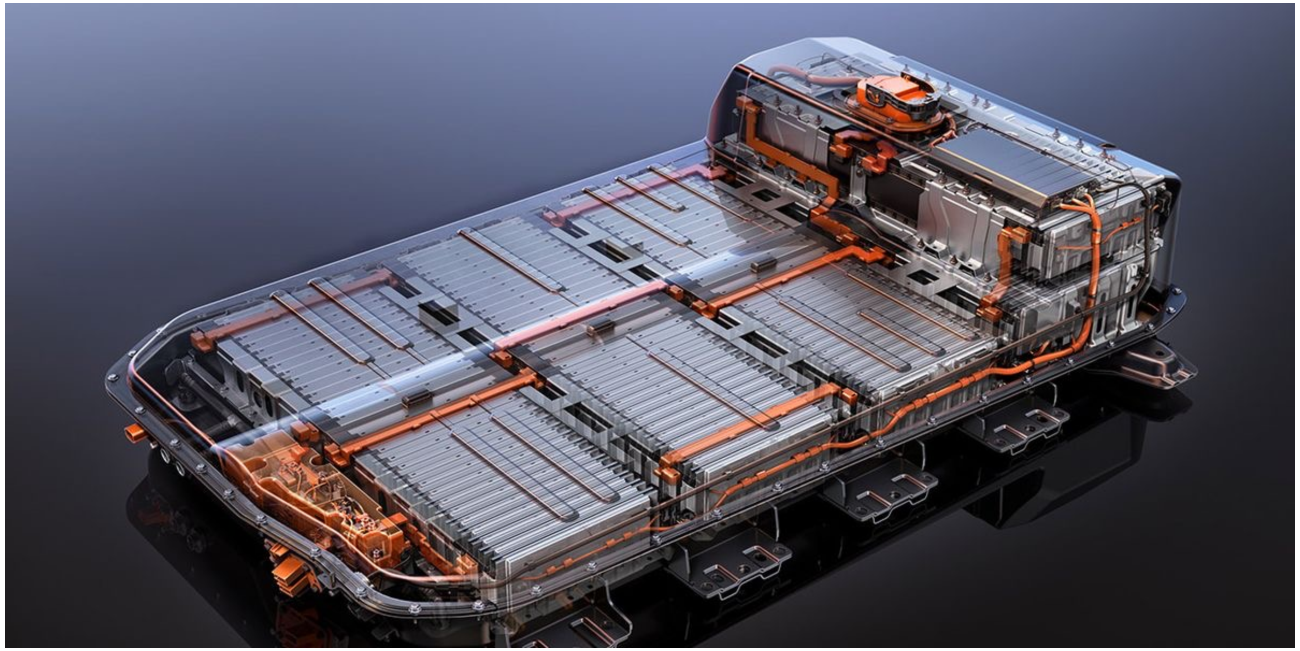
Pt Au Pb

Alkaline batteries

Cd Ni

Mn Zn

EV Battery

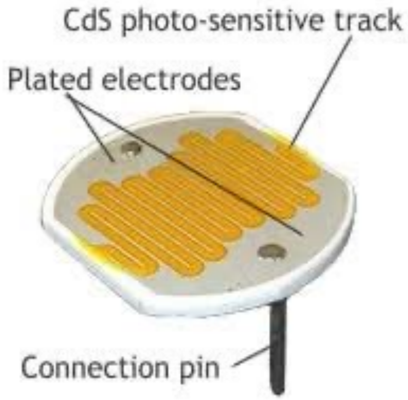


Cu Ni Mn Co

Photovoltaic cells, photoresistors, infrared detectors all use heavy metals

Infrared detectors & Photoresistors

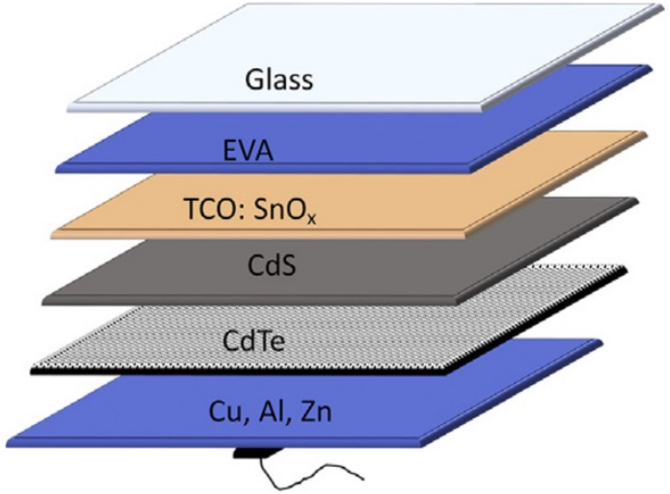
Cd Pb Cu Hg



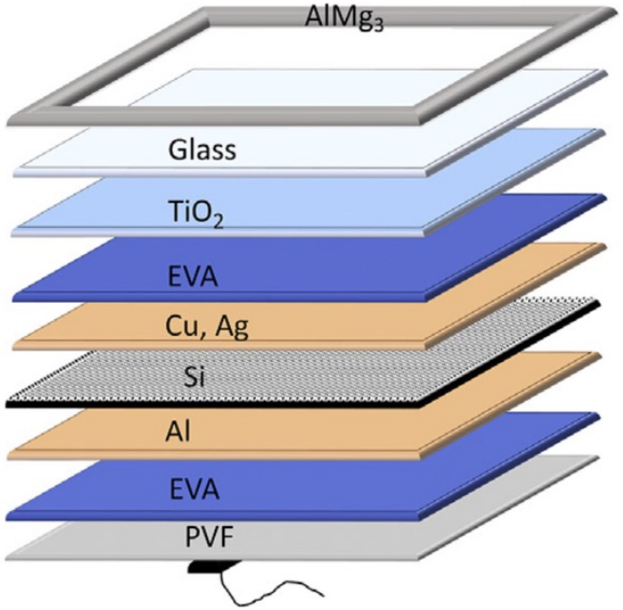
Cu Cd Ag Zn Pt

Solar photovoltaic cells

Cadmium-Telluride



crystalline-Silicon



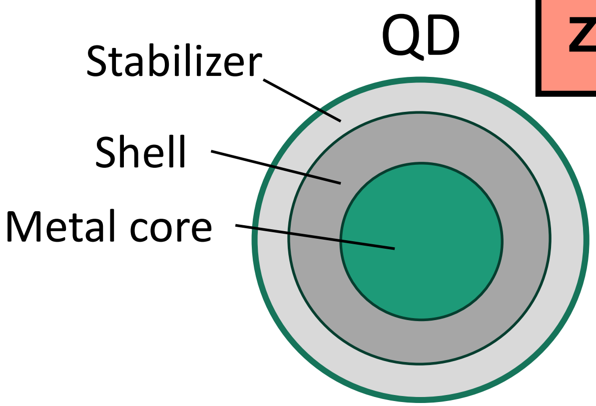
Metal core quantum dots are valuable technology

- Semiconductor crystal

