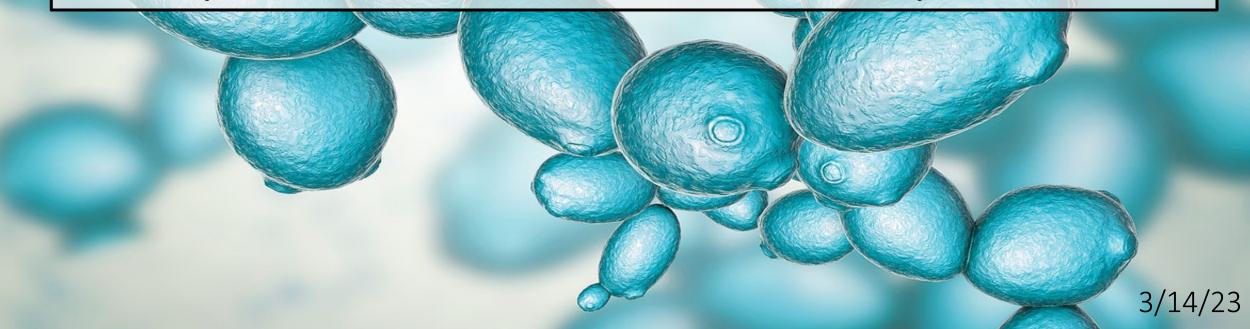
Module 2: Protein engineering to create a model system for bioremediation of heavy metals



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

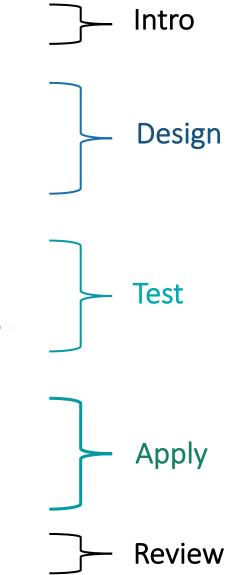
Functional assays:

Elemental analysis of metal uptake

Cell tolerance of metal uptake

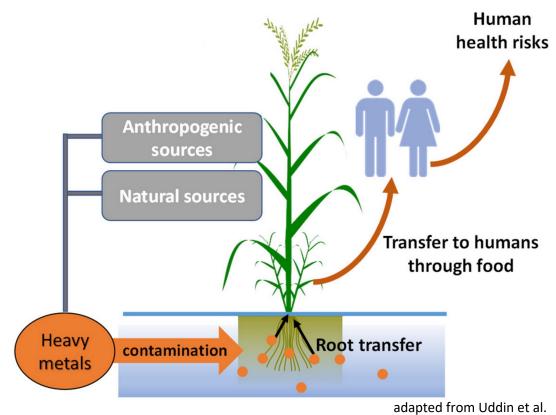
Module Outline

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



Overview of today's lecture

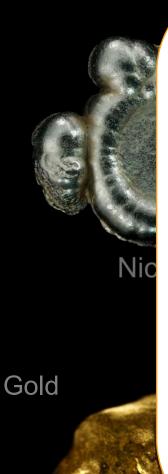
- Heavy metals
 - What are they?
 - What are their uses?
- How do heavy metals get into environment?
 - Geogenic sources
 - Anthropogenic sources
- What happens after heavy metal exposure
 - To soil
 - To plants
 - To humans
- How can we mitigate heavy metal contamination?



Heavy metals and their uses



Heavy metals



Heavy metals is poorly defined as a term

- Relatively high atomic density (greater than 5 g/cm³)
- Atomic number > 20
- Exhibit metal-like properties



cury

Commonly encountered heavy metals have multiple uses

8A **18**

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

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

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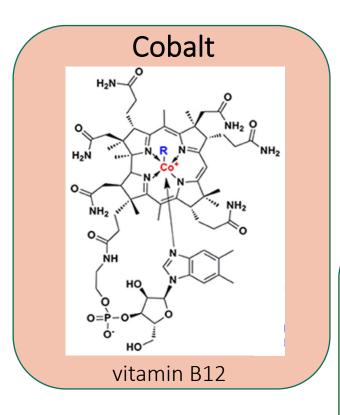
Metals can act as protein co-factors in human biology

