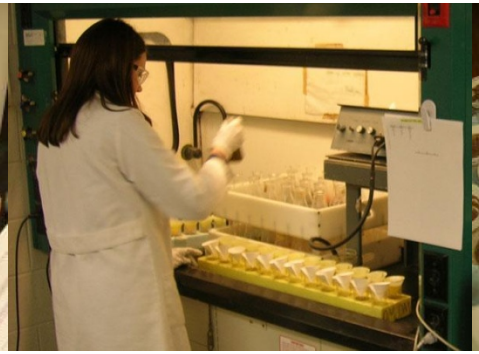
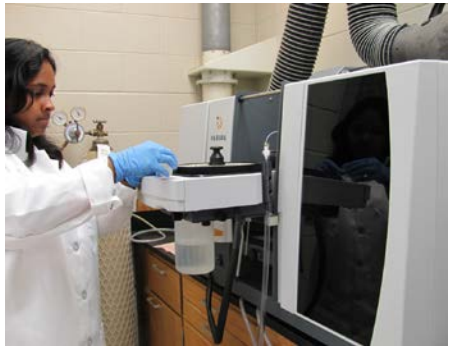
Analytical Methods to Monitor Phytoremediation



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Outline

- Overview of Phytoremediation
  - for inorganic and organic contaminants
- Overview of Soil Analysis
  - Sampling, processing, analysis, calculations, interpretations (with emphasis on inorganics)
- Overview of Plant Analysis
  - Sampling, processing, analysis, calculations, interpretations (with emphasis on inorganics)
- Challenges

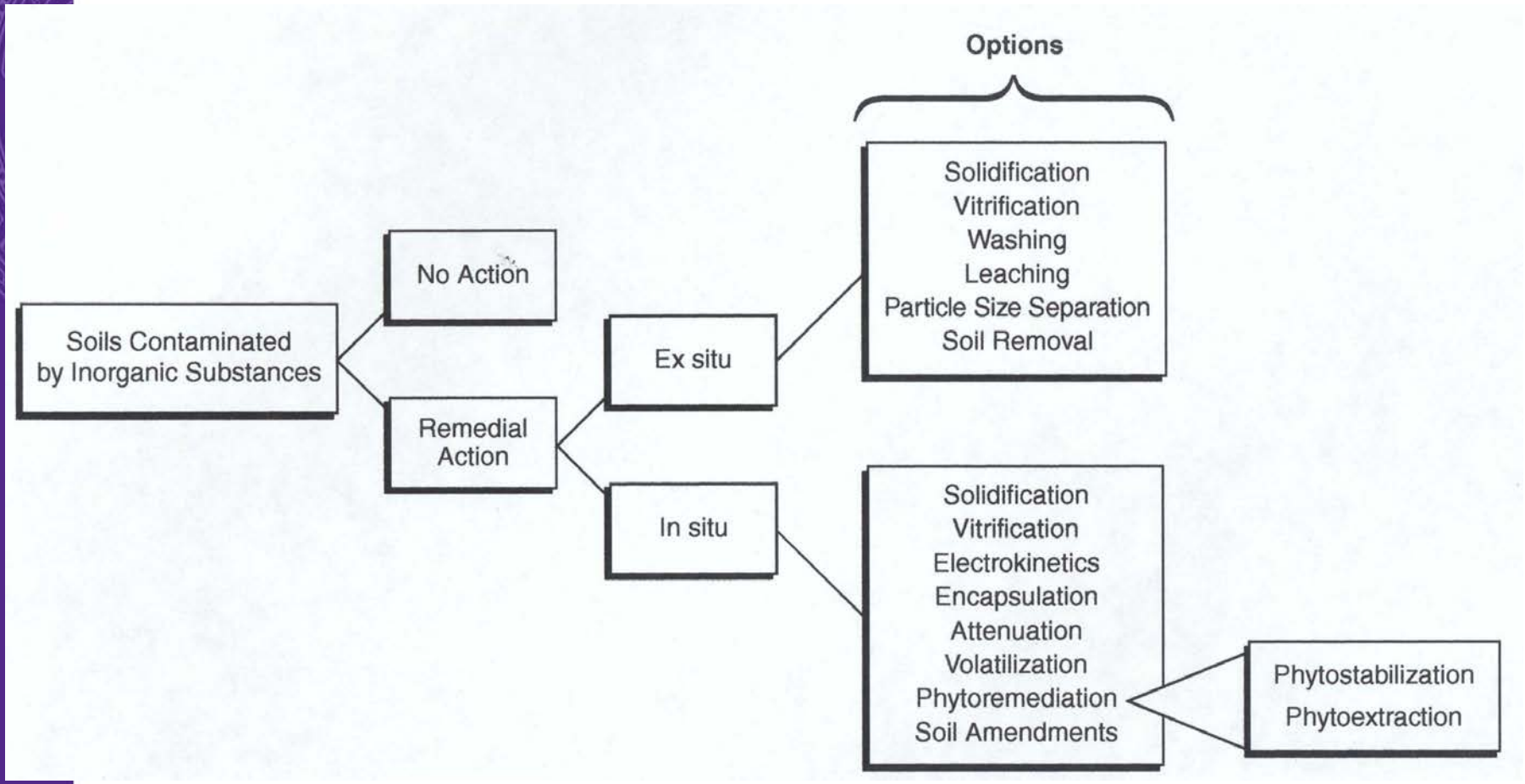
Definitions

- Remediation – processes or methods for treating contaminants in soil or water such that they are contained, removed, degraded, or rendered less harmful
  - Site remediation: processes that contain (i.e., restrict movement) a contaminant but do not necessarily affect the contaminant
  - Soil or water remediation: generally refers to processes that directly treat the soil or water (contaminated medium) and affect the contaminant

Definitions

- *In situ* remediation: treatment of soil or water in place
- *Ex situ* remediation: physical removal and treatment of soil or water

Options for contaminated soils- Inorganic substances



Different technologies

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General soil remediation approaches separated by the net effect on the contaminant

Remediation technology effect	Technologies for Inorganic contaminants
Reduce contaminant concentration	Washing, leaching, particle size separation, attenuation, volatilization, phytoextraction, electrokinetics
Encapsulate contaminant in an inert matrix	Solidification, vitrification
Reduce contaminant bioavailability without reducing total concentration	Soil amendments Altering redox conditions
Containment	Encapsulation Phytostabilization
Removal	Soil excavation

Reduce Bioavailability of Contaminant

- Inorganics - Soil amendments
  - Promote sorption
  - Change pH
  - Change redox conditions
  - Promote chelation by organics

Example amendments – P, oxides of Fe and Mn, zero valent Fe, zeolites, clays, animal waste, biosolids

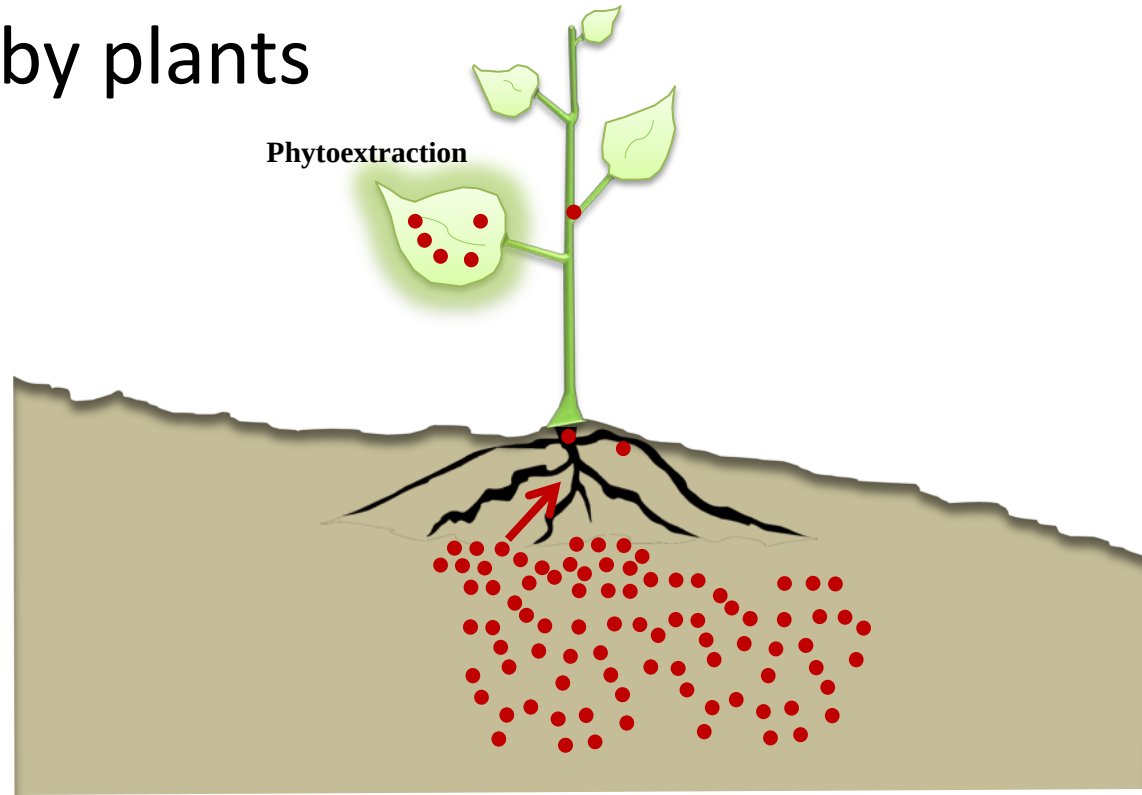
Phytoremediation

- What is phytoremediation? “plant”-based remediation. Use of plants to remediate a site, soil or ground water
- Confusion on the use of the term
- Goals are different depending on whether the contaminant is inorganic or organic

Phytoremediation – Inorganic Contaminants

- Phytoremediation for inorganics – soil only, two kinds

1. Phytoextraction – removal of contaminants by plants



Phytoremediation – Inorganic Contaminants

1. Phytoextraction –

   Requires transfer of metals from soil to above-ground portion of plant, and then harvest and removal of metal-rich biomass

