Remediation and Restoration of Hydrocarbon and Brine Contaminated Soils

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The goal of this course is empowerment! I want to empower you to:

- Be able to manage your own soil remediation project
- If you use a contractor, be able to tell when the contractor knows what he or she is doing
- Be able to evaluate vendor claims for soil remediation products

Bioremediation of hydrocarbon contaminated soils

Bioremediation is not the first response to this problem!

Bioremediation can be used to treat the oil-stained soil after fluids have been properly disposed of.

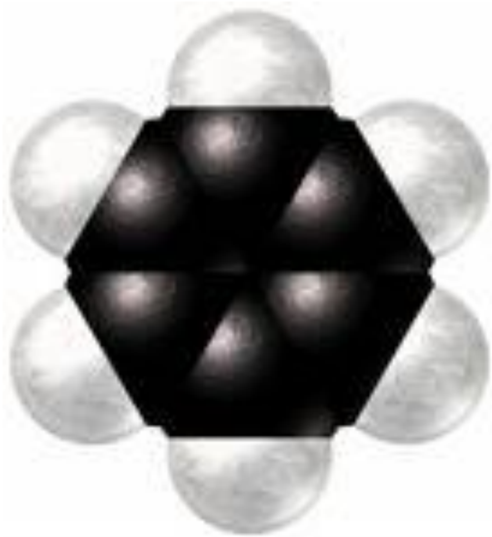
Why choose bioremediation for treatment of hydrocarbon contaminated soils?

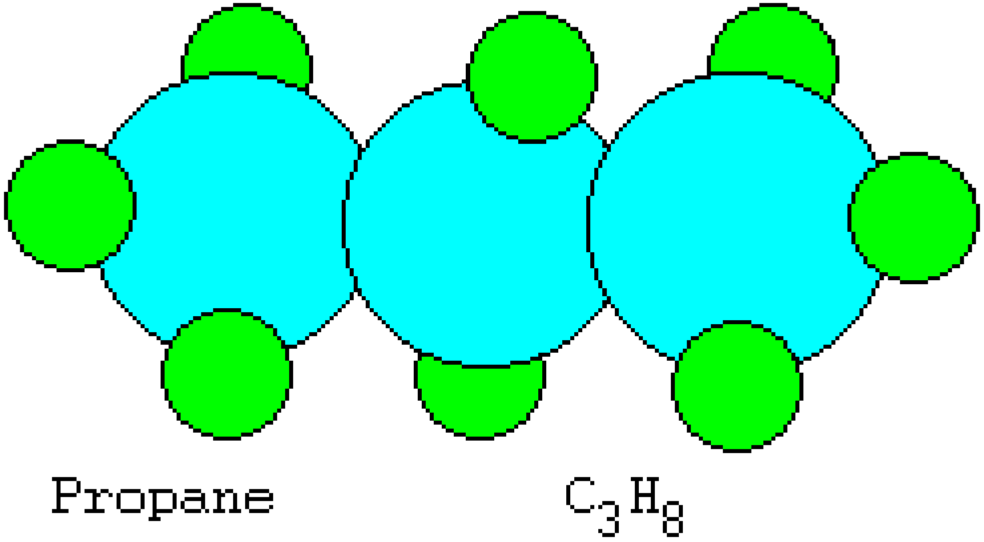
- Bioremediation is usually the most cost-effective solution for the treatment of hydrocarbon contaminated soils because
  - Can often treat contamination in place
    - No removal or disposal costs
    - Minimal site disturbance and reduced post-cleanup costs
- Waste toxicity is permanently eliminated; long-term liability risks are eliminated
- Positive public perception

Bioremediation is a powerful tool but…

- The #1 reason for failure of a bioremediation project is a basic lack of understanding of the unique needs and limitations of the microorganisms that are responsible for the process