- Used in many industries:
  - Medical diagnostics
  - Solar cells
  - Electronics (TVs, etc)

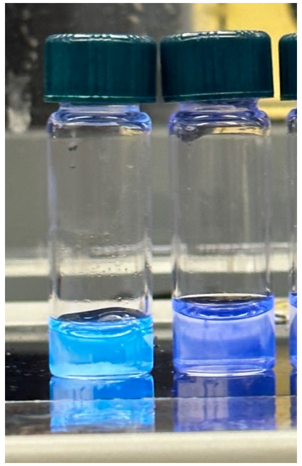
- Nano-scale structure which can be "tuned" by changing the size and material of the metal core
  - Larger particles emit longer wavelength light
  - Other properties are optimized for signal strength, etc…

- Quantum dots often have Cadmium selenide or Cadmium sulfide cores
  - Because of toxicity issues, non-toxic cores have been developed

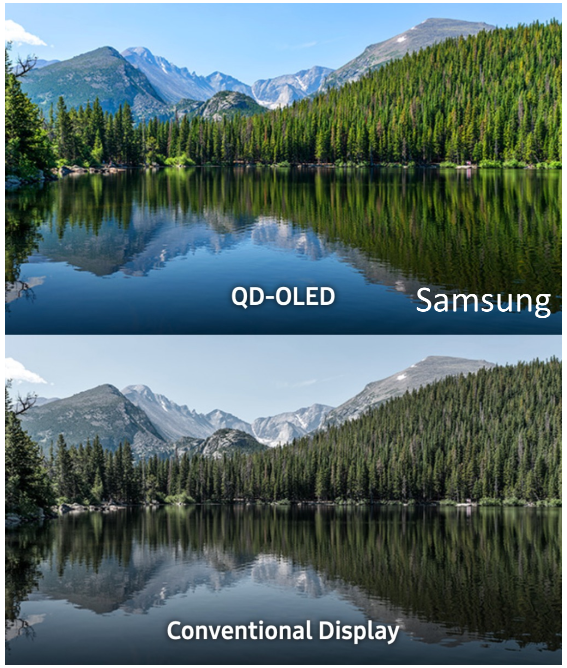


Cd

Zn



Zn QDs
(Bill Pinney)