   – Natural hyperaccumulators

   – Induced hyperaccumulation

Hyperaccumulators

Alpine pennycress. (K60548)

Plants that accumulate metals to levels that would be toxic to most other plants

Most hyperaccumulators take up only one metal, but some accumulate up to 3 metals to very high concentrations

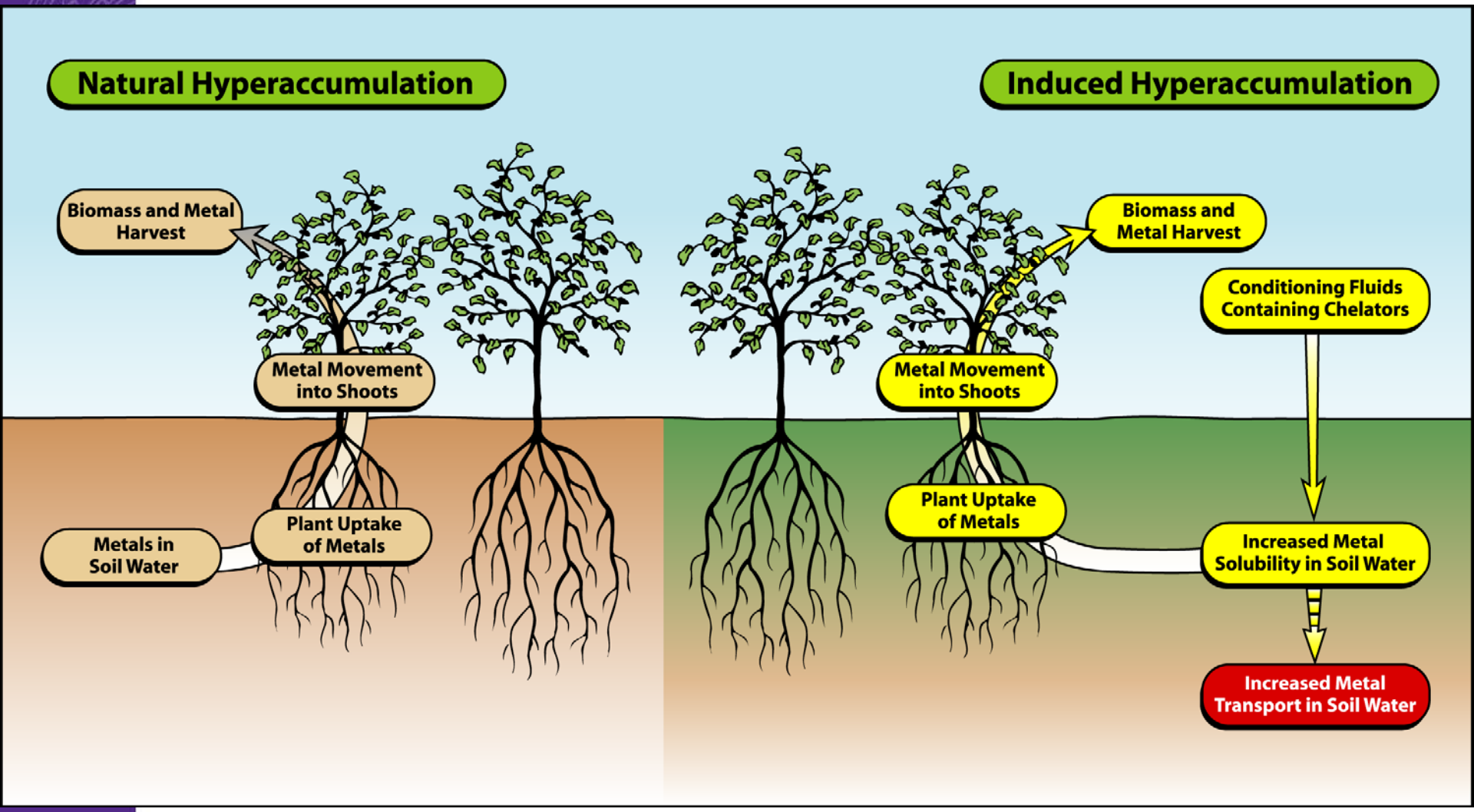
e.g. *Thlaspi caerulescens* (Alpine pennycress)

Hyperaccumulators could be used to extract metals from soils

- Plant material as source of metals (phytomining)

Most hyperaccumulators grow very slowly
Research to increase growth and/or to find other, faster-growing hyperaccumulators

Phytoextraction



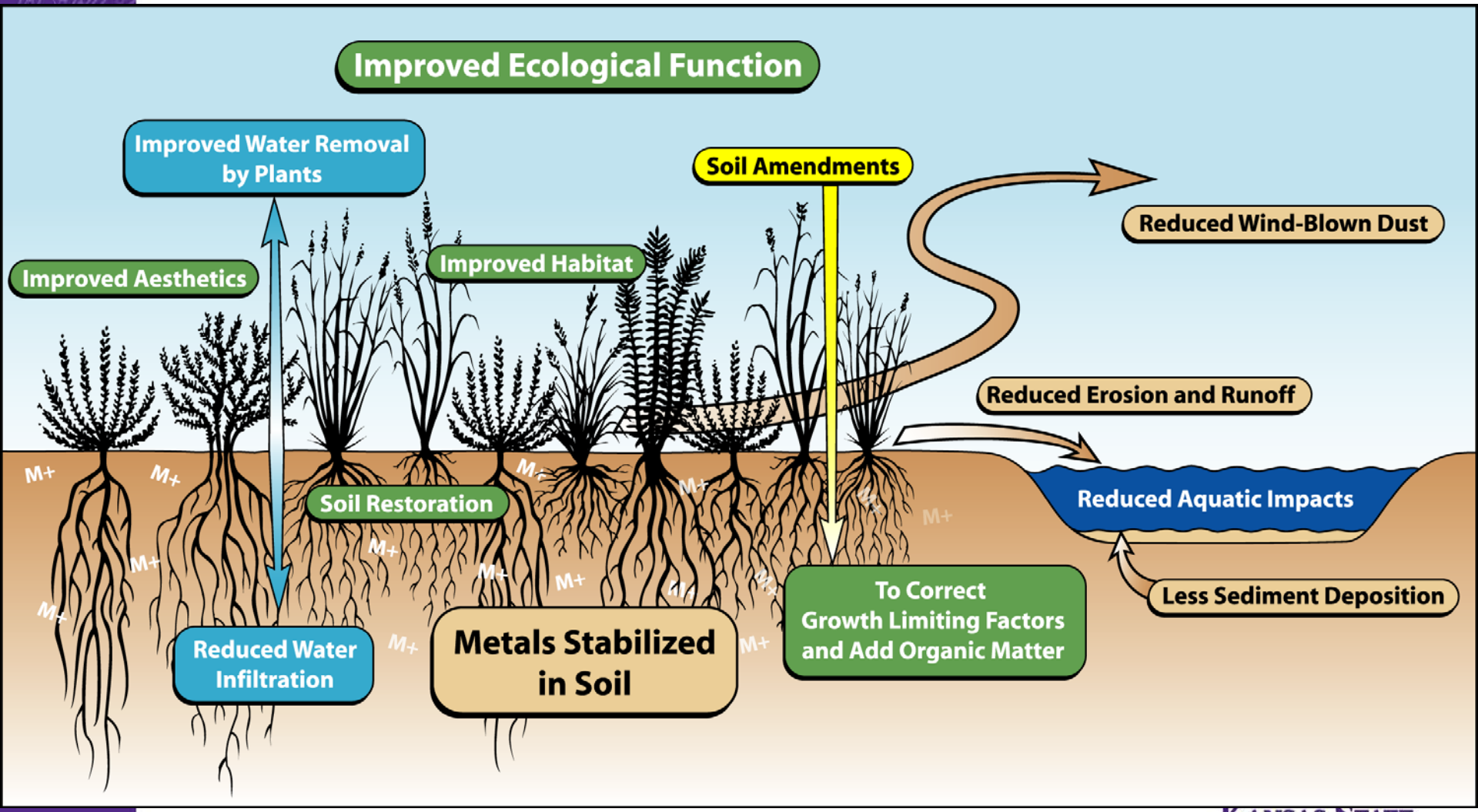
Source: Pierzynski et al., 2005

Phytoremediation – Inorganic Contaminants

2. Phytostabilization – establish permanent vegetative cover to minimize movement of soil and contaminants via wind and water erosion.

- Involves the use of soil amendments to correct for factors that limit plant growth.

Phytostabilization



Source: Pierzynski et al., 2005

Phytostabilization of mine impacted areas
Site: Galena, Kansas, U.S.A.