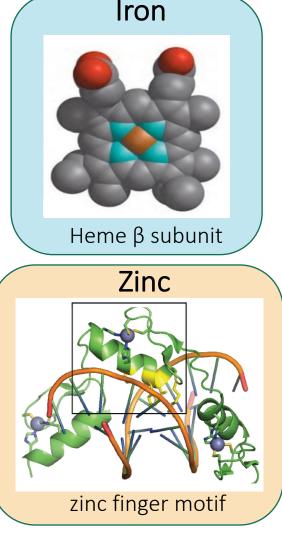
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6.94	9.01											10.81	12.01	14.01	16.00	19.00	20.18
11	12											13	14	15_	16	17	18
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22.99	24.31	3	4	5	6	7	8	9	10	11	12	26.98	28.09	30.97	32.07	35.45	39.95
19	20	21	22	23	24	25	26	27	28	29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

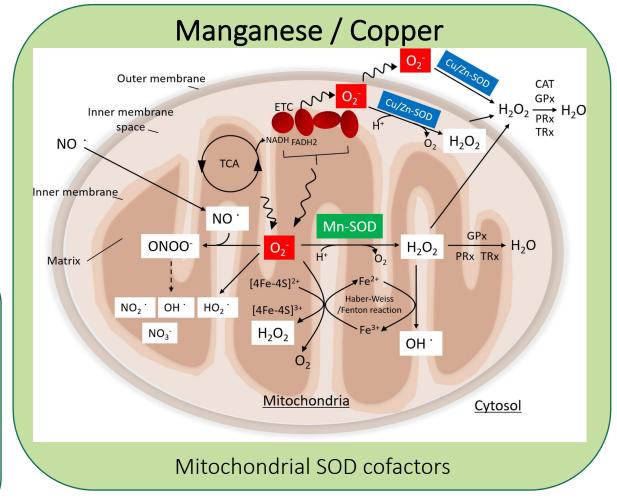
58	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
140.1	140.9	144.2	(145)	150.4	152.0	157.3	158.9	162.5	164.9	167.3	168.9	173.0	175.0
90	91	92	93	94	95	96	97	98	99	100	101	102	103
90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	⁹⁶ Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

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Metals crucial for metabolic activity are also known as essential elements







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3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 0 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31	зв З	4B 4	5B 5	6B 6	7В 7	8	— 8B— 9	10	1B 11	^{2В} 12	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	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
39.10	40.08	44.96	47.87	50.94	52.00	54.94	55.85	58.93	58.69	63.55	65.38	69.72	72.64	74.92	_{78.96}	79.90	83.80
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te		Xe
85.47	87.62	88.91	91.22	92.91	95.96	(98)	101.1	102.9	106.4	107.9	112.4	114.8	118.7	121.8	127.6	126.9	131.3
55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	TI	Pb	Bi	Po	At	Rn
132.9	137.3	138.9	178.5	180.9	183.8	186.2	190.2	192.2	195.1	197.0	200.6	204.4	207.2	209.0	(209)	(210)	(222)
87	88	89	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118
Fr	Ra	Ac	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Nh	Fl	MC	Lv	Ts	Og
(223)	(226)	(227)	(261)	(262)	(266)	(264)	(277)	(268)	(281)	(281)	(285)	(286)	(289)	(289)	(293)	(293)	(294)

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

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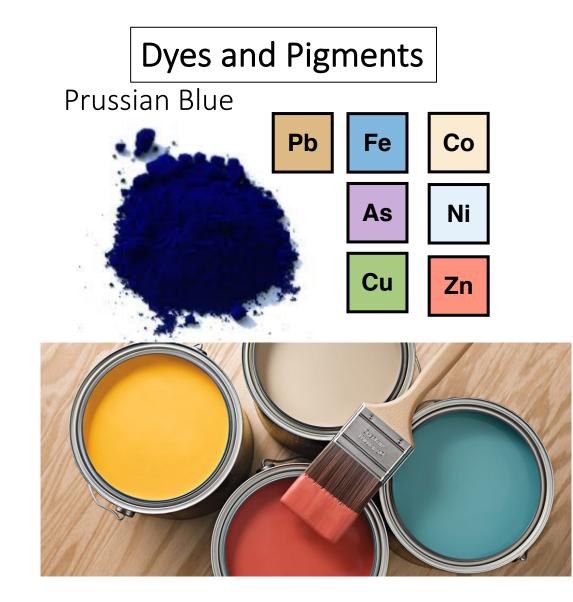