Some hydrocarbon chemistry





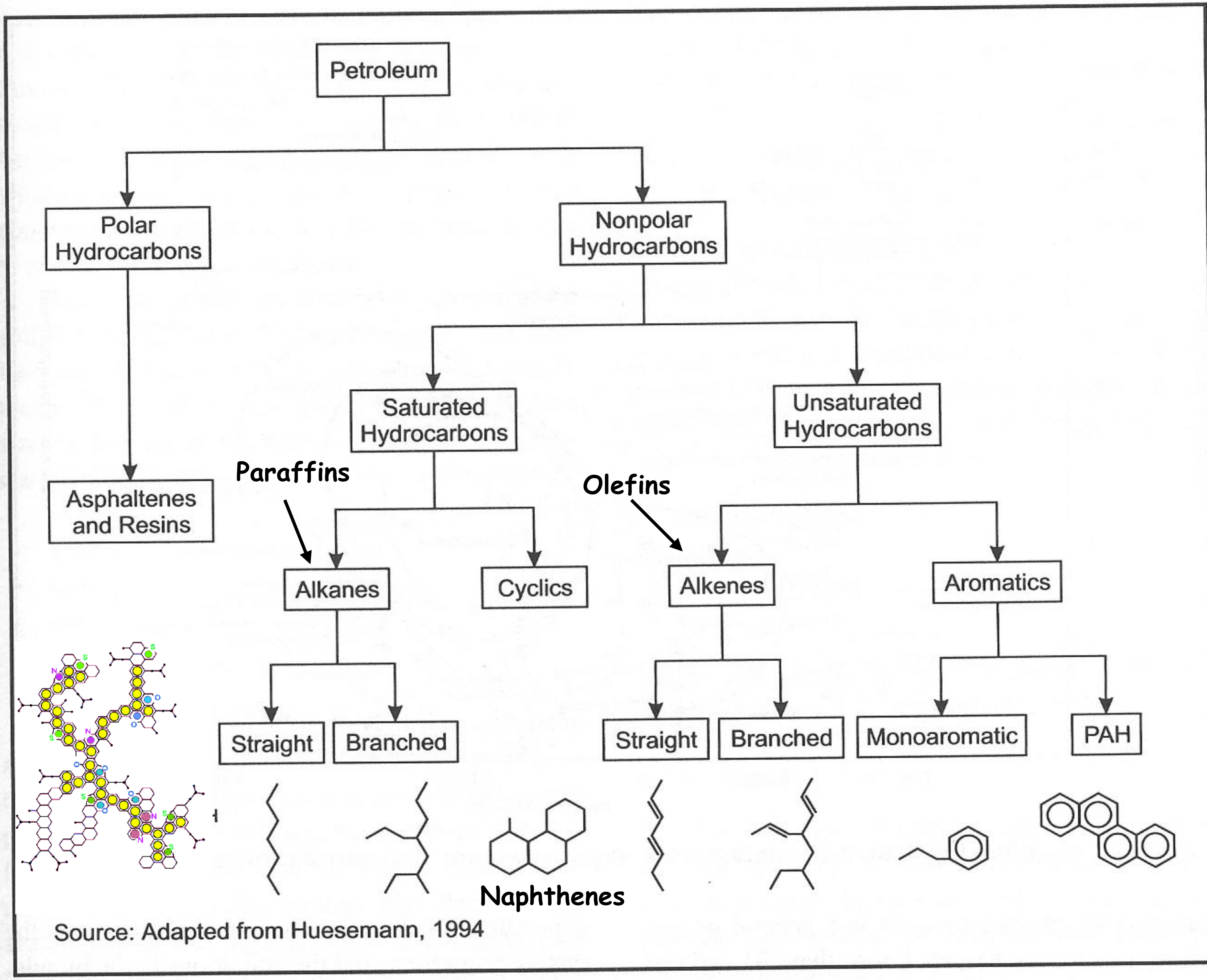
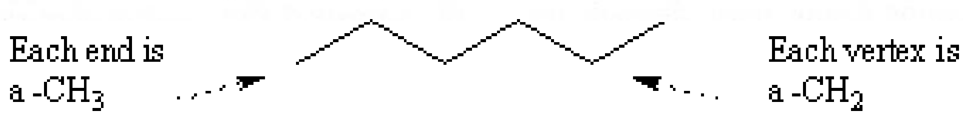
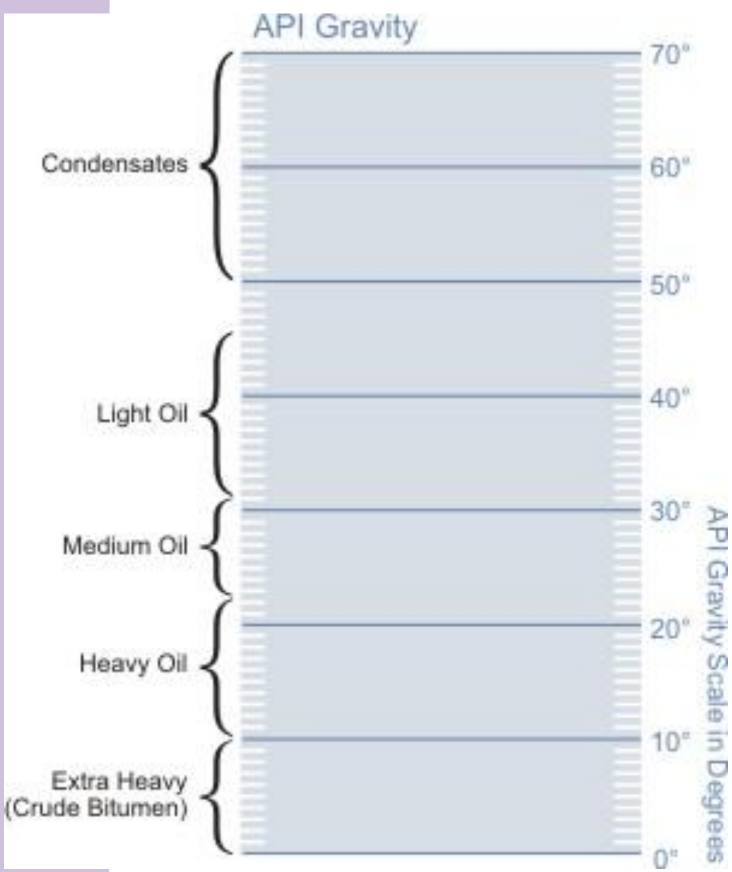