Funded by KDHE/KLA

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Phytostabilization of military contaminated sites with second generation biofuel crops

Site: Fort Riley, Kansas, U.S.A.
08/29/2017

Funded by NATO

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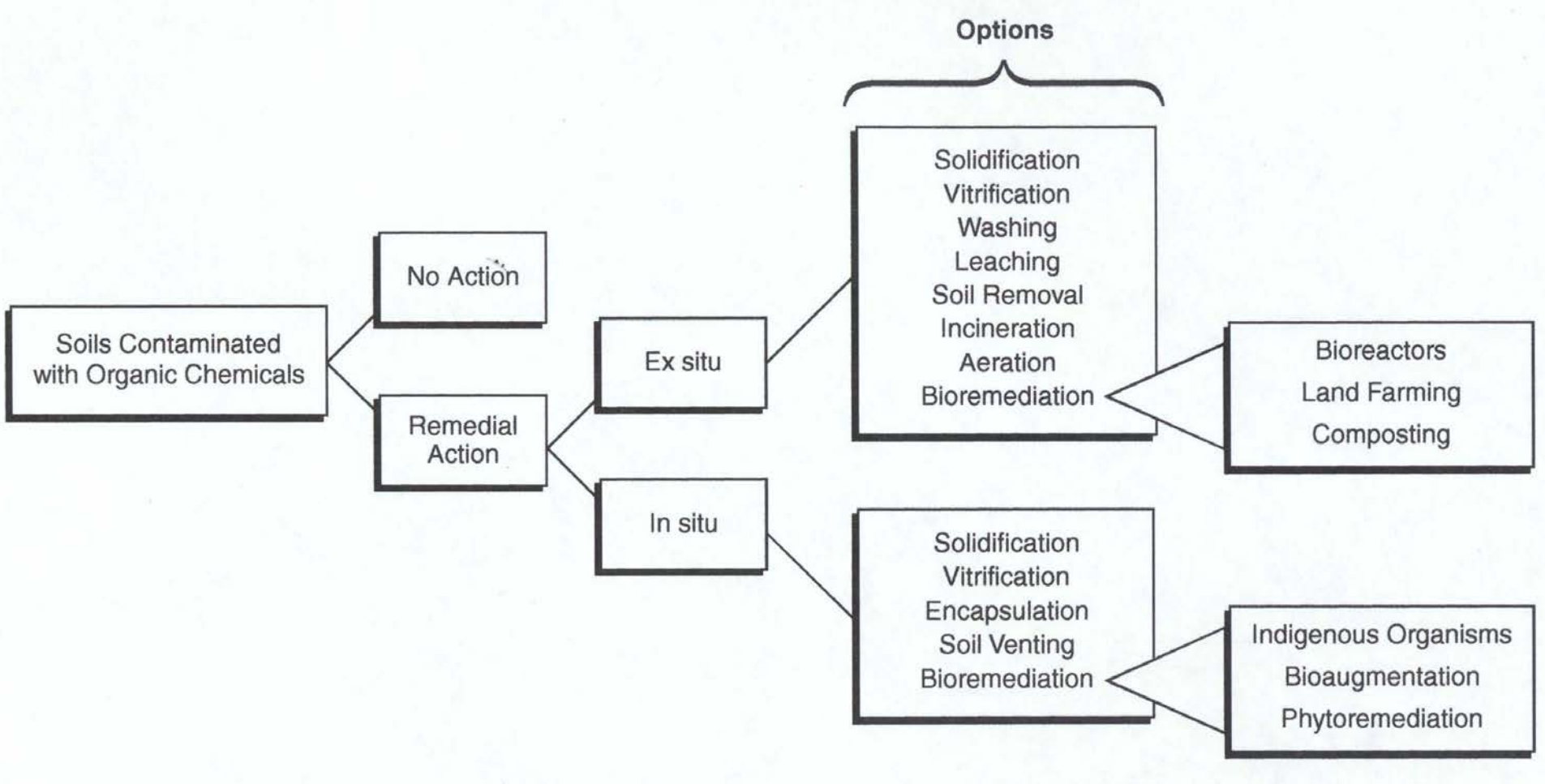
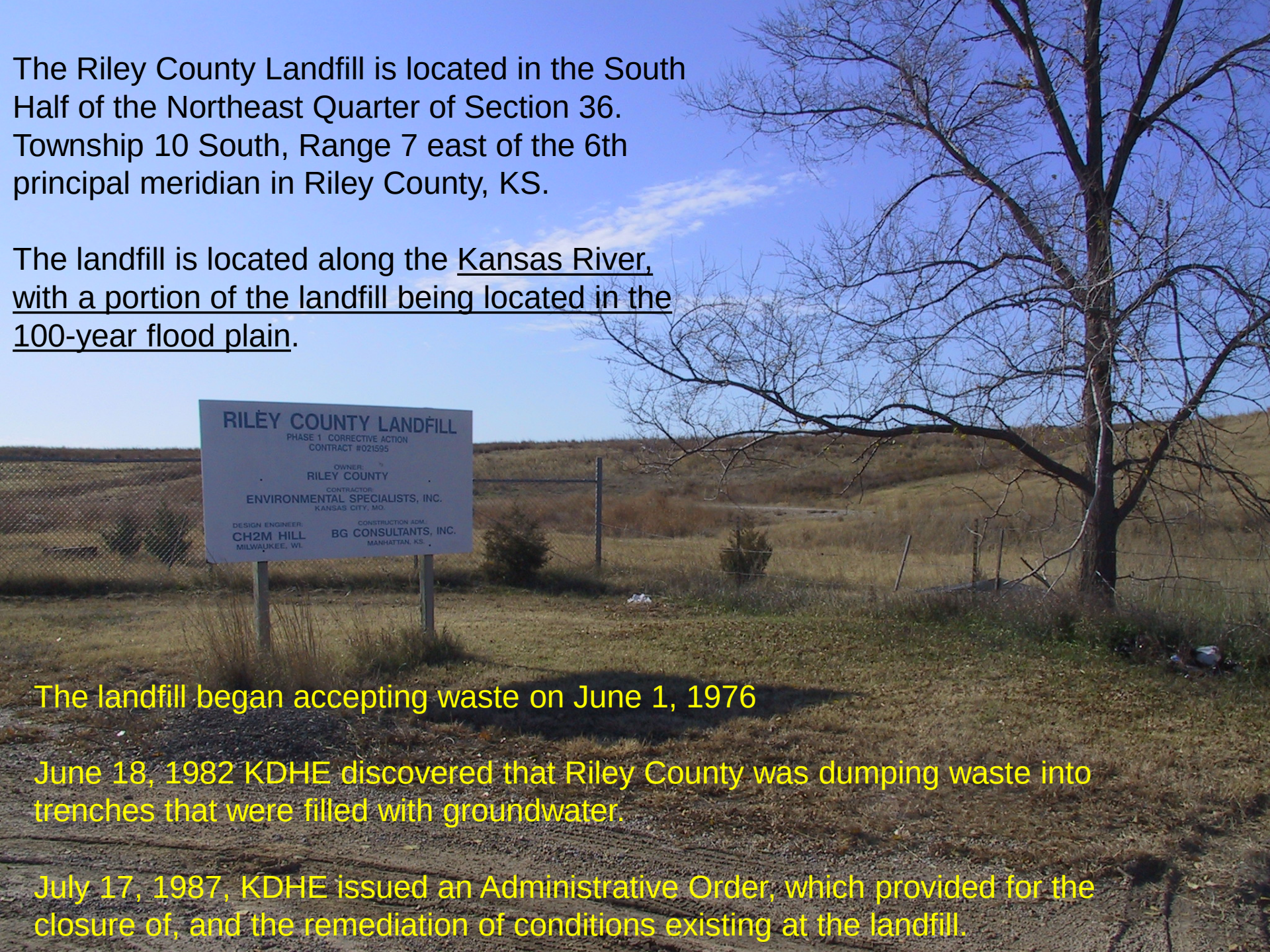
Options for contaminated soils-organic chemicals



In situ – Bioremediation Organic Contaminants

- Phytoremediation for organics

1. Phytovolatilization – the use of deep rooted plants to transpire and volatilize VOCs in shallow ground water
   - Riley County Landfill



The Riley County Landfill is located in the South Half of the Northeast Quarter of Section 36. Township 10 South, Range 7 east of the 6th principal meridian in Riley County, KS.

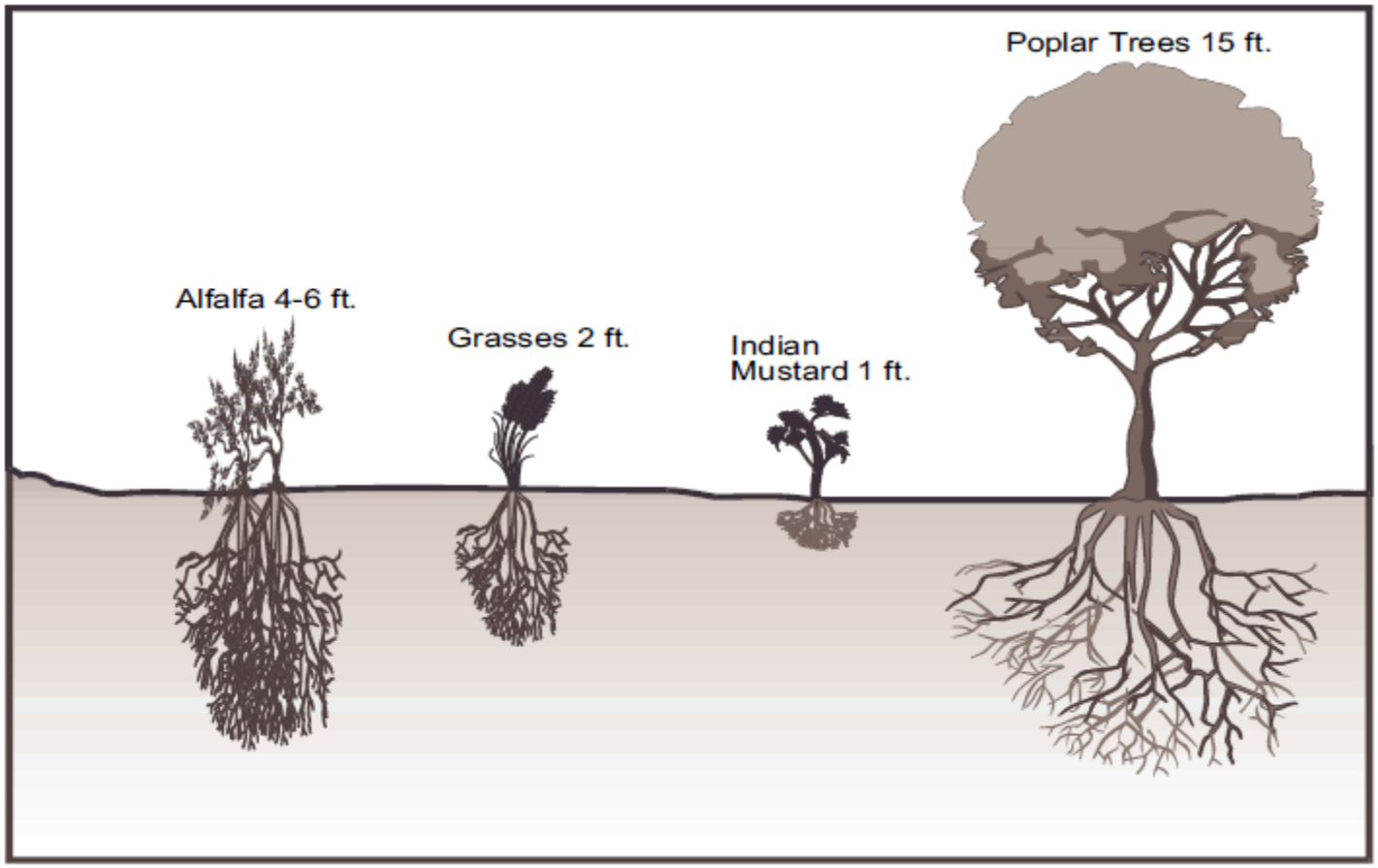
The landfill is located along the Kansas River, with a portion of the landfill being located in the 100-year flood plain.