Some heavy metals are highly toxic at low exposure levels

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Environmental contamination with heavy metals

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

Geogenic sources

Weathering of rocks

Volcanoes

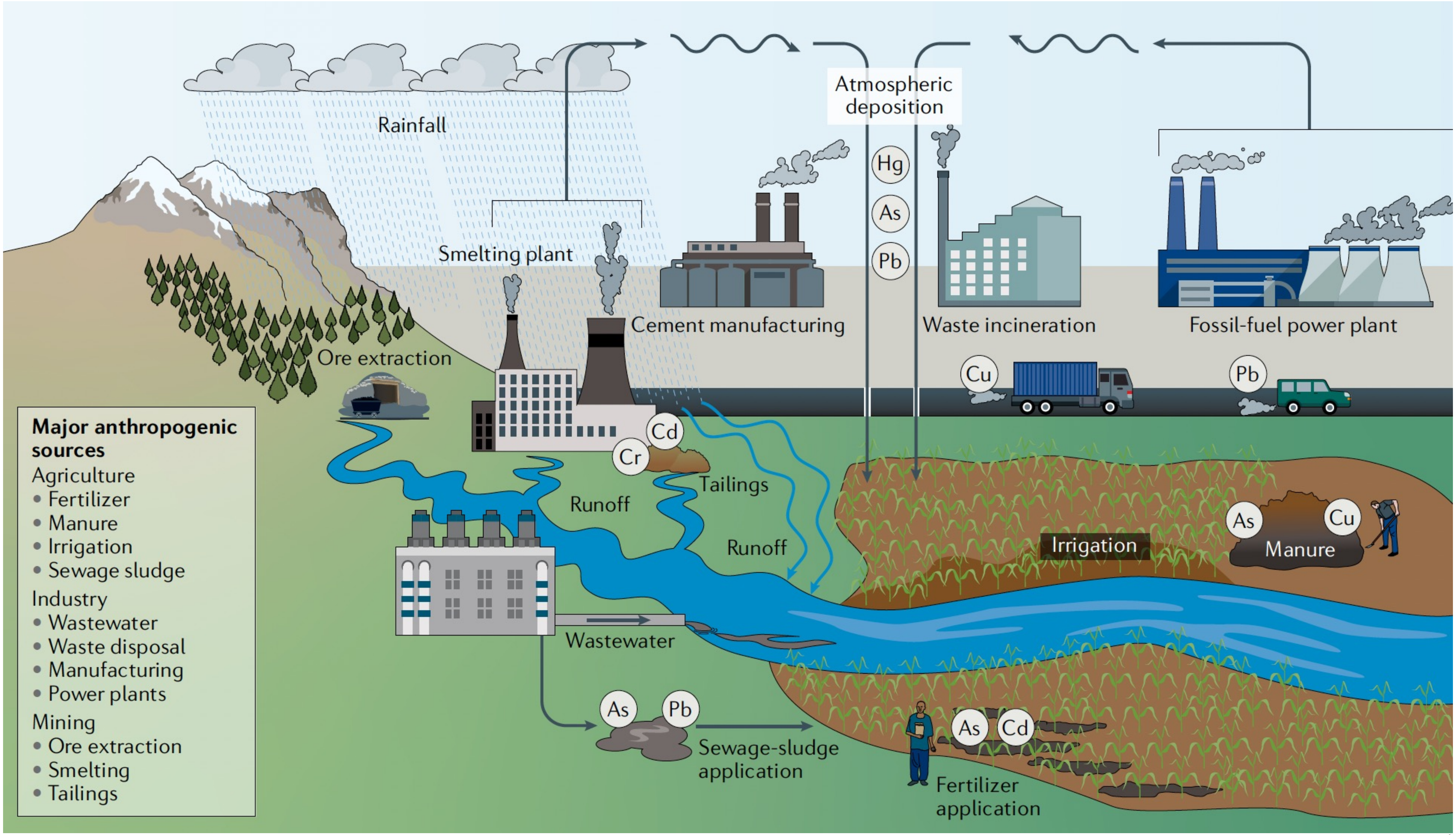
Anthropogenic sources

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