FIGURE 7. Illustration of the types of organic compounds in petroleum



$$API\ gravity = \left(\frac{141.5}{specific\ \ gravity}\right) - 131.5$$



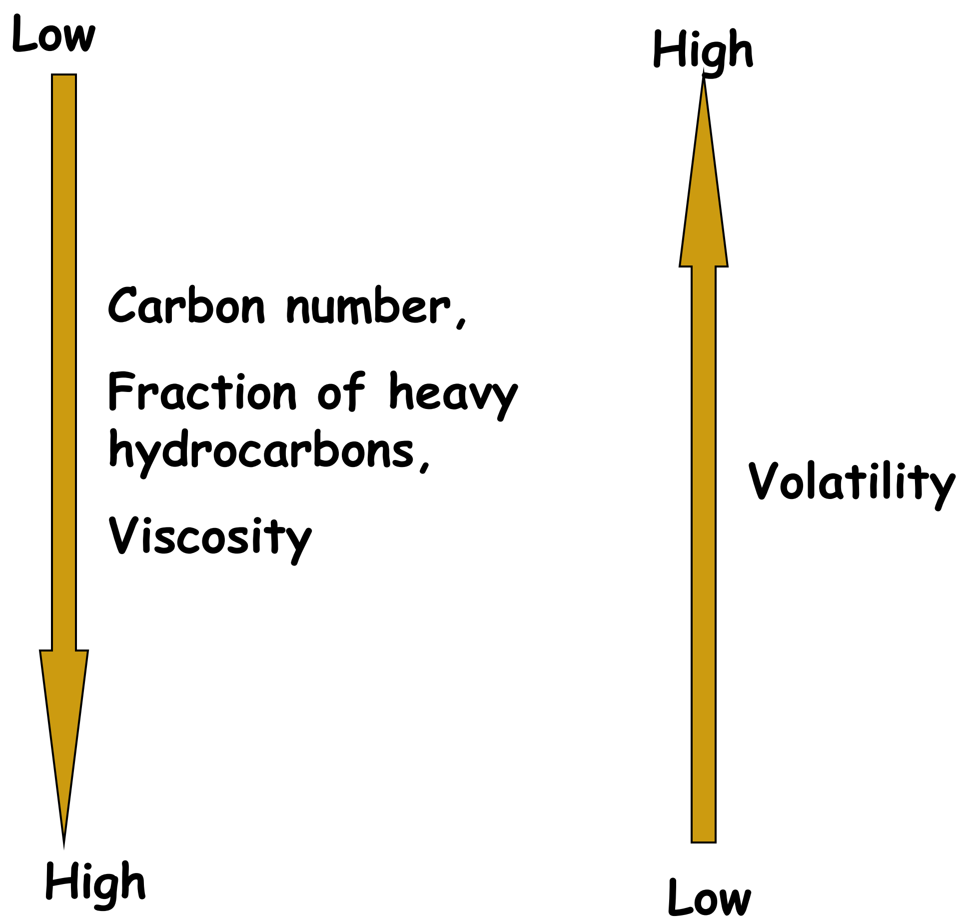
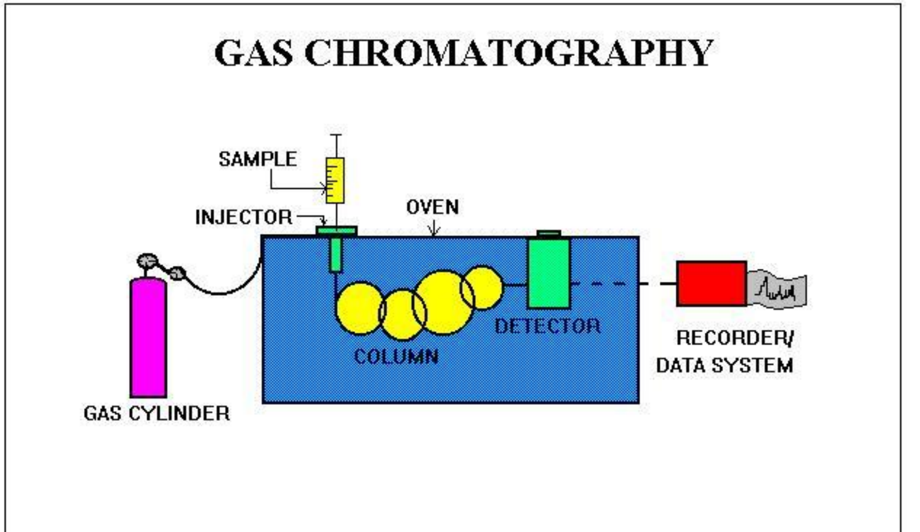


Table 11.3 Names and Formulas of the First Ten Straight-Chain Alkanes

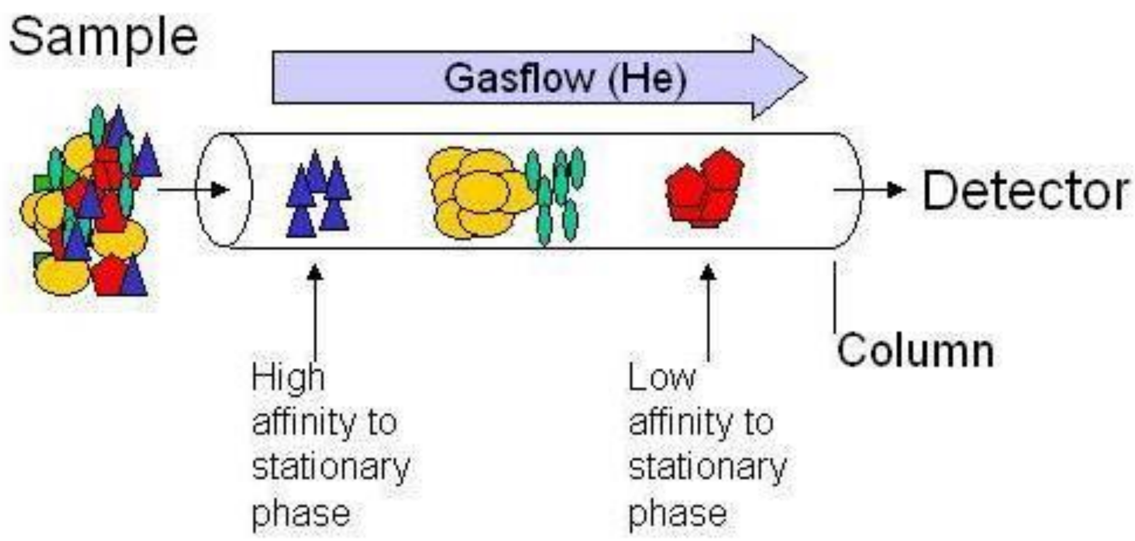
Name	Molecular Formula	Condensed Formula	Melting Point, °C	Boiling Point, °C
Alkanes	C_nH_{2n+2}			
Methane	CH_4	CH_4	−182.5	−160
Ethane	C_2H_6	CH_3CH_3	−183.6	−88.7
Propane	C_3H_8	$CH_3CH_2CH_3$	−187.6	−42.2
Butane	C_4H_{10}	$CH_3CH_2CH_2CH_3$ or $CH_3(CH_2)_2CH_3$	−139.0	−0.4
Pentane	C_5H_{12}	$CH_3CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_3CH_3$	−129.9	36.0
Hexane	C_6H_{14}	$CH_3CH_2CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_4CH_3$	−94.5	68.8
Heptane	C_7H_{16}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_5CH_3$	−90.6	98.4
Octane	C_8H_{18}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_6CH_3$	−56.9	125.6
Nonane	C_9H_{20}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_7CH_3$	−53.6	150.7
Decane	$C_{10}H_{22}$	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or $CH_3(CH_2)_8CH_3$	−29.7	174.0