The landfill began accepting waste on June 1, 1976

June 18, 1982 KDHE discovered that Riley County was dumping waste into trenches that were filled with groundwater.

July 17, 1987, KDHE issued an Administrative Order, which provided for the closure of, and the remediation of conditions existing at the landfill.

Example rooting depths



Phytoremediation – Organic Contaminants

2. Enhanced degradation of organics – the presence of plants accelerates the degradation of organic chemicals in soils and shallow ground water
   - Rhizosphere effect
   - Plant metabolism

Craney Island Fuel Facility, Virginia

Treatment	-- % Petroleum Degradation -- 6 months	24 months
Unvegetated	12a	31c
Clover	11a	50a
Fescue	9a	45ab
Bermuda	13a	40b

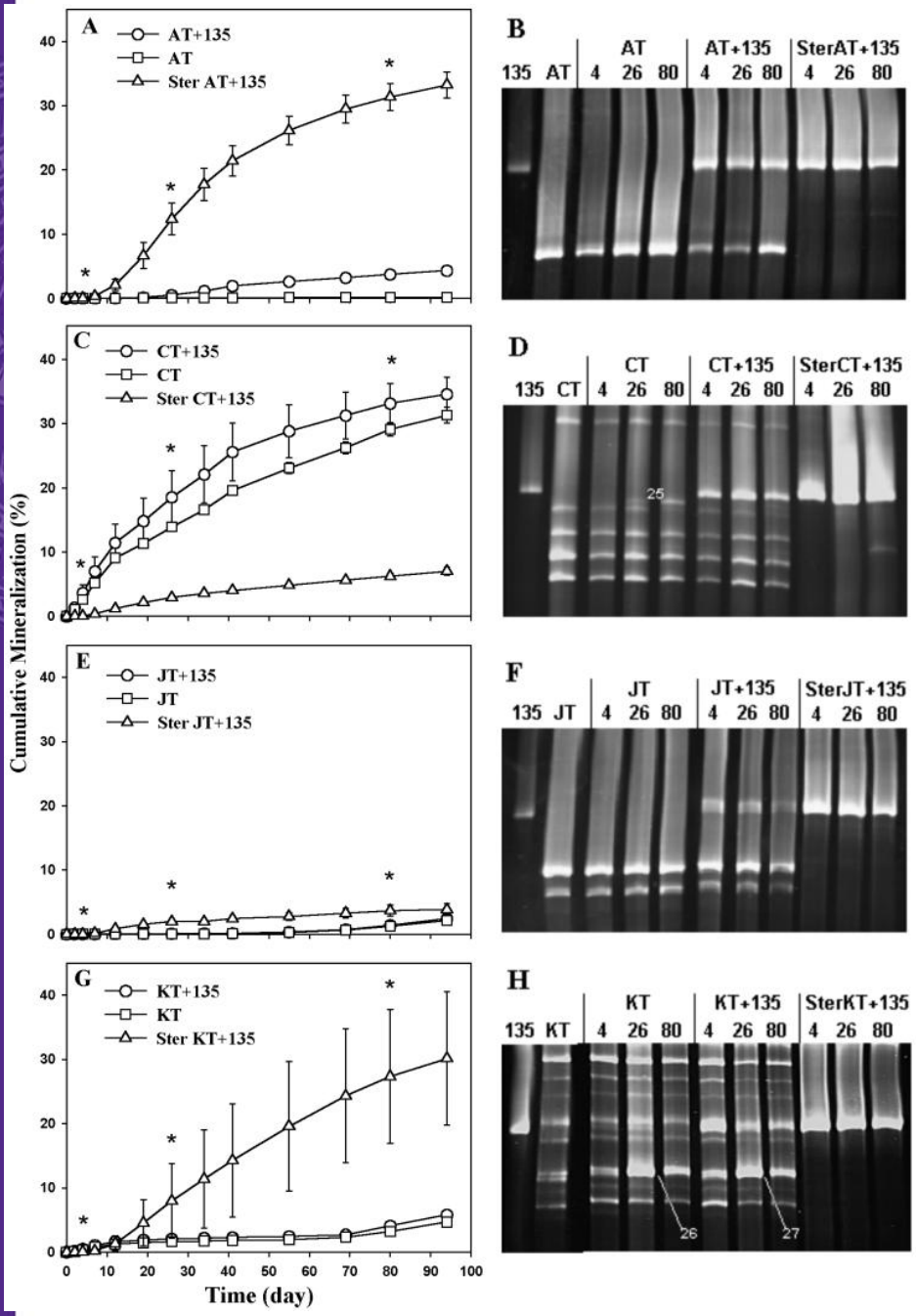
Soil: 60% sand, 21 % silt, 19 % clay

Started in 1995, reseeded fescue and clover plots in 1996

Source: https://clu-in.org/products/phyto/search/phyto_details.cfm?ProjectID=121

Other In situ bioremediation methods

- Indigenous organisms: Degradation of some organic contaminants will proceed by the action of indigenous microorganisms
- Bioaugmentation: the inoculation of soil/water using microorganisms with an enhance ability to degrade the organic contaminant (can be either isolated from native populations or be genetically engineered)



Pyrene mineralization curves and TGGE analysis of contaminated soils. (A, C, E, and G) Pyrene mineralization curves. AT, CT, JT, and KT are soils amended with pyrene only. AT+135, CT+135, JT+135, and KT+135 are soils amended with pyrene and *Mycobacterium* sp. strain 135. Ster AT+135, Ster CT+135, Ster JT+135, and Ster KT+135 are sterilized soils amended with pyrene and strain 135. Soils were sampled for TGGE analysis at days 4, 26, and 80 (asterisks). Error bars represent standard errors from three replicates. (B, D, F, and H) TGGE analysis. Lanes AT, CT, JT, and KT, unamended soil at day 0; lanes, 4, 26, and 80, pyrene-amended soils extracted at days 4, 26, and 80, respectively. Bands CT-25, KT-26, and KT-27 were excised for sequencing and phylogenetic analysis. Electrophoresis was carried out with a temperature gradient of 55 to 65°C for 16 h 40 min in a 9 M urea–6% gel. TGGE: temperature gradient gel electrophoresis (TGGE).

Cheung PY, Kinkle BK.
Appl Environ Microbiol. 2001 May;67(5):2222-9

Soil Analysis

- Soil analysis to assess selected soil properties, plant-available nutrient concentrations, salinity, contaminant concentrations, and contaminant bioavailability in soil

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Five Basic Components

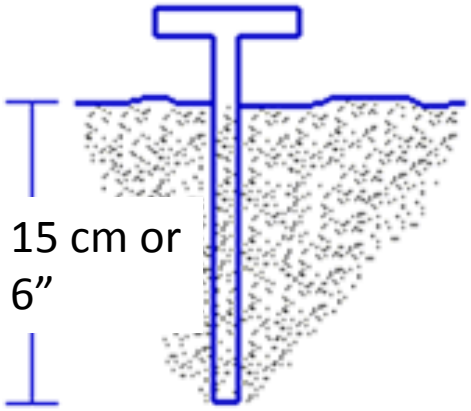
- Collecting the sample
- Sample processing
- Analysis
- Relevant calculations
- Interpretation

Collecting the Sample

- Sample must be representative of the material you are wanting to analyze

soil probe
trowel or shovel
clean plastic bucket

Soil



Mostly focus on surface soils, ~15 cm depth (most relevant rooting depth)

Sample Collection

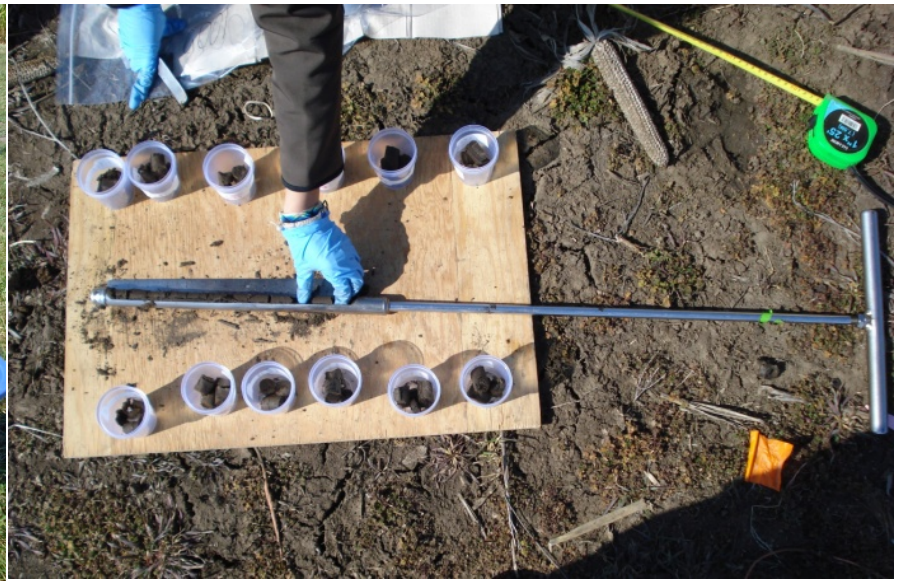
Step 1

- Identify uniform areas to be tested
- Avoid sampling areas that might give misleading results. If necessary, obtain a separate sample for these areas