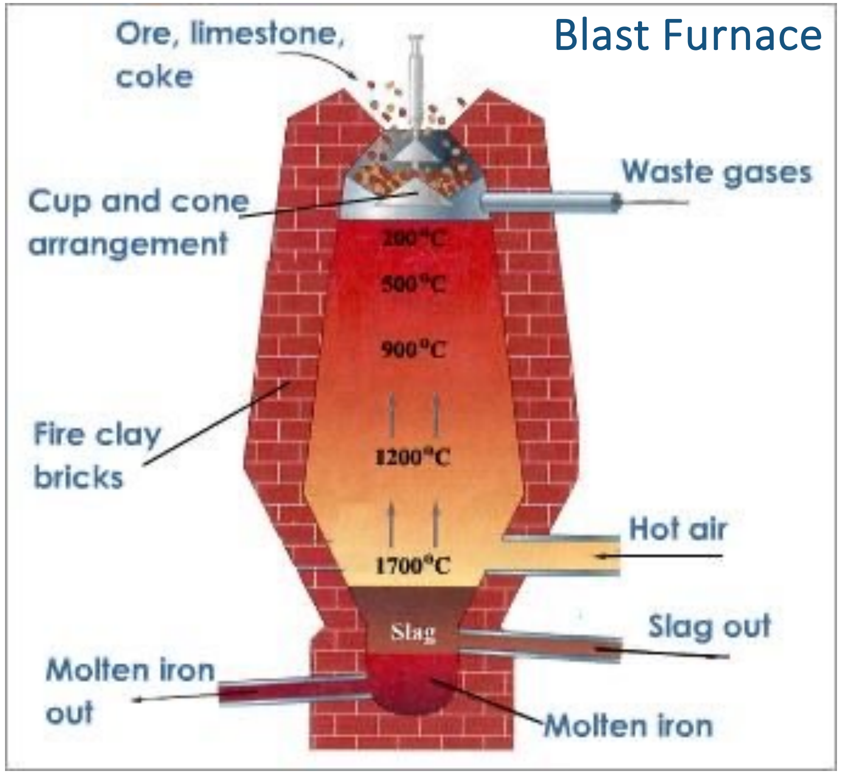
Smelting and mining activity produce metal contaminants

Mining

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

Smelting

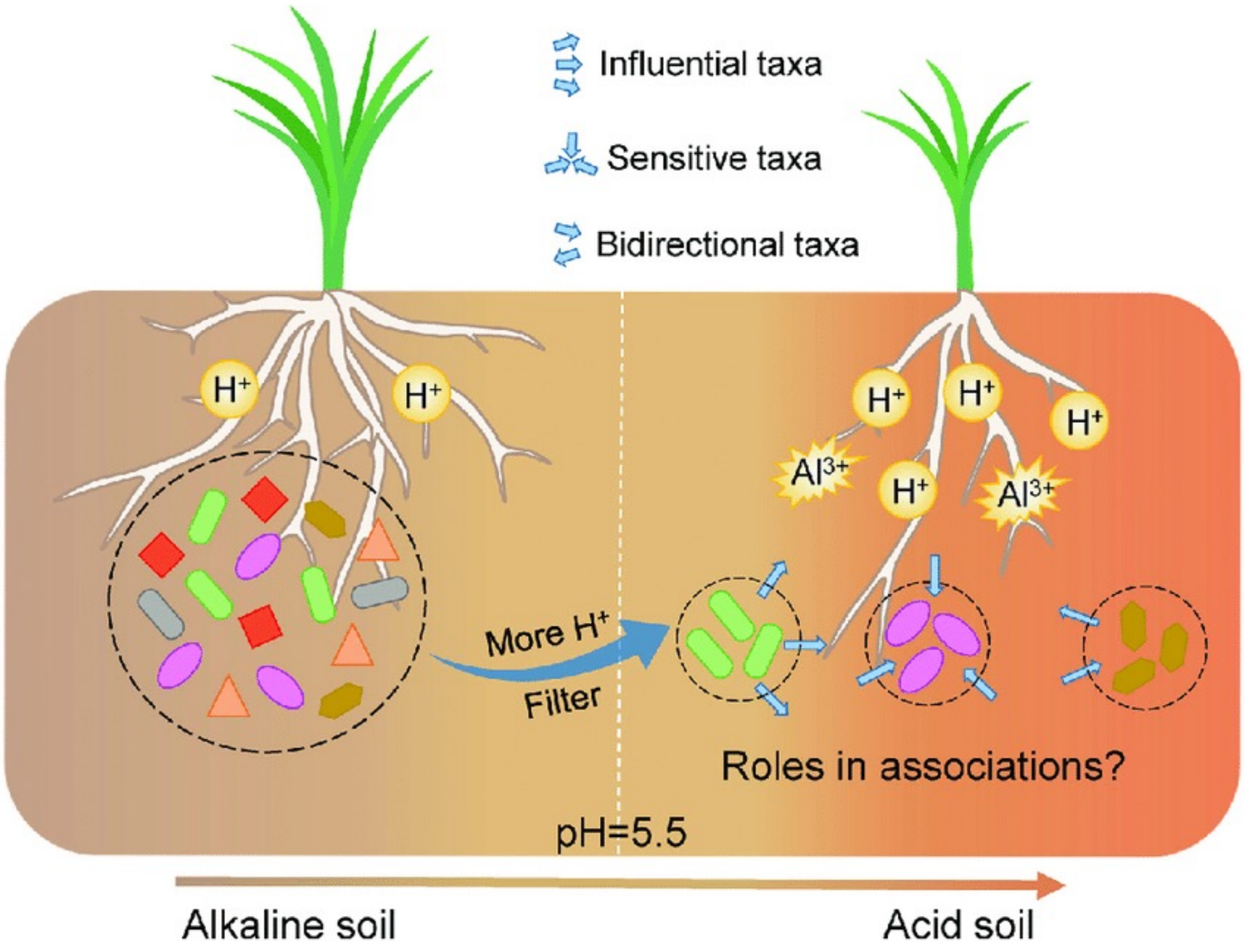
- Slag generated from refinement of metal can contain contaminants
    - Smelting zinc produces slag containing lead and cadmium
- Heavy metal particulates are also released