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3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 0 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31	зв З	4B 4	5B 5	6B 6	7В 7	8	— _{8B} — 9	10	1B 11	^{2В} 12	13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.07	17 Cl 35.45	18 Ar 39.95
19 K 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	53 126.9	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 TI 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)	(203) 116 LV (293)	(210) 117 Ts (293)	118 Og (294)

58	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
140.1	140.9	144.2	(145)	150.4	152.0	157.3	158.9	162.5	164.9	167.3	168.9	173.0	175.0
00	0.4		1										
90	91	92	93	94	95	96	97	98	99	100	101	102	103
Th	⁹¹ Pa	92 U	93 Np	94 Pu	95 Am	⁹⁶ Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

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Heavy metals are used to manufacture common materials





Fe Cr Ni

Stainless Steel



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

Chrome plating

Ni

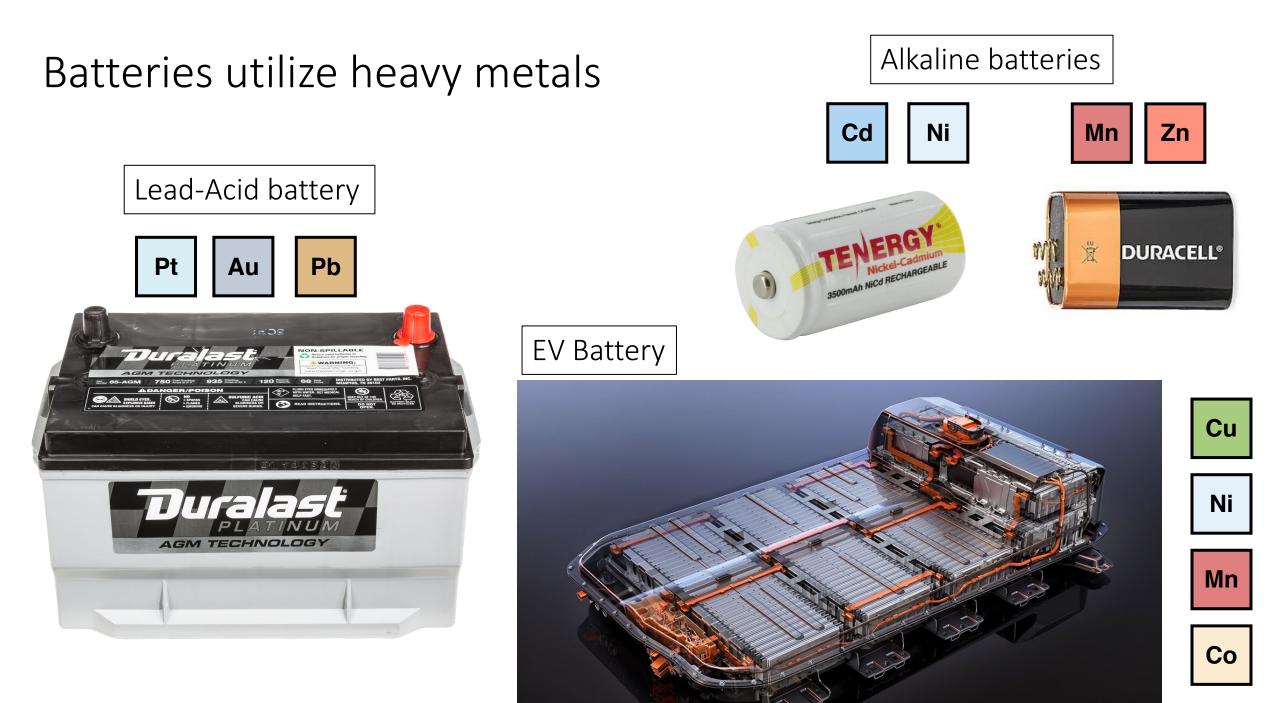
Au

Cd

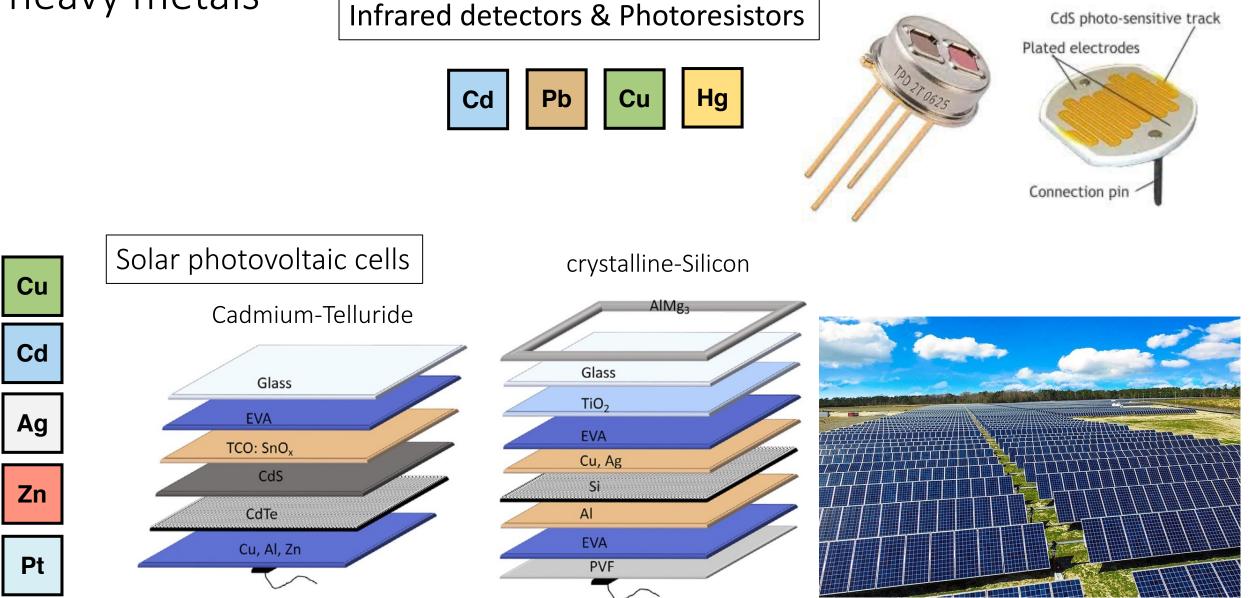
Aa







Photovoltaic cells, photoresistors, infrared detectors all use heavy metals