Source:
http://www.agronomy.ksu.edu/soiltesting/p.aspx?tabid=40

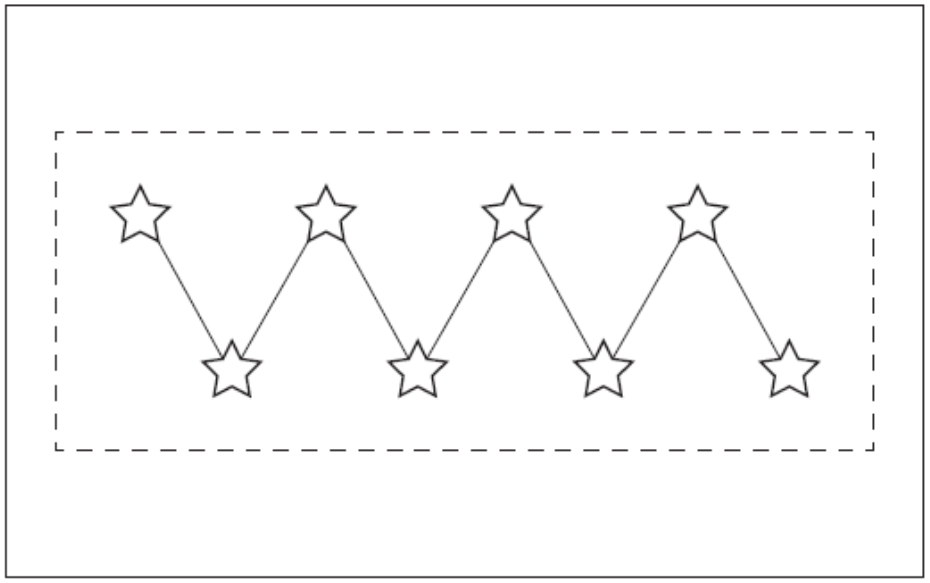
Sample Collection (cont.)

- Take enough samples to properly represent the area (for example, one plot unit)
- Collect a vertical sample starting at the surface of the soil and digging ~ 6 inches (~15 cm) deep or take long core samples and separate depth-wise
- Mix all the samples thoroughly in the bucket



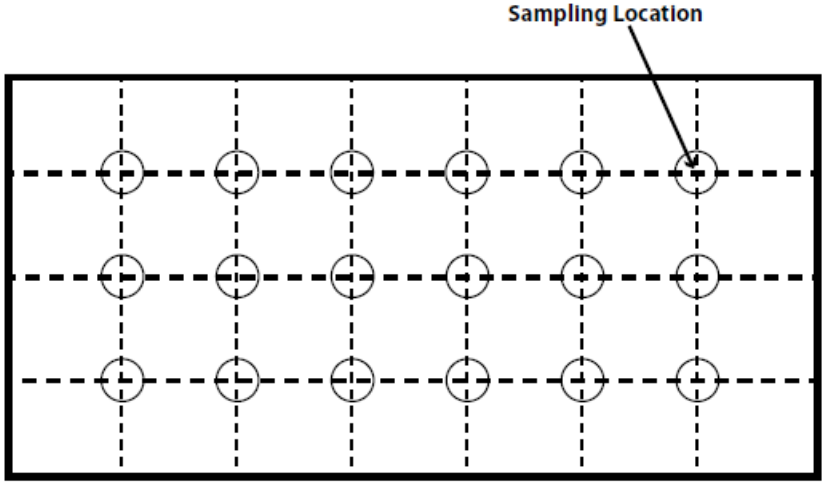
Limitations

- Soils vary continuously within sites. Surface (topographical) variation may be easily seen, but nutrient or contaminant variability is usually not obvious



When taking a composite soil sample, use a zig-zag pattern of locations (stars) to get a representative sample within the test area (dotted line).

Figure 1. *Example of a sampling grid. Samples are obtained at the intersection of the grid lines (dashed lines).*