Consequences of heavy metal contamination

Heavy metals fundamentally change soil microbial richness and damage plants

- 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**
- Accumulates in plant roots and causes overall **cell stress**



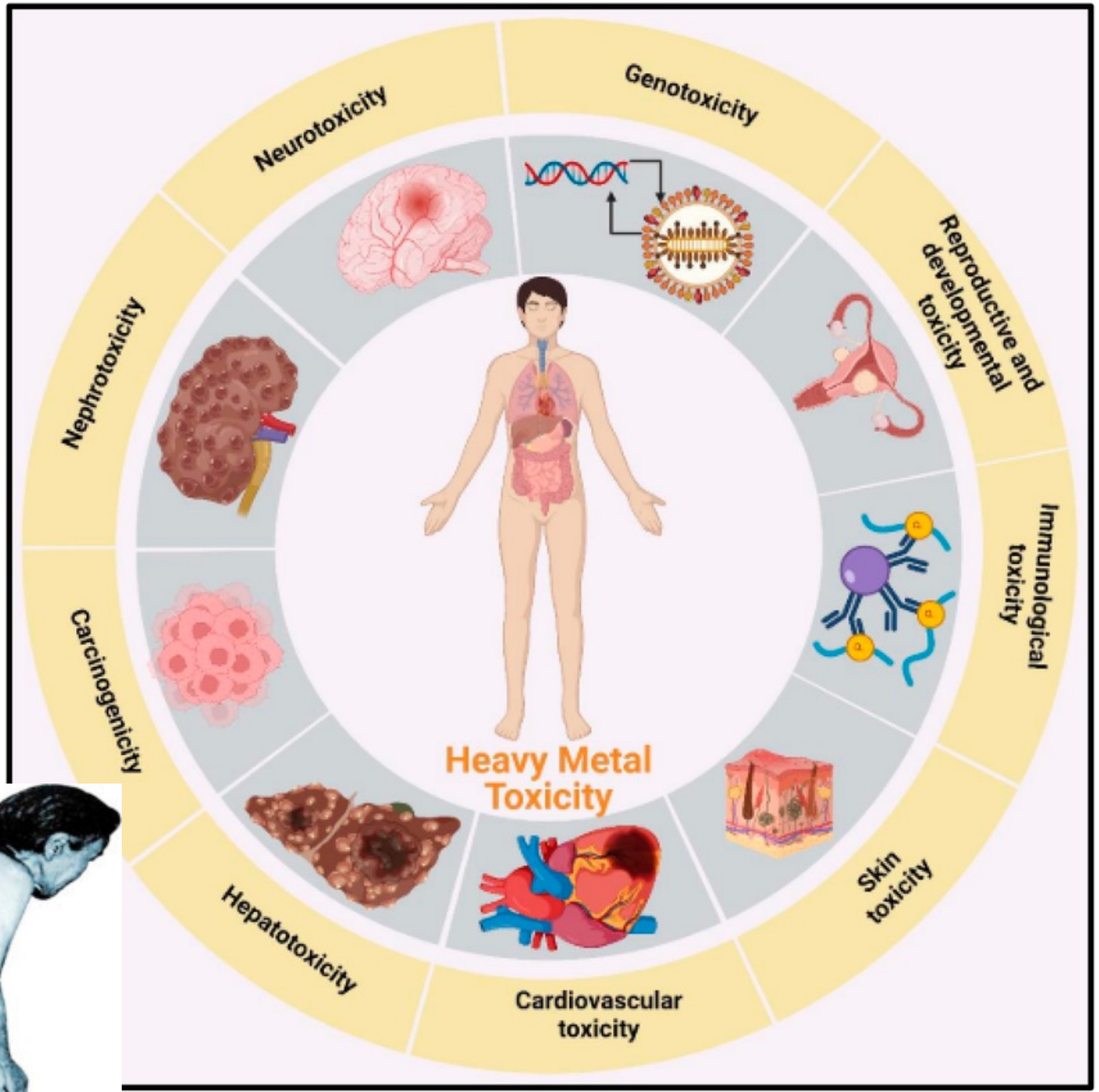
Zhang et al. 2022

Heavy metal exposure has wide ranging effects on human health