1A 1	S	om	e he	eavy	' me	etals	sare	e hig	ghly	tox	ic at	t lov	v ex	pos	ure		^{8A} 18
1 H								leve	els								2
□ 1.008	2											13	14	15	10	1 ^A	He 4.00
3 Li	⁴ Be											5 B	6 C	7 N	8 0	9 F	10 Ne
6.94	9.01											10.81	12.01	14.01	16.00	19.00	20.18
11 Na 22.99	12 Mg 24.31	зв З	4B 4	5B 5	6B 6	7B 7	8	— 8В— 9	10	1B 11	2B 12	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	24.51 20	21	22		24	25	26	27	28	29	30	31	32	33	34	35	39.95
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
39.10	40.08	44.96	47.87	50.94	52.00	54.94	55.85	58.93	58.69	63.55	65.38	69.72	72.64	74.92	78.96	79.90	83.80
37 Rb	³⁸ Sr	39 Y	40 Zr	41 Nb	42 Mo	43 TC	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 	54 Xe
85.47	87.62	88.91	91.22	92.91	95.96	(98)	101.1	102.9	106.4	107.9	112.4	114.8	118.7	121.8	127.6	126.9	131.3
55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg		Pb	Bi	Po	At	Rn
132.9 87	137.3 88	138.9 89	178.5 104	180.9	183.8 106	186.2 107	190.2	192.2 109	195.1 110	197.0	200.6 112	204.4 113	207.2 114	209.0 115	(209) 116	(210) 117	(222) 118
Fr	Ra	Ac	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Ĉn	Nh	FI	Mc	Lv	Ts	Ög
(223)	(226)	(227)	(261)	(262)	(266)	(264)	(277)	(268)	(281)	(281)	(285)	(286)	(289)	(289)	(293)	(293)	(294)