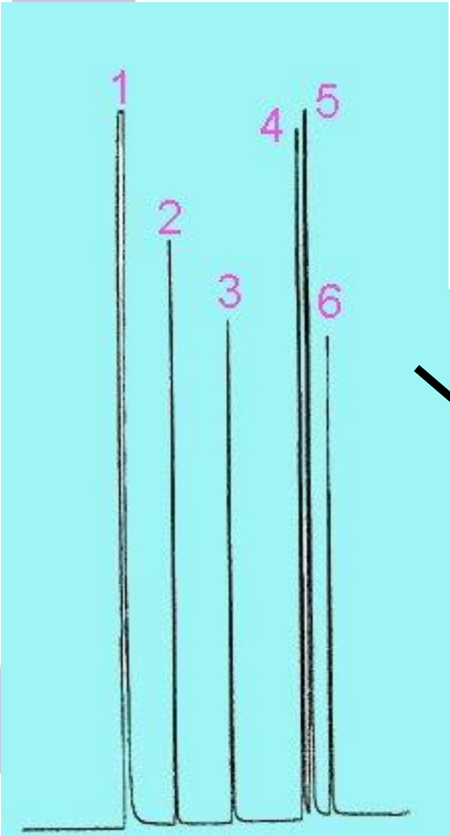
Gas chromatography
(Both a quantitative and qualitative tool)

Gas chromatographic methods (with FID), such as modifications of EPA Method 8015, are the most popular means of identification of petroleum type.



Gas chromatography: how it works

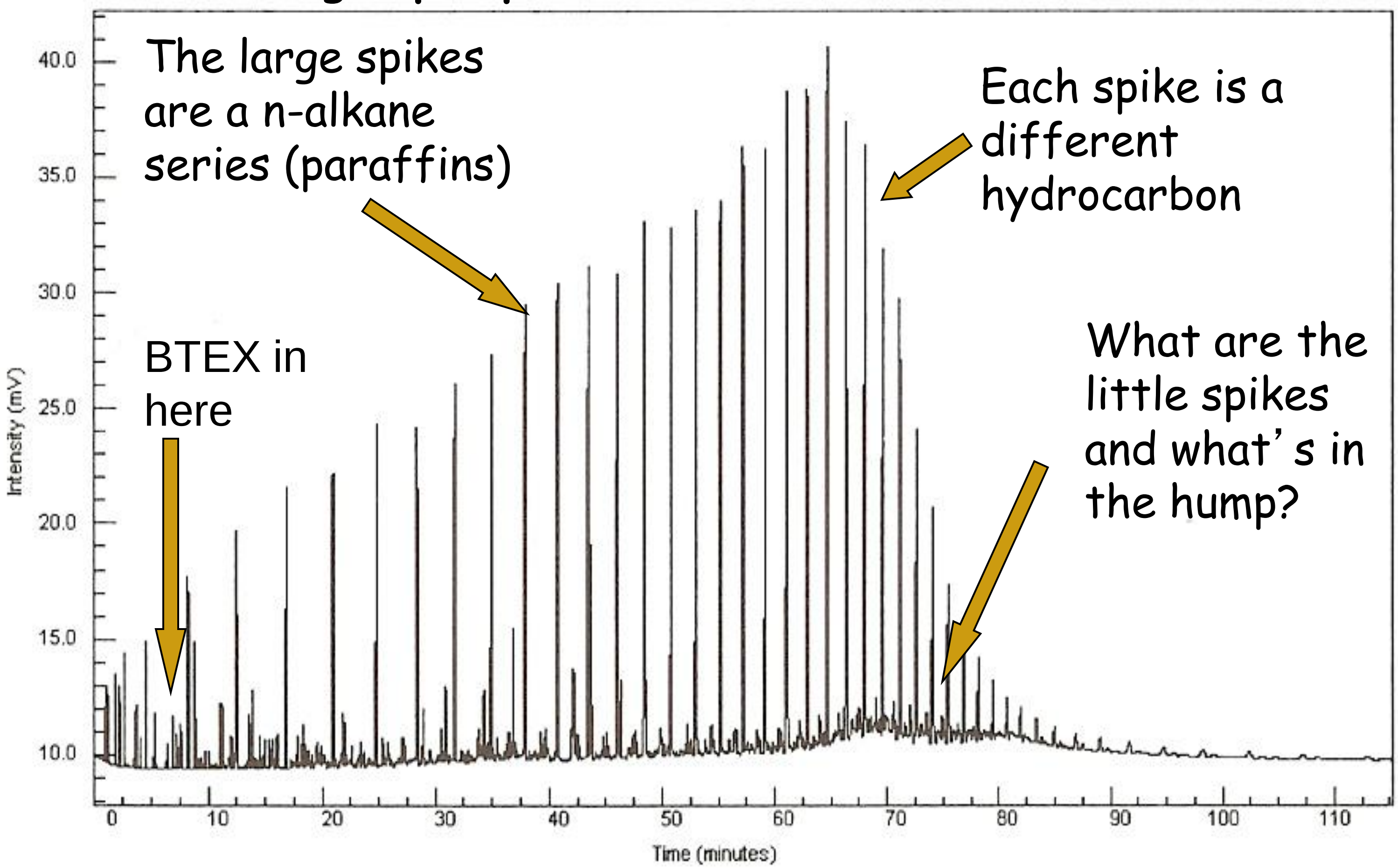




Peak #	Compound	Retention Time
1	Methanol	2.244 min
2	Benzene	3.260 min
3	Toluene	4.455 min
4	Ethyl Benzene	5.941 min
5	Para-xylene Meta-xylene	6.115 min
6	Ortho-xylene	6.524 min