- Systemic toxicity
- Damage of multiple organs
- Genomic damage

  - Carcinogenic (Cr)
  - Skin ulcers (Cr)
  - Neurotoxic (Pb, Hg)
  - Bone and kidney deterioration (Cd)
  - Cardiovascular (Pb, Cd)

Itai-Itai disease



Bayou et al. 2023

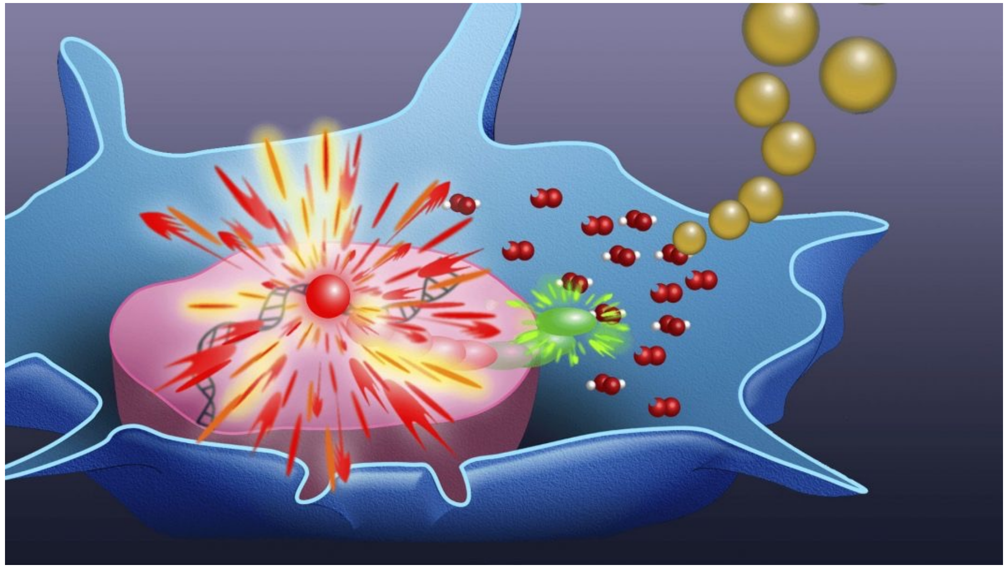
There are multiple proposed mechanisms for metal toxicity