58	59	60	61	62	63	64	65	66	67	68	69	70	71
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
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90	91	92	93	94	95	96	97	98	99	100	101	102	103
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr
	I G	<u> </u>				•••• •••							

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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 rocks

Volcanoes



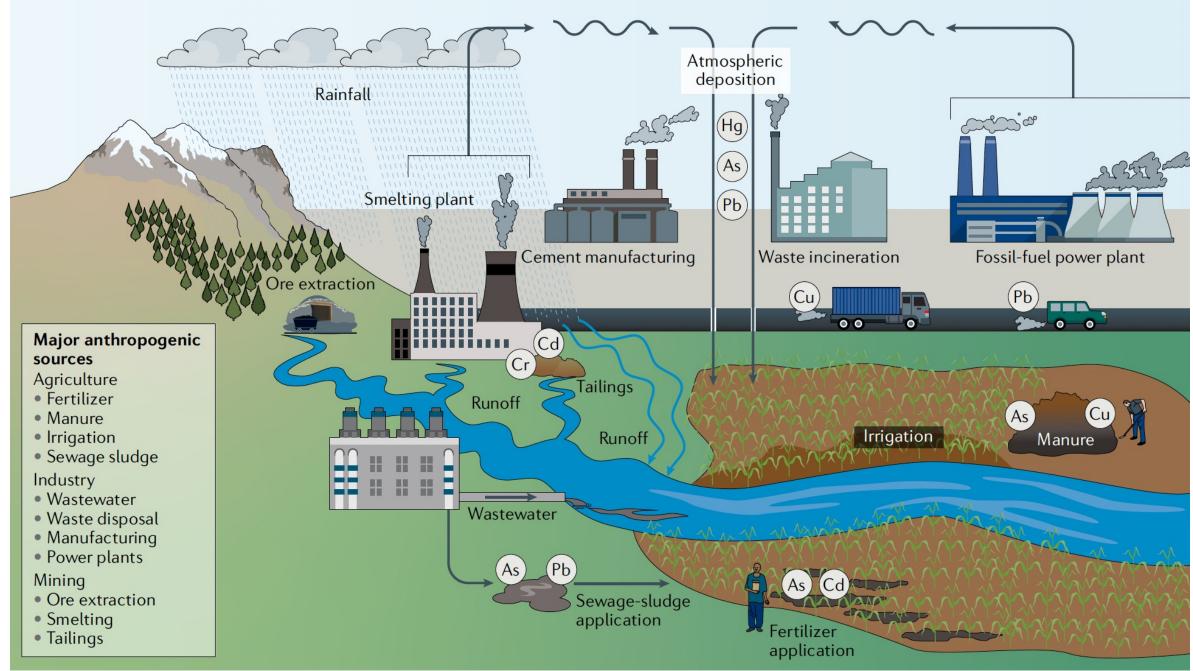
Anthropogenic sources

Agrochemicals

Industrial activity

Smelting and mining activity

Sewage and waste disposal



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