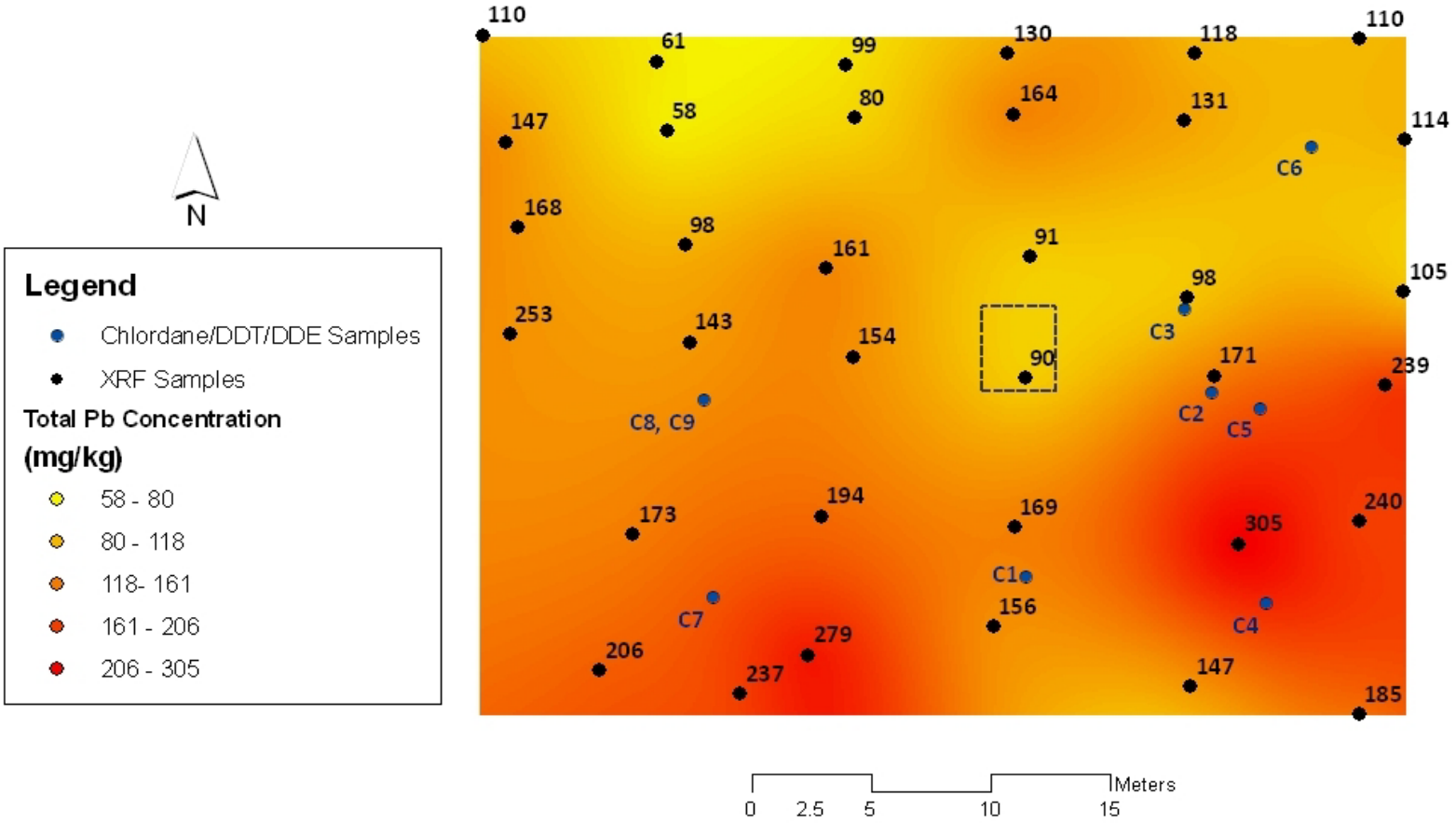


Sources: https://www.bookstore.ksre.ksu.edu/pubs/MF3192.pdf

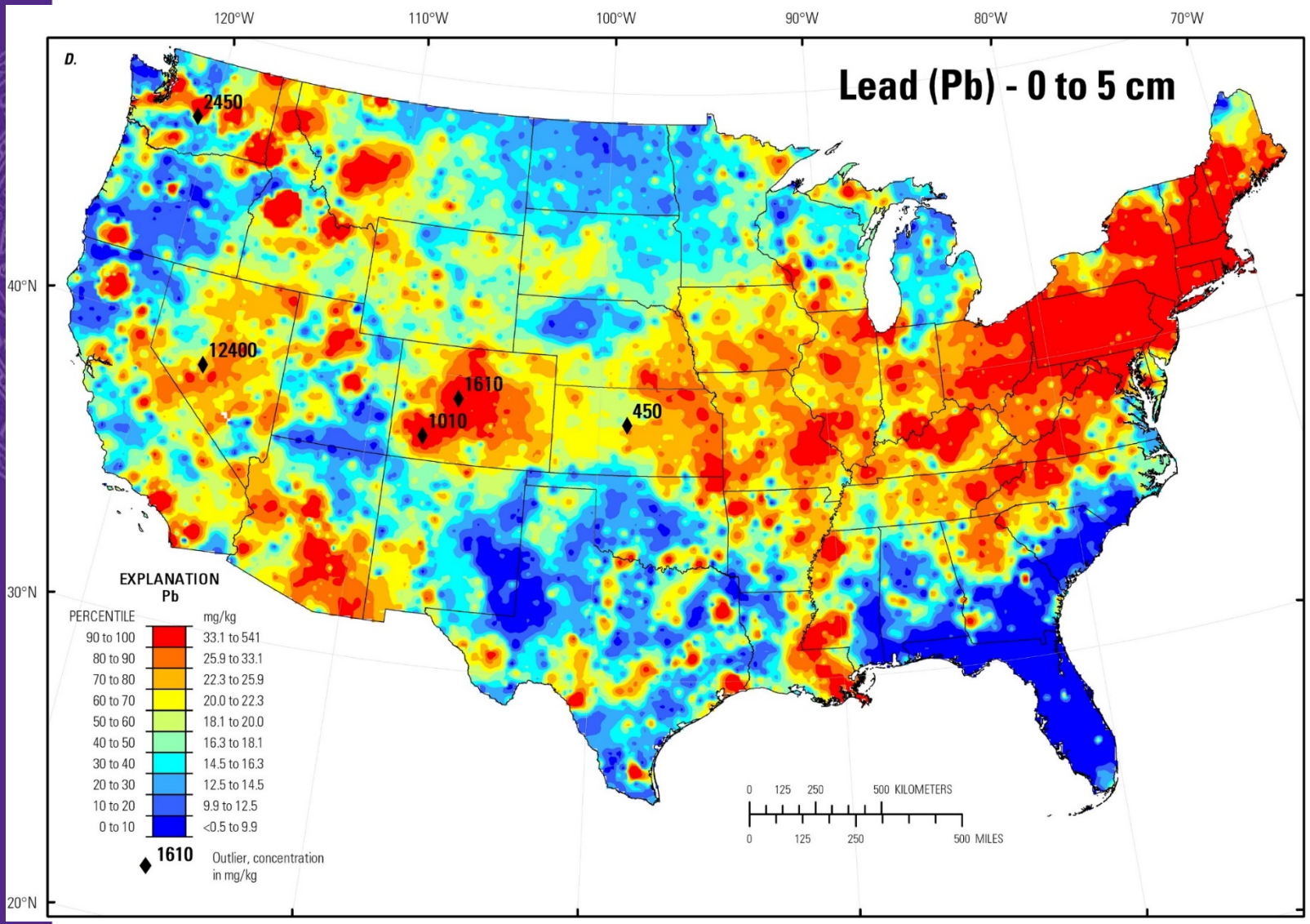
Limitations

Heterogeneity

Example: Washington Wheatley site, Kansas City, MO



Soil Pb distribution map



Source: Smith, D.B., Cannon, W.F., Woodruff, L.G., Solano, Federico, and Ellefsen, K.J., 2014, Geochemical and mineralogical maps for soils of the conterminous United States: U.S. Geological Survey Open-File Report 2014–1082, 386 p., *http://dx.doi.org/10.3133/ofr20141082*.

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X-ray fluorescence spectrometry

- Two types: Laboratory-based, Hand-held
- Hand-held type is ideal for initial soil screening for recognizing trace element contamination

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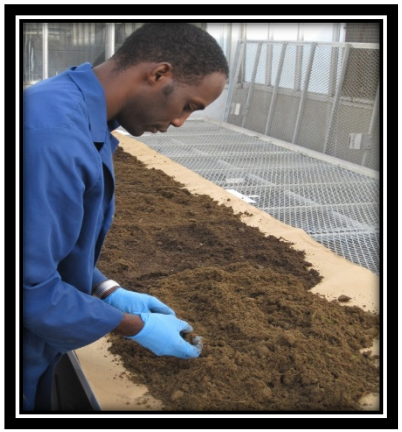
Sample Processing

- Preservation- moist versus air-drying (quick)
- Reduce heterogeneity- gentle grinding, sieving



- Convert to a form suitable for analysis
  - Digestion

    Digest soils with mixture of concentrated acids and hydrogen peroxide

  - Extraction

Soil Digestion

Microwave assisted digestion unit:
Closed system

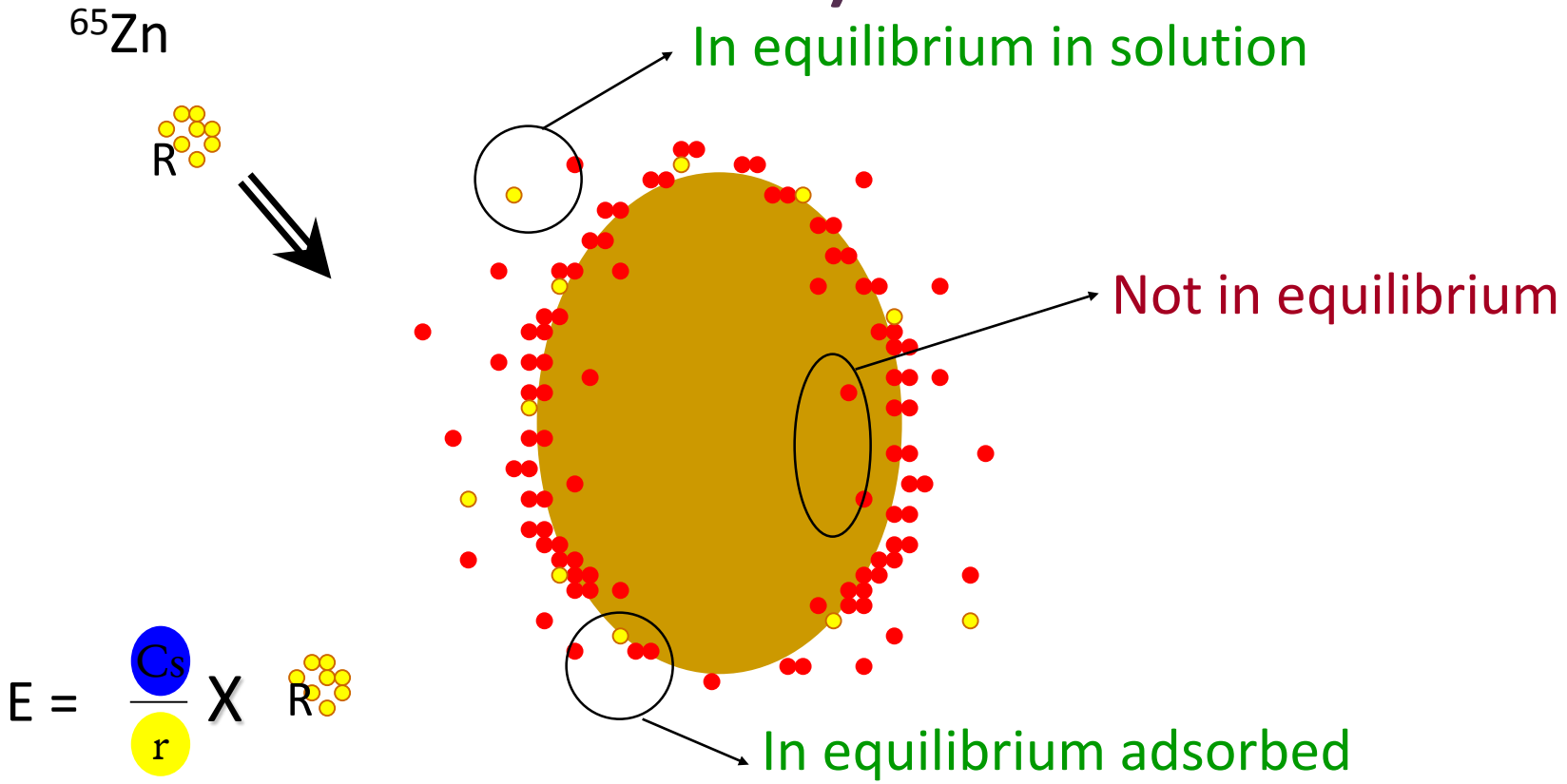
Block digestion unit:
Open system

USEPA Method 3051A (USEPA, 2007)
USEPA. 2007. Method 3051A Microwave assisted acid digestion of sediments, sludges, soils, and oils. www.epa.gov/waste/hazard/testmethods/sw846/pdfs/3051a.pdf

Soil Extractions

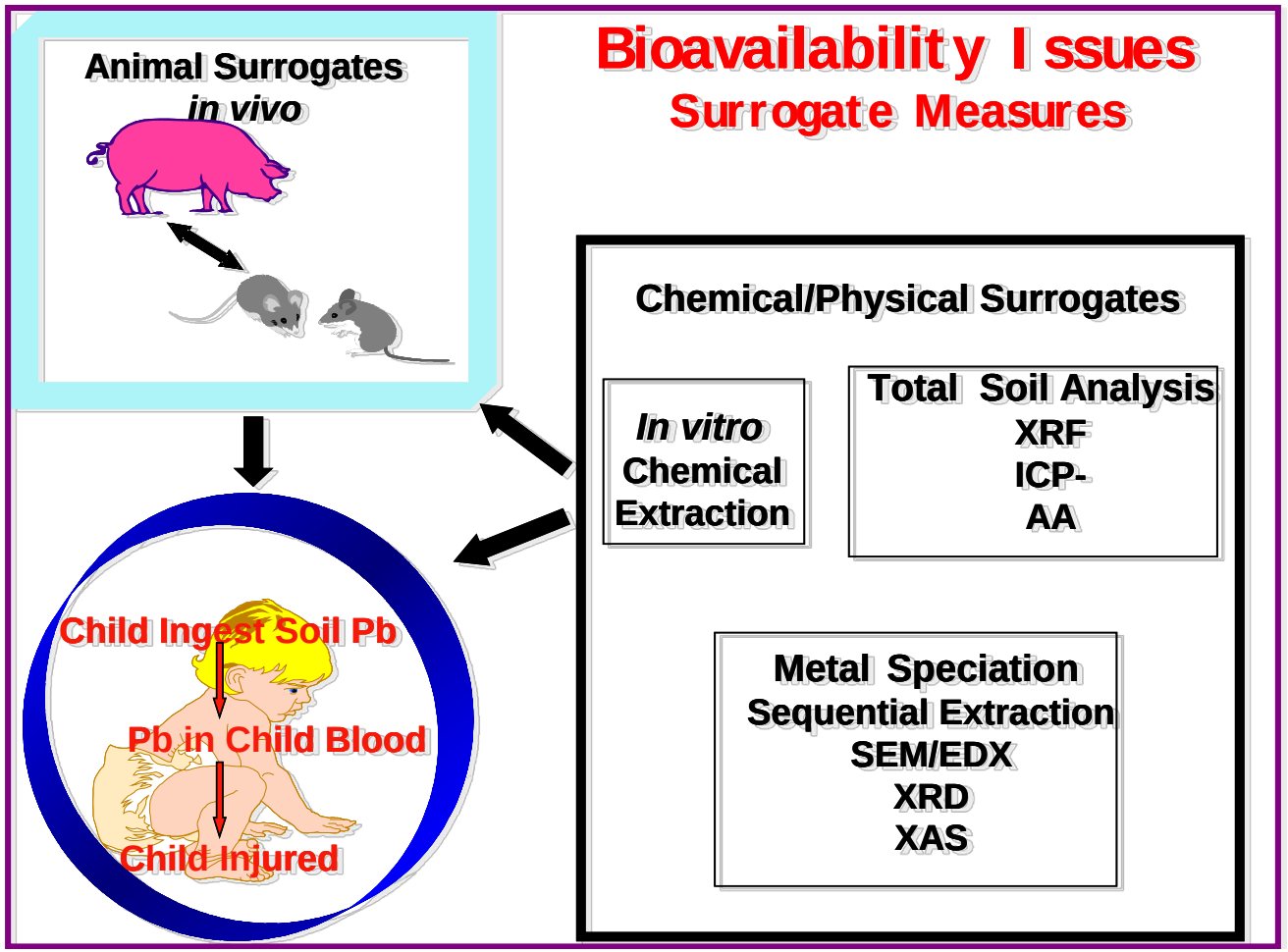
Extractable concentration of a substance or element - the amount that can be extracted, a subfraction of the total. Expressed as g/kg, mg/kg, or µg/kg.