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

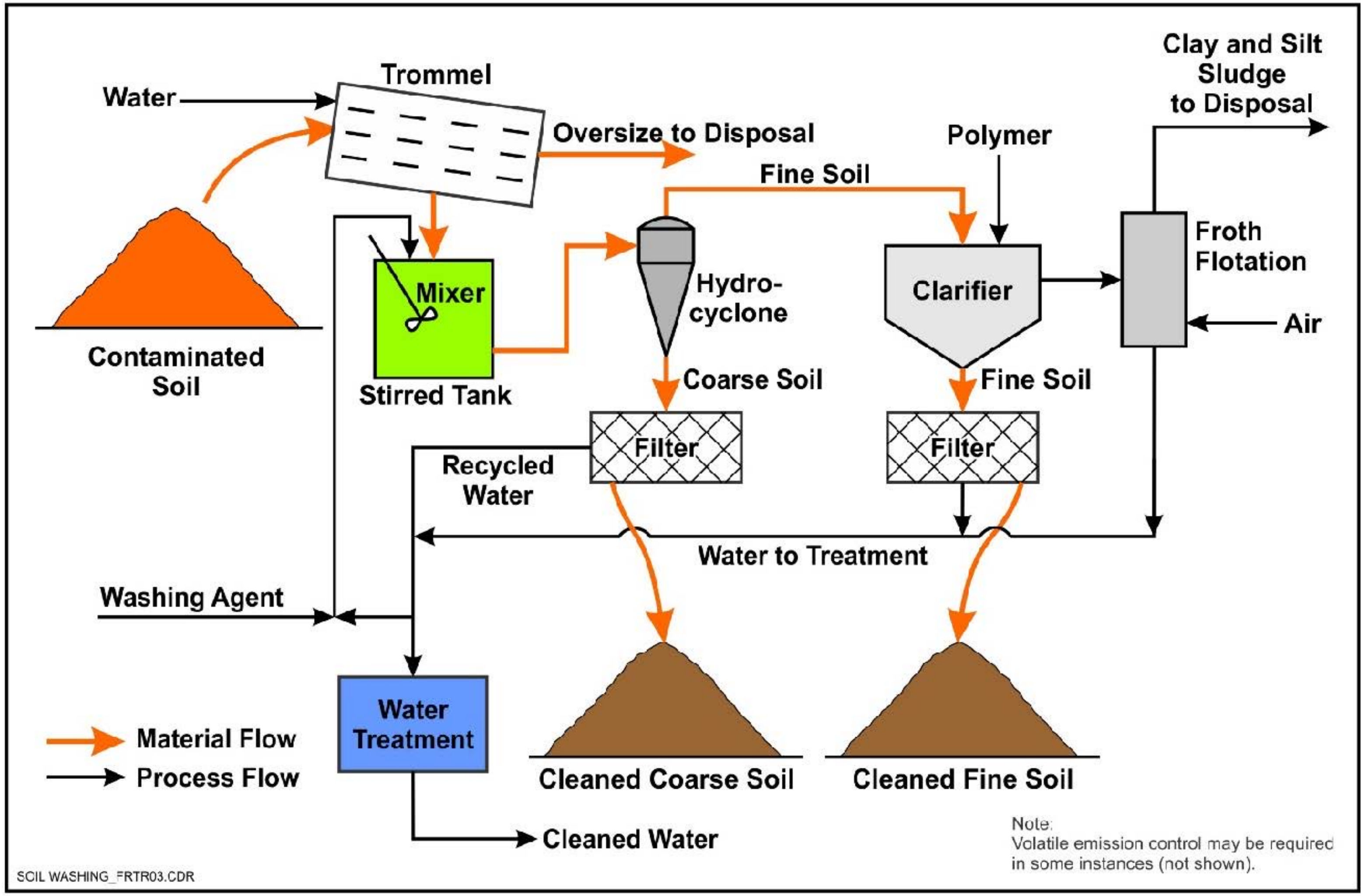
Oxidative stress

- Disrupt mitochondrial function
- Generate reactive oxygen species



What can we do to mitigate this issue?

Physical and chemical mitigation of heavy metal contamination



www.frtr.gov

Soil excavation / soil washing

- Physically filter soil since contaminants can cause clumps
- Wash the remaining soil with
  - Surfactants
  - Solvents
  - Acids