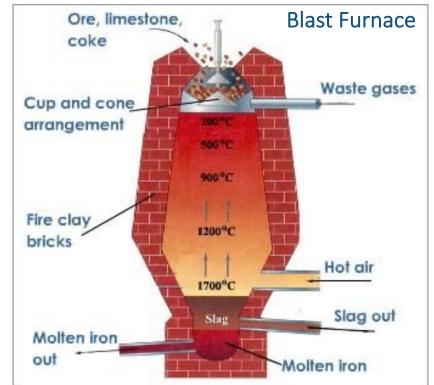


Mining

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

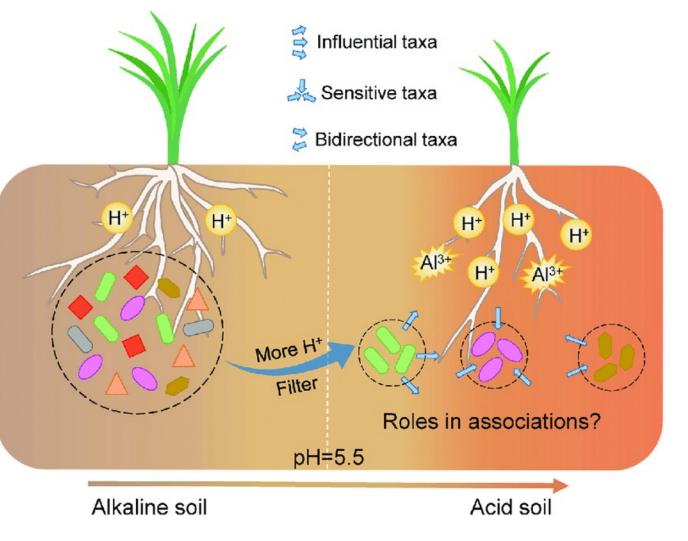
Smelting

- 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

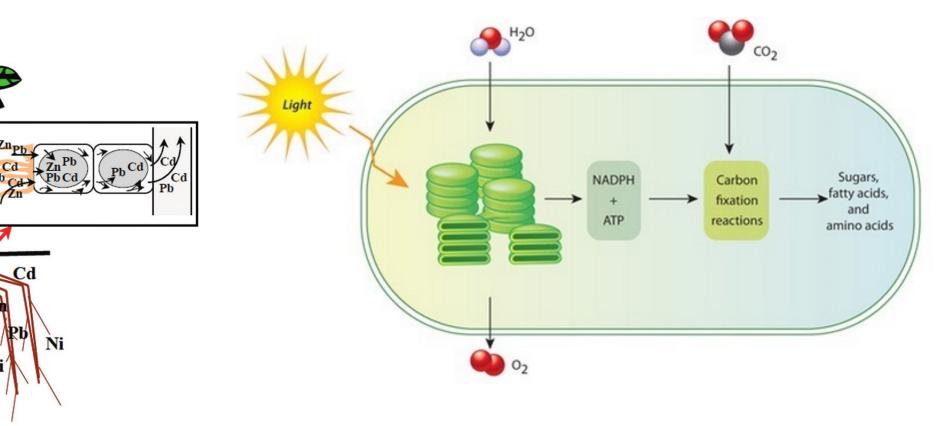


Heavy metal accumulates in plants and disrupts essential biology

• Most heavy metal enters the plant through the **roots** and accumulates there

Ni

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



Cd

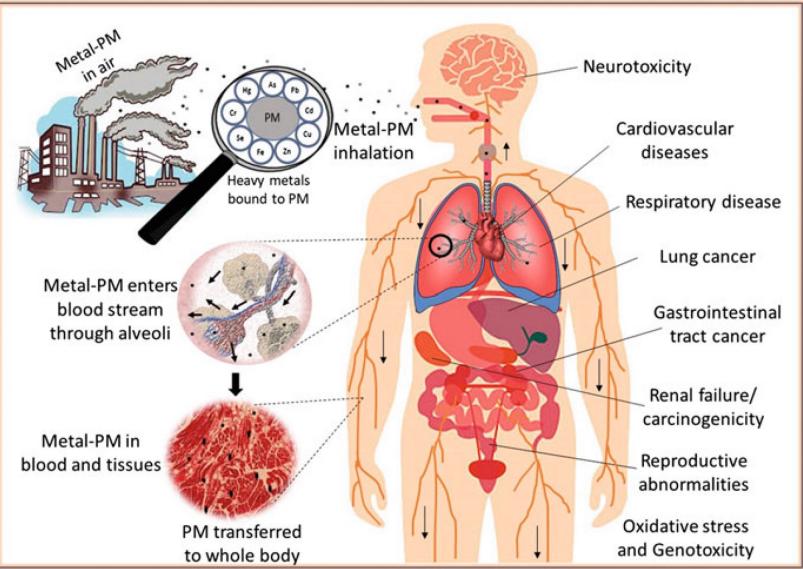
Heavy metal exposure has wide ranging effects on human health