Example 1: Potentially available element conc. measured by an isotopic dilution technique (E value)



where *E* represents the amount of isotopically exchangeable Zn (mg/kg), *Cs* is the concentration of Zn in the extract (mg/L), and *R* and *r* are, respectively, the initial amount of radioactivity ^{65}Zn introduced to the system and the radioactivity of ^{65}Zn remaining in the solution after 3 days.

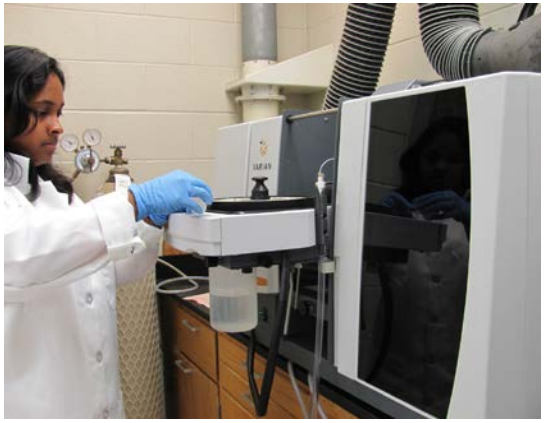
Example 2:
In vitro Bioaccessible lead



USEPA. 2013. Method 1340 In vitro bioaccessibility assay for lead in soil. SW-846 hazardous waste test methods. www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/1340.pdf

Some Selected Methods of Chemical Analysis: Inorganic contaminants

1. Inductively Coupled Plasma- Optical (atomic) Emission Spectrometry
2. Inductively Coupled Plasma- Mass Spectrometry
3. Graphite Furnace- Atomic Absorption Spectrophotometry (GF-AAS)
4. Hydride Generation Atomic Absorption Spectrophotometry



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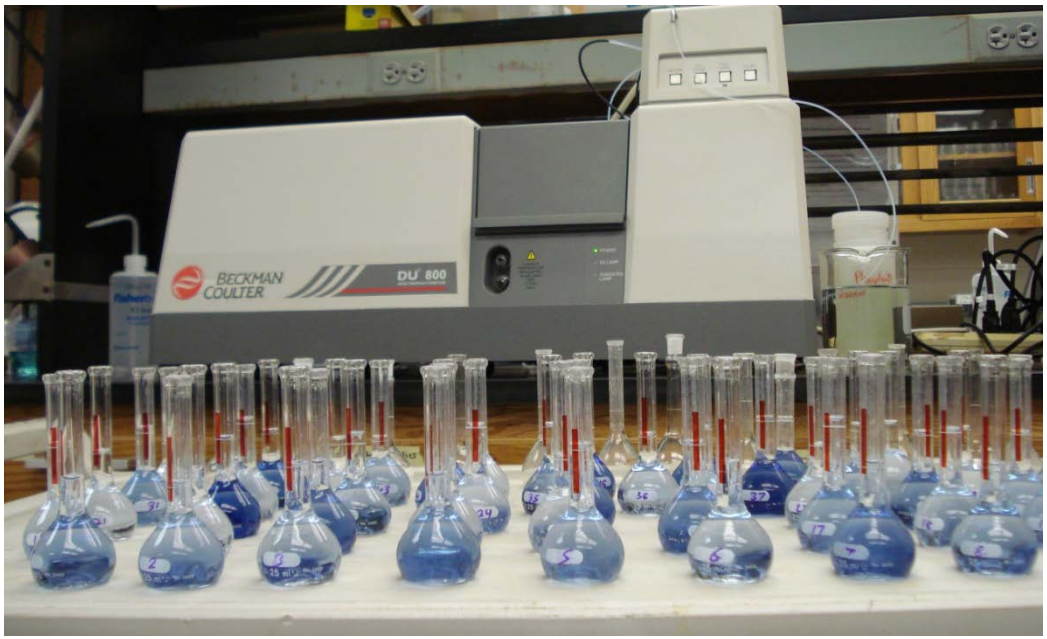
5. Colorimetry

- For some elements colorimetric methods are acceptable

Example: Phosphorus (molybdate reactive or orthophosphate), Nitrogen (NO_3^--N or NH_4^+-N)

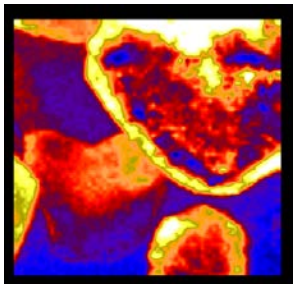


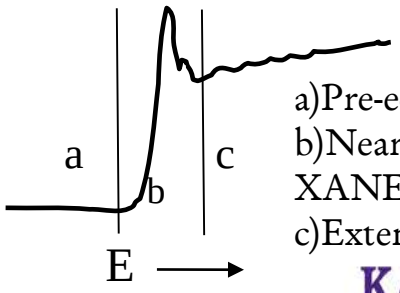
Spectrophotometer

Other Techniques

- Solution speciation
  - Chromatopgraphy-ICP-MS
  - Ion selective electrode
  - Ion chromatography
  - Colorimetric Methods
- Elemental mapping (micron to sub micron)
  - X-ray fluorescence spectroscopy
- Elemental speciation
  - X-ray absorption spectroscopy
  - X-ray diffraction

Arsenic on Bangladesh Biotite





a)Pre-edge region
b)Near-edge region-XANES
c)Extended region-EXAFS

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Relevant Calculations

- Putting raw data into a usable form
- Final forms
  - Concentration: mass per unit mass (%, mg/kg, etc.)
  - Mass per unit area: kg/ha, etc.

Interpretations
Agronomic parameters

Soil Test	Low	Medium	High
Available N	0-25 ppm	25-50 ppm	50-80 ppm
Available P	0-25 ppm	25-100 ppm	> 100 ppm
Available K	0-125 ppm	125-250 ppm	>250 ppm

Source: Fertilizing garden in Kansas, KSU Agricultural Experiment Station and Cooperative Extension Service

Interpretations (cont.)

Selected Trace Element Concentrations in soils

Element	"Normal" Range (mg/kg)
Arsenic (As)	< 5 to 40
Cadmium (Cd)	<1 to 2
Copper (Cu)	2 to 60
Molybdenum (Mo)	< 1 to 5
Nickel (Ni)	2 to 100
Lead (Pb)	10 to 150
Selenium (Se)	< 1 to 5
Zinc (Zn)	25 to 200

Source: Bowie, S.H.U. and I. Thornton. 1985. Eds. Environmental Geochemistry and Health. Kluwer Academic, Hingham, MA.

Other useful references:

Essignton, M.E. 2015. *Soil and Water Chemistry: An Integrative Approach*.
Lindsay, W.L. 1979. *Chemical Equilibria in Soils.*

Principles of Plant Analysis

Plant Analysis

"the determination of the elemental composition of plants or a portion of plant for elements essential for plant growth. It can also include determining elements that are detrimental to growth of animals and humans through our food chain"

Munson and Nelson, 1990

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Plant Analysis: Contaminants

- Accurate interpretation of the results of a plant analysis requires
  - Maximum limit allowable in plant
- Calibration data that relate concentration of the element in soil to concentration in the plant (site specific, crop specific, variety specific)

Five Basic Components

Same 5 components as for soils

- Collecting the sample
- Sample processing
- Analysis
- Relevant calculations
- Interpretation

Sample Collection

- Avoid leaves/fruits/tubers damaged by insects or disease or contaminated with fertilizers, dusts, or sprays
- Samples needs to be collected at the same time, from the same plant parts

Sample Collection (cont.)

- Samples needs to be decontaminated (remove soil or dust particles) by gently washing with a dilute detergent solution (~0.1%) – followed by a thorough rinsing with deionized water- followed by a rinsing with Milli-Q (ultra-pure) water

Sample Preparation

For inorganics

- Dry samples immediately at about 65 to 80°C (150 to 175°F) in paper, cotton, or plastic mesh bags
- Store them under refrigeration (~ 4°C) to avoid molding or OM decomposition- if samples cannot be dried immediately
- Grind dry samples to reduce the particle size and homogenize the samples

Sample Analysis

- Analysis of a plant sample requires that the organic fraction of the samples to be destroyed
- Can be accomplished by two ways
  - Wet Digestion
  - Dry Ashing

Sample Analysis (cont.)

Wet digestion:

Plant samples are dissolved in concentrated acids or mixtures of concentrated acids at high temperatures

The solution is filtered to removed undissolved solids

Microwave assisted digestion unit:
Closed system

Block digestion unit:
Open system

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Sample Analysis (cont.)

Dry Ashing

- High temperature (500°C) oxidation of a plant sample in a muffle furnace or high-temperature oven
- Dissolved ash in an acid solution
- Filter before analysis to remove any undissolved solids

Extraction

for organics

Cleaned fresh or dried/freeze-dried plant samples are extracted using methods such as the QuEChERS (“Quick, Easy, Cheap, Effective, Rugged, and Safe”) proposed by Anastassiades et al. (2003) and modified by Slizovskiy et al. (2010)

Sample Analysis (cont.)