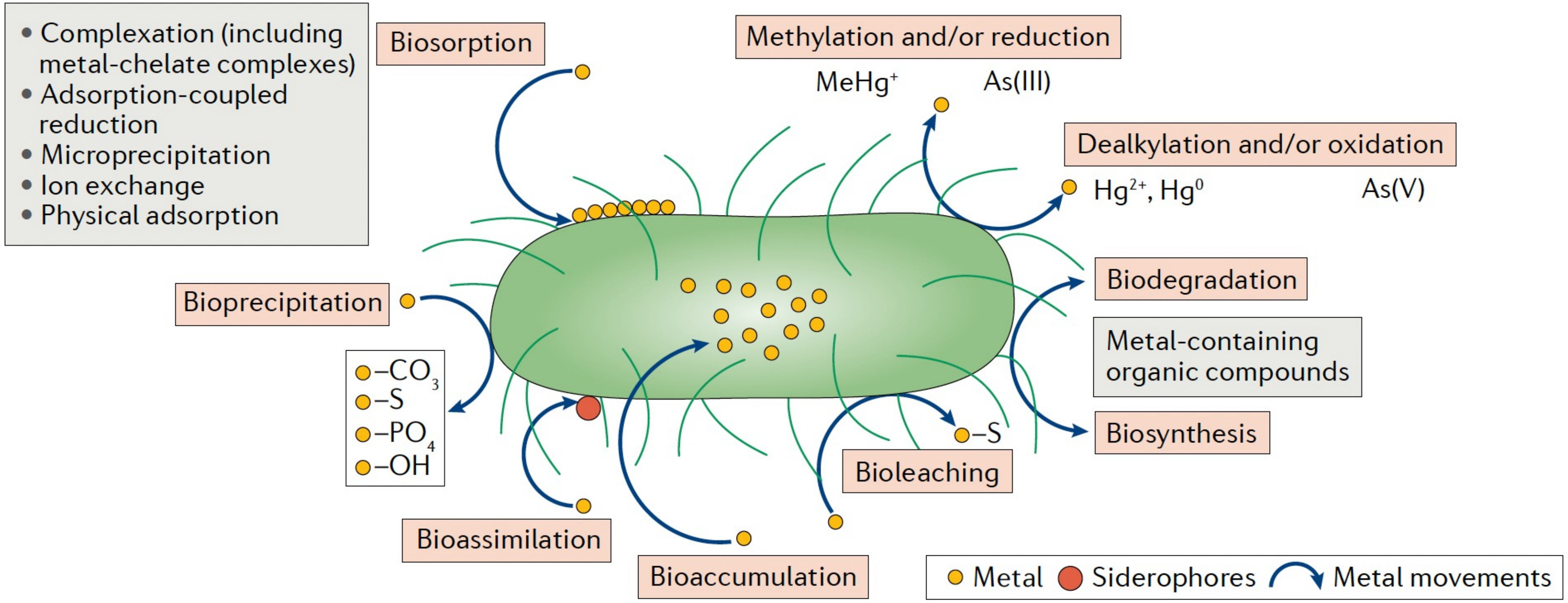
Chemical precipitation of contaminants from wastewater

- Water treatment provides cleaner water
- Concentrated contaminants can be contained

Pros / Cons

Bioremediation is a useful tool to mitigate heavy metal contamination

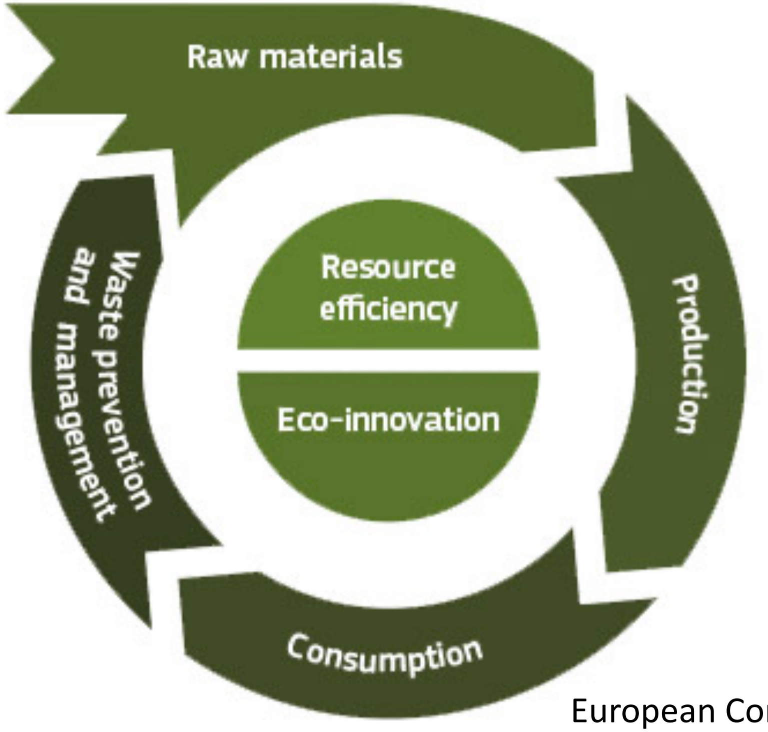
- Bacteria, yeast, and plants have **natural defenses** against heavy metal damage
- These defenses can be engineered to create effective **remediation models** for pollutants



Then what?

Circular economy (applied to metals in manufacturing)

- Promote sustainability by keeping resources in circulation for as long as possible
  - Maximize use of precious materials
  - Reduce waste
- Efficiency in production
- Mindful consumption
- Waste management
  - Recycling
  - Containment of waste in production so that material can be reused
    - Closed-loop manufacturing



European Commission

How does this all relate to your Mod2 project?

- Design elements to create a more optimized bioremediation model
- Use Saccharomyces cerevisiae
  - Previously modified to produce H_2S gas to help precipitate contamination metals in media
- Use rational peptide design to create a cell surface display peptide capable of capturing cadmium sulfide
  - Base design on literature regarding amino acid binding to cadmium
- Explore idea of capturing cadmium sulfide capable of being recycled as quantum dots