Routes of exposure

- Inhalation
- Ingestion
- Dermal

Health effects

- Systemic toxicity
- Damage of multiple organs



Reviews of Environmental Contamination and Toxicology. Volume 253

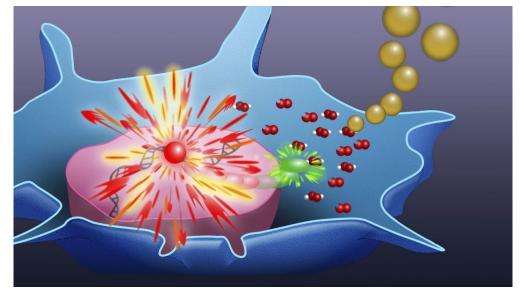
There are multiple proposed mechanisms for metal toxicity

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

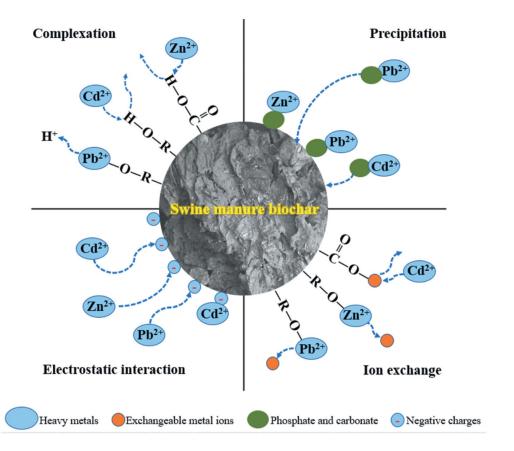
- Disrupt mitochondrial function
- Generate reactive oxygen species



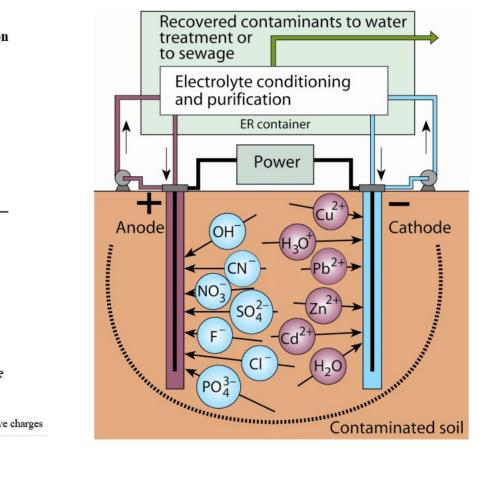
What can we do to mitigate this issue?

Physical and chemical mitigation of heavy metal contamination

Soil Amendment with Biochar



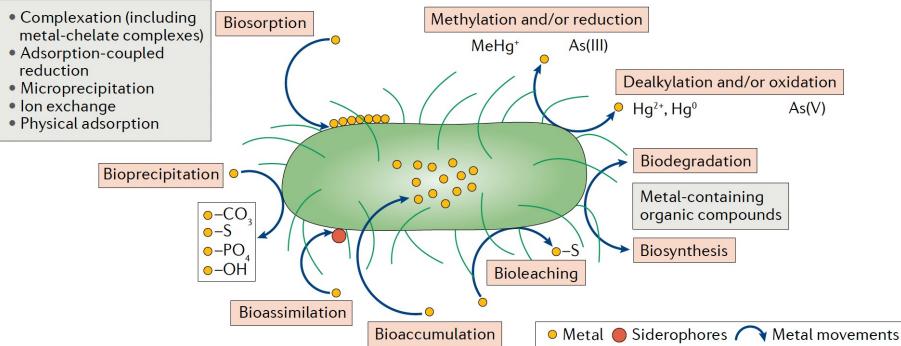
Electrokinetic remediation



- 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