- Once contaminant of interest in solution, contaminant concentration can be determined by a variety of instrumentation
- Similar techniques as for soil analysis

Examples:

- Graphite furnace-AAS
- ICP- MS

Suitable for μg/kg (ppb) and/or sub-ppb concentration (most trace elements in plants)

Other methods

Speciation and localization of elements in plants using μ-X-ray fluorescence spectroscopy and X-ray absorption spectroscopy

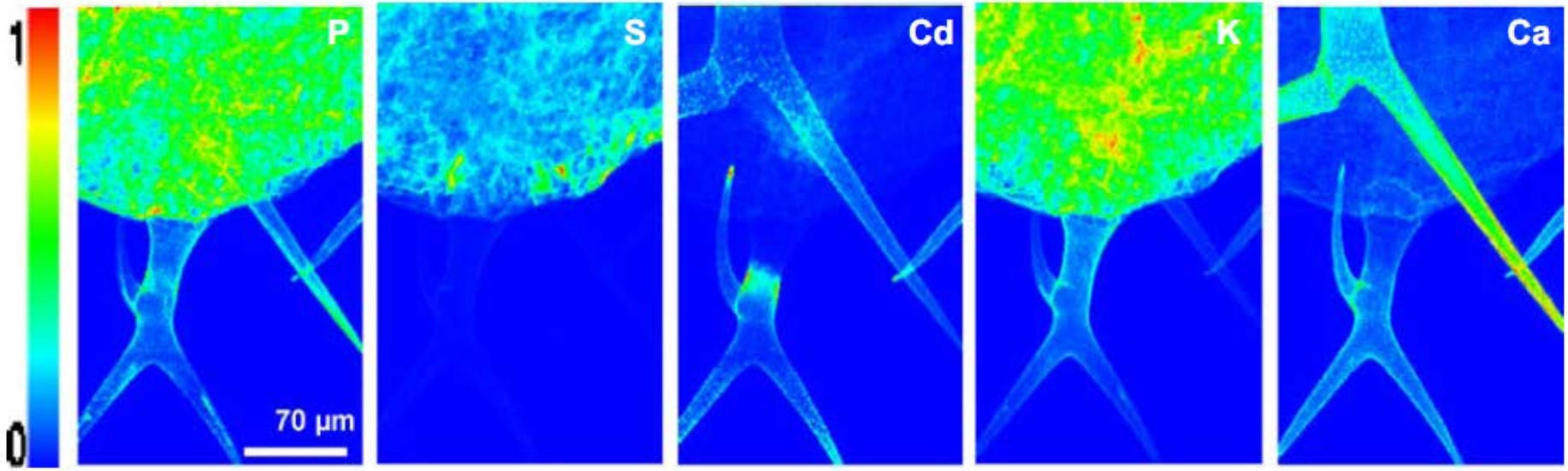

Fig. 7 False-colour μ-XRF elemental maps recorded on a leaf containing trichomes in *A. thaliana*. Beam size: 0.9×0.3 μm^2 (H×V) and exciting energy: 3550 eV. Reprinted from Isaure et al. 2006a with permission from Elsevier

Interpretations- Plant analysis

CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOODS

CODEX STAN 193-1995

1. PREAMBLE

1.1 SCOPE

This Standard contains the main principles and procedures which are used and recommended by the Codex Alimentarius in dealing with contaminants and toxins in foods and feeds, and lists the maximum levels of contaminants and natural toxicants in foods and feeds which are recommended by the CAC to be applied to commodities moving in international trade.

1.2 DEFINITION OF TERMS

1.2.1 General

The definitions for the purpose of the Codex Alimentarius, as mentioned in the Procedural Manual, are applicable to the General Standard for Contaminants and Toxins in Foods (GSCTF) and only the most important ones are repeated here. Some new definitions are introduced, where this seems warranted to obtain optimal clarity. When reference is made to foods, this also applies to animal feed, in those cases where this is appropriate.

1.2.2 Contaminant

Codex Alimentarius defines a contaminant as follows:

"Any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter".

This standard applies to any substance that meets the terms of the Codex definition for a contaminant, including contaminants in feed for food-producing animals, except:

1) Contaminants having only food quality significance, but no public health significance, in the food(s).

2) Pesticide residues, as defined by the Codex definition that are within the terms of reference of the Codex Committee on Pesticide Residues (CCPR). Pesticide residues arising from pesticide uses not associated with food production may be considered for inclusion in the GSCTF if not dealt with by the CCPR.

3) Residues of veterinary drugs, as defined by the Codex definition, that are within the terms of reference of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF).

4) Microbial toxins, such as botulinum toxin and staphylococcus enterotoxin, and microorganisms that are within the terms of reference of the Codex Committee on Food Hygiene (CCFH).

5) Processing aids (that by definition are intentionally added to foods).

WHO/FAO
general standards for contaminants and toxins in foods.

Many amendments to 1995 document are available online.

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Interpretations- Plant Analysis (cont.)

LEAD

Reference to JECFA:	10 (1966), 16 (1972), 22 (1978), 30 (1986), 41 (1993), 53 (1999)
Toxicological guidance:	PTWI 0.025 mg/kg bw (1987 for infants and young children, extended to all age groups in 1993, maintained 1999)
Residue definition:	Lead, total
Synonyms:	Pb
Related Code of Practice:	Code of Practice for the Prevention and Reduction of Lead Contamination in Foods (CAC/RCP 56-2004) Code of Practice for Source Directed Measures to Reduce Contamination of Foods with Chemicals (CAC/RCP 49-2001)

Commodity/Product Code	Name	Level mg/kg	Suffix	Type	Reference	Notes/Remarks for Codex Alimentarius
FT 0026	Assorted (sub)tropical fruits, edible peel	0.1		ML		
FI 0030	Assorted (sub)tropical fruits, inedible peel	0.1		ML		
FB 0018	Berries and other small fruits	0.2		ML		
FC 0001	Citrus fruits	0.1		ML		
FP 0009	Pome fruits	0.1		ML		
FS 0012	Stone fruits	0.1		ML		
VB 0040	Brassica vegetables	0.3		ML		Excluding kale
VA 0035	Bulb vegetables	0.1		ML		
VC 0045	Fruiting vegetables, Cucurbits	0.1		ML		
VO 0050	Fruiting vegetables, other than Cucurbits	0.1		ML		Excluding mushrooms
VL 0053	Leafy vegetables	0.3		ML		Including Brassica leafy vegetables but excluding spinach.
VP 0060	Legume vegetables	0.2		ML		
VD 0070	Pulses	0.2		ML		
VR 0075	Root and tuber vegetables	0.1		ML		Including peeled potatoes

CODEX STAN 193-1995. ***CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD*** AND FEED. Adopted 1995; Revised 1997, 2006, 2008, ***2009***; Amended ***2009***.

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Interpretations- Plant Analysis (cont.)

- Dry weight basis versus fresh weight basis
- Conversion of fresh weight based ML to dry weight based ML using produce moisture content

  Example: Assuming dry weight of lettuce is 5%
  - 0.3 mg/kg X 100/5 = 6 mg/kg

Challenges

Numerous

- Quality Assurance/ quality control (QA/QC)

No number is significant or worthy of being recorded, without an estimate of its uncertainty

Buffington (1978)

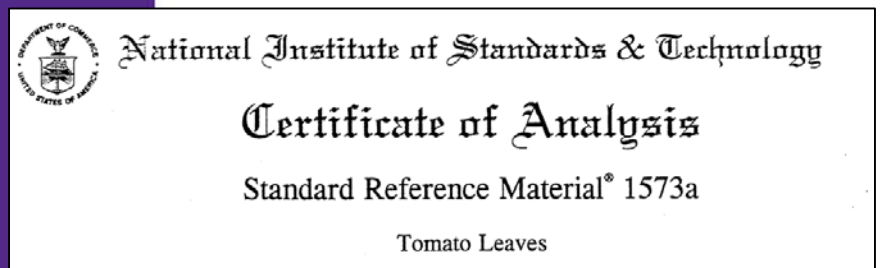
Quality Assurance and Quality Control Program

- Data quality
  - Precision
  - Accuracy
  - Completeness
  - Representativeness
  - comparability

Quality Assurance/Quality control measures

Common for both soil and plant analysis….

- Duplicate samples
- Duplicate/multiple digestions
- Standard Reference Materials
- Laboratory check samples
- Blanks
- Spike samples, etc.
- Interlaboratory comparisons


National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1573a

Tomato Leaves


National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2711a

Montana II Soil

Moderately Elevated Trace Element Concentrations

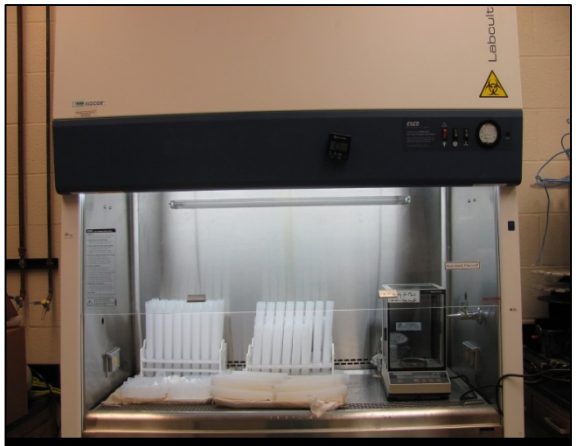
Quality Assurance/Quality control measures

- Standard addition: solution of known concentration of analyte is added to the unknown solution so any impurities in the unknown are accounted for in the calibration.

Challenges

- Contamination

  Need a “clean room” to process plant samples



“ultra-clean lab”
Biosafety cabinet

The “clean room” in many laboratories- biosafety cabinet

Dust and dirt do not have a chance to sneak in

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Challenges:
Lab and lab ware- How clean is clean enough?

- All glasses except quartz contain metals that may leach into samples- use only plastic containers
- Special cleaning/washing procedures for lab ware