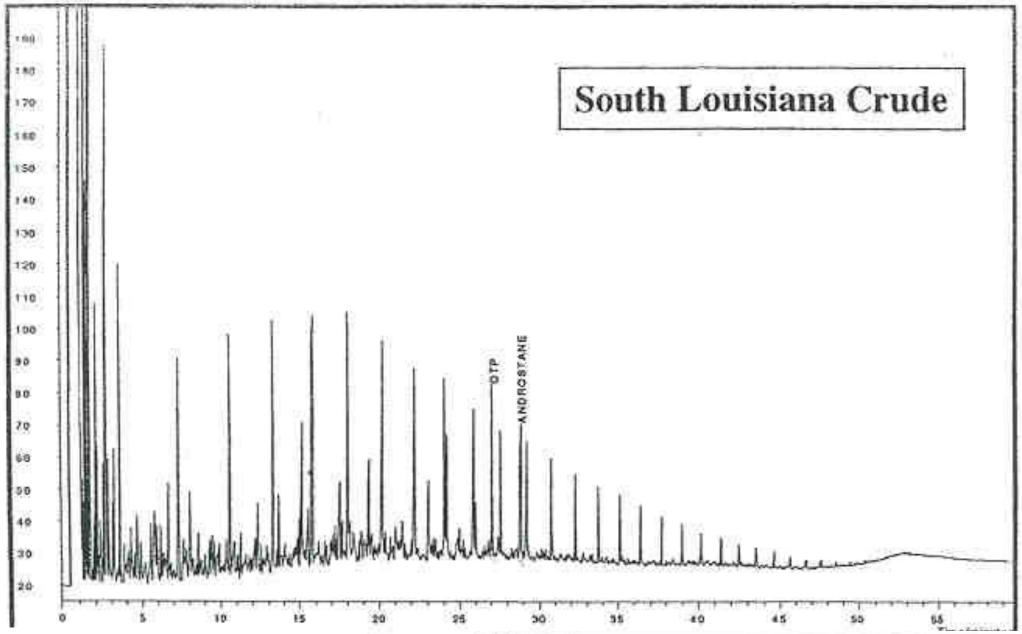
GC analysis of a crude oil

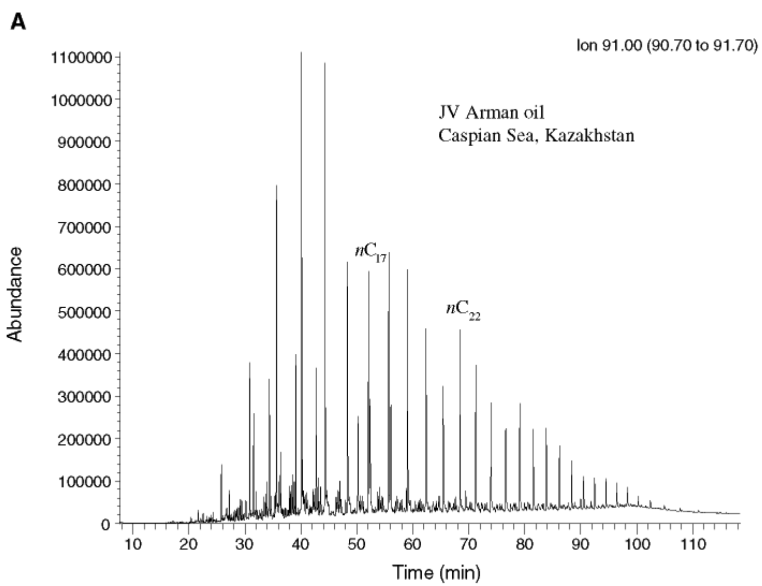
Increasing carbon number → up to ~C45
Increasing boiling point →
Decreasing vapor pressure →

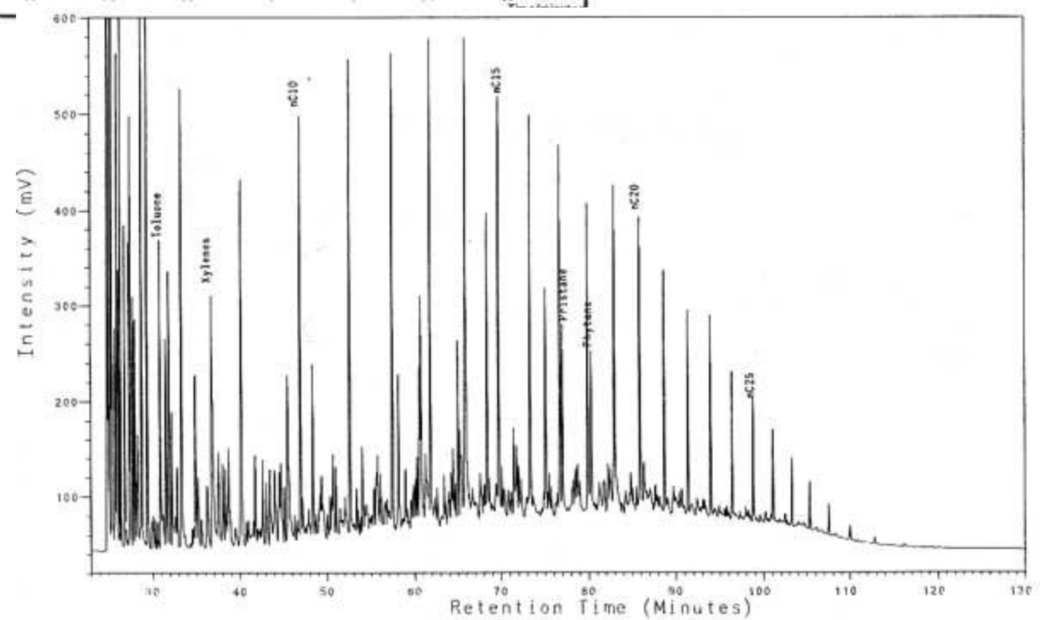


Crude oil contains C4 to C45+ hydrocarbons

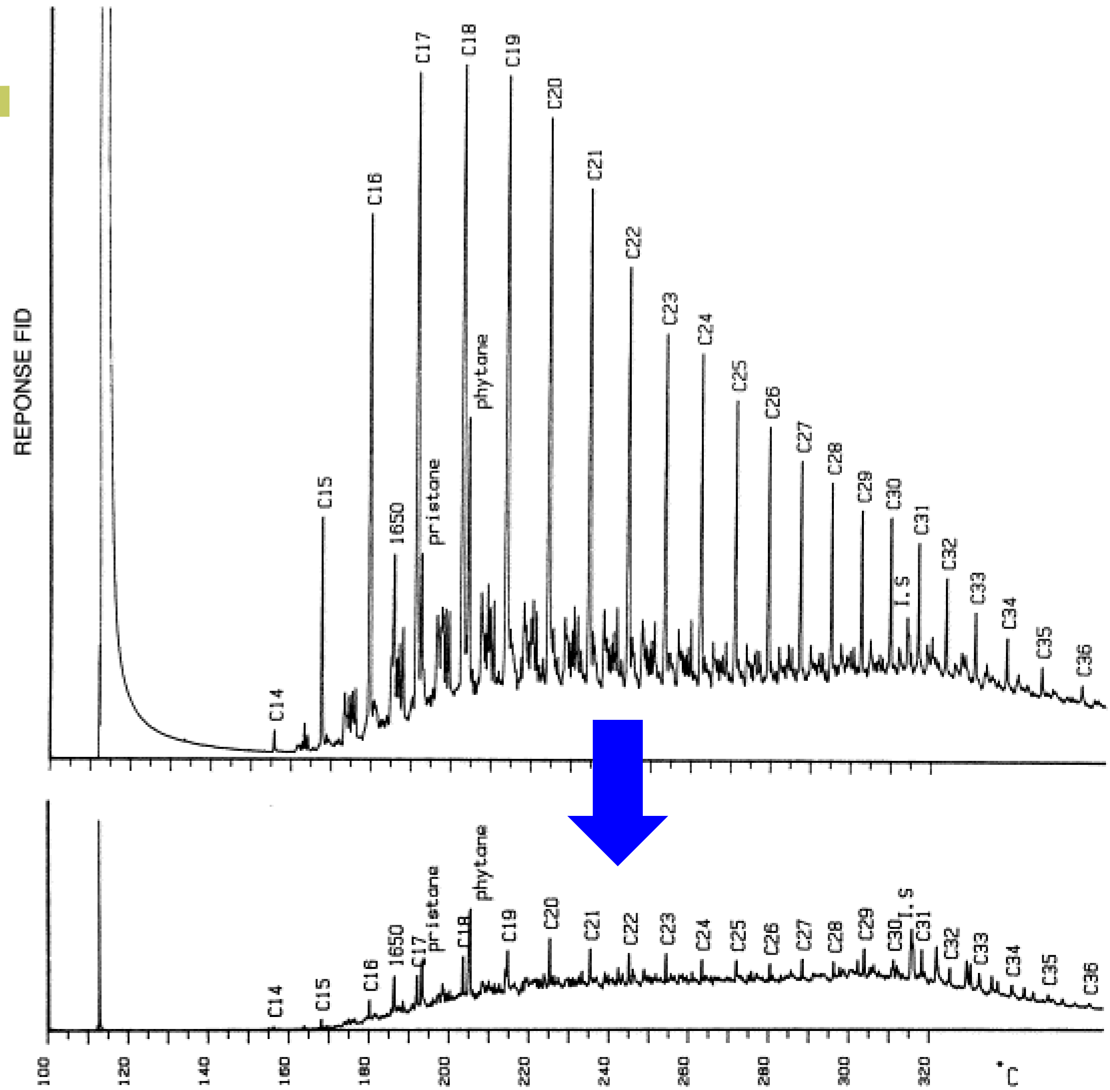
Other crude oils



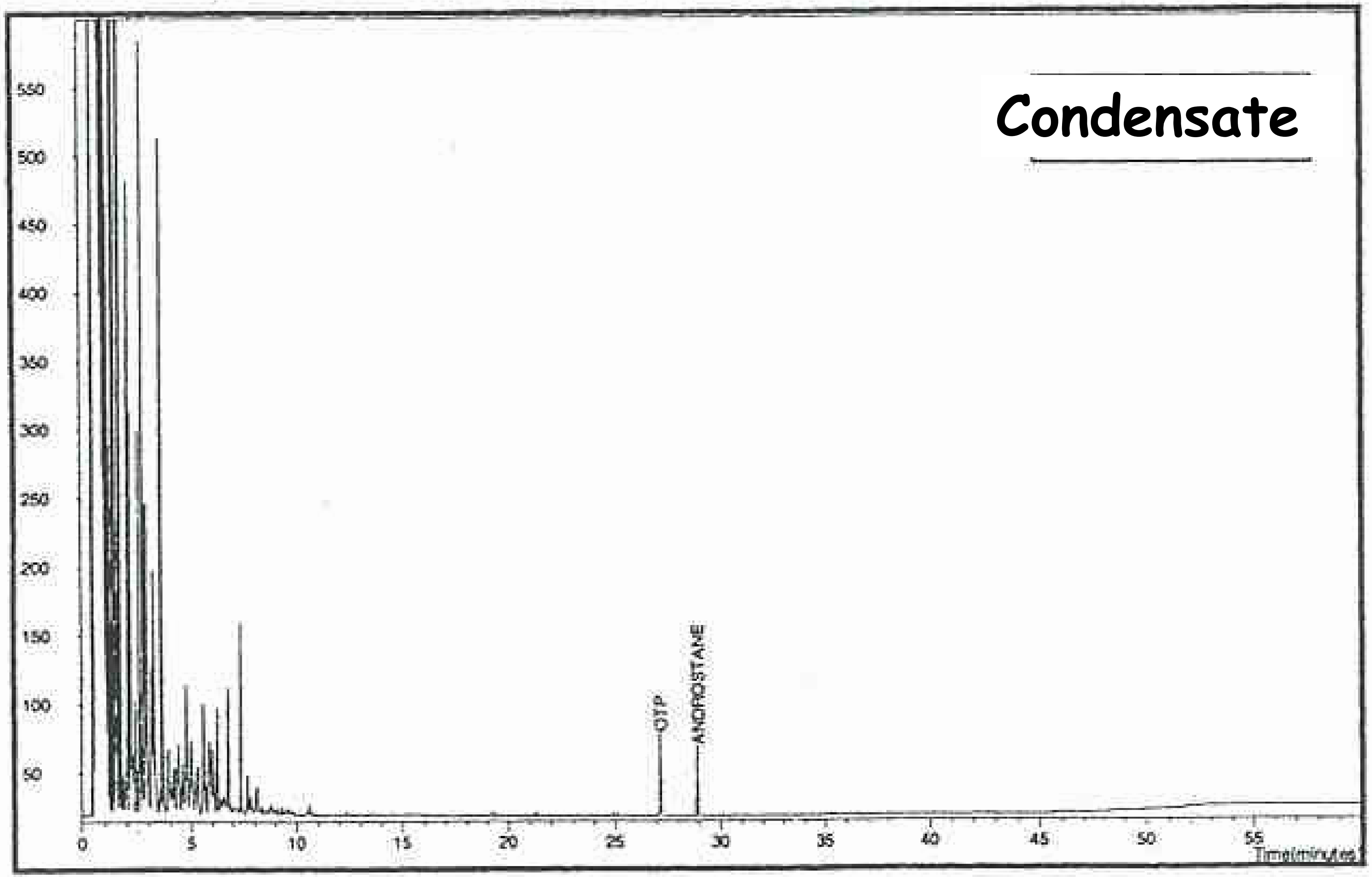




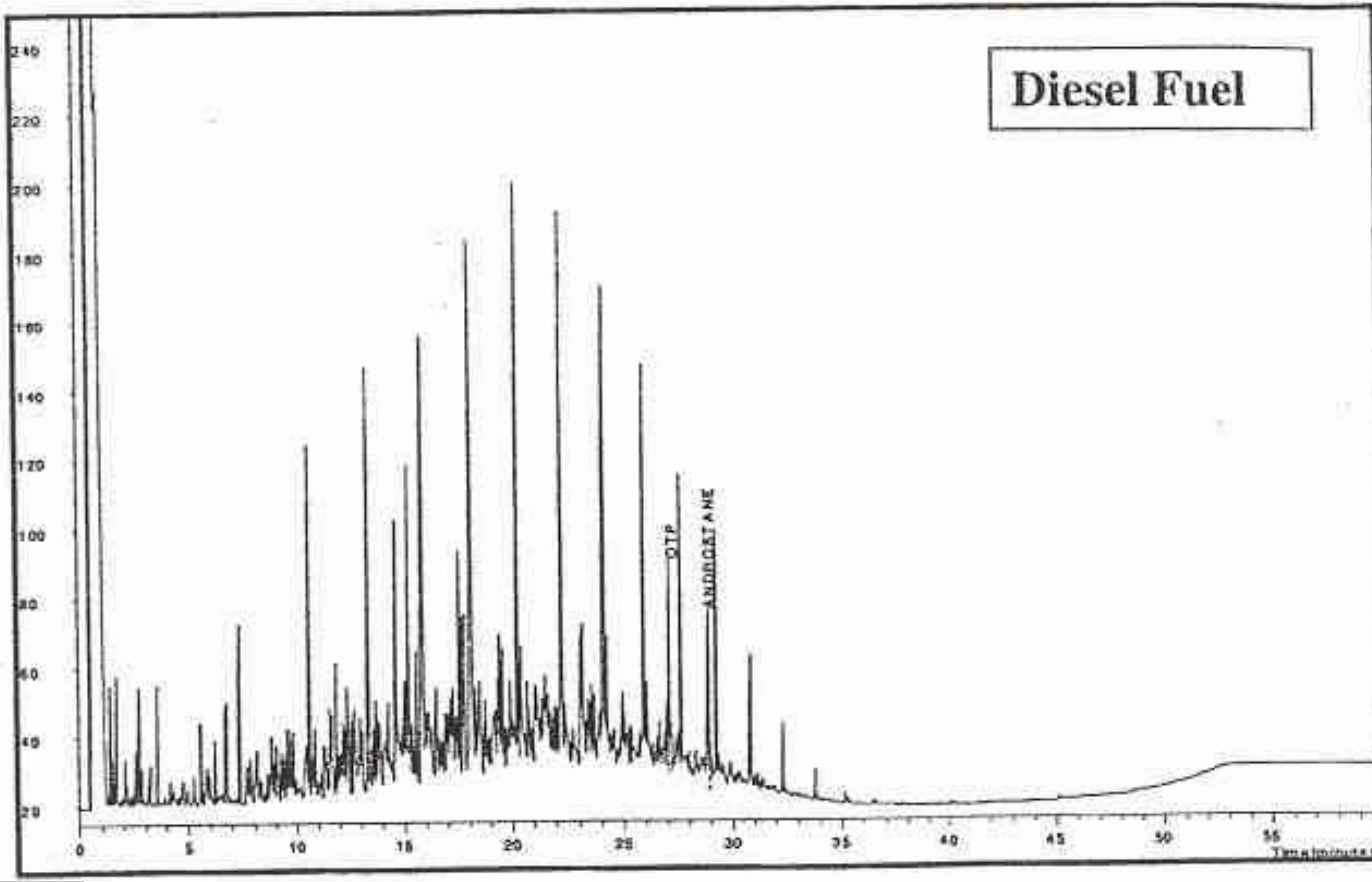
Partially biodegraded crude oil



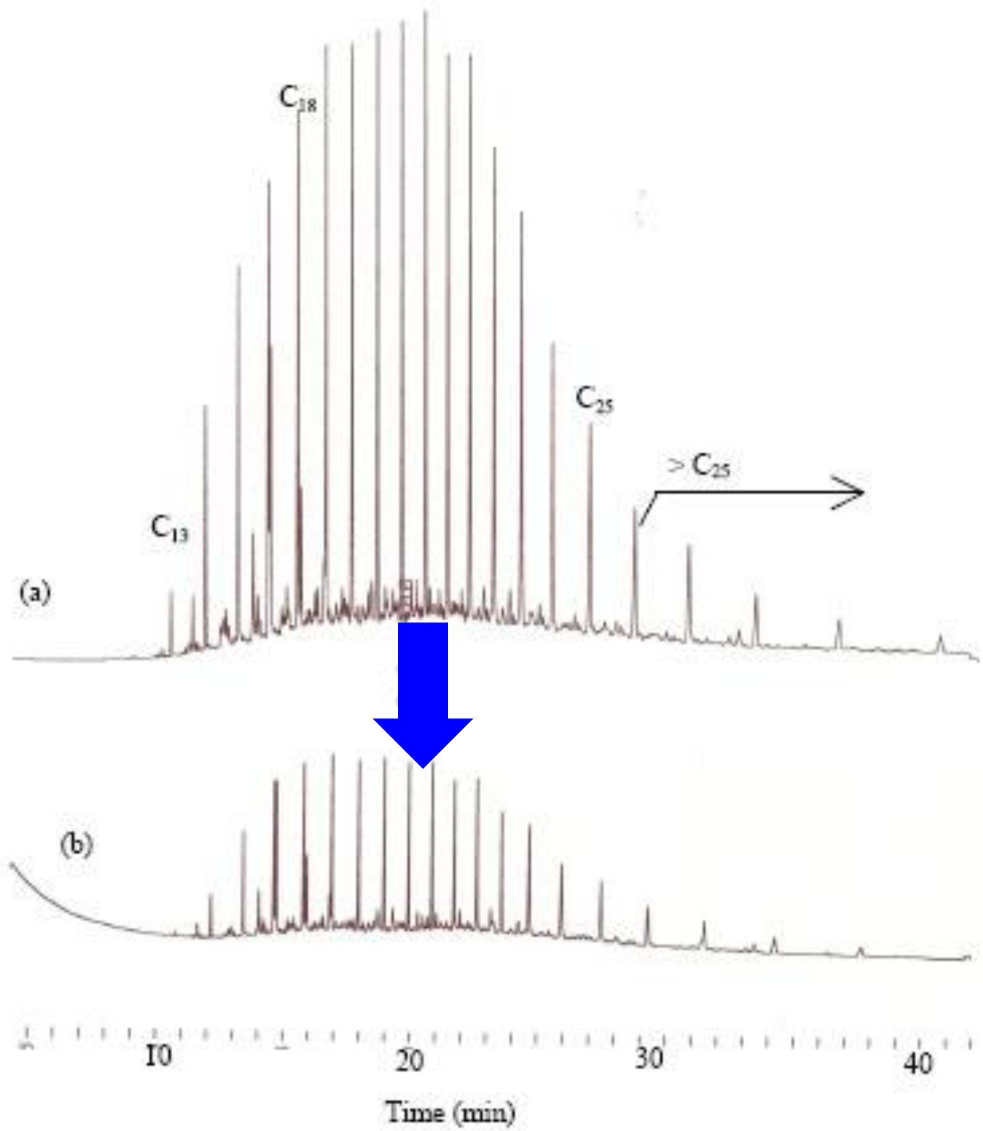
Condensate



550
500
450
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
40
45
50
55
OTP
ANDROSTANE
Time(minutes)

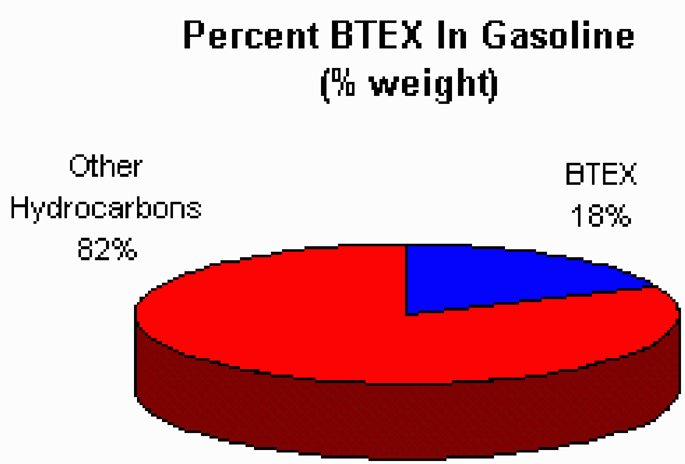

Diesel Fuel
OTP
ANDROSTANE
240
220
200
180
160
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
45
50
55
Time (minutes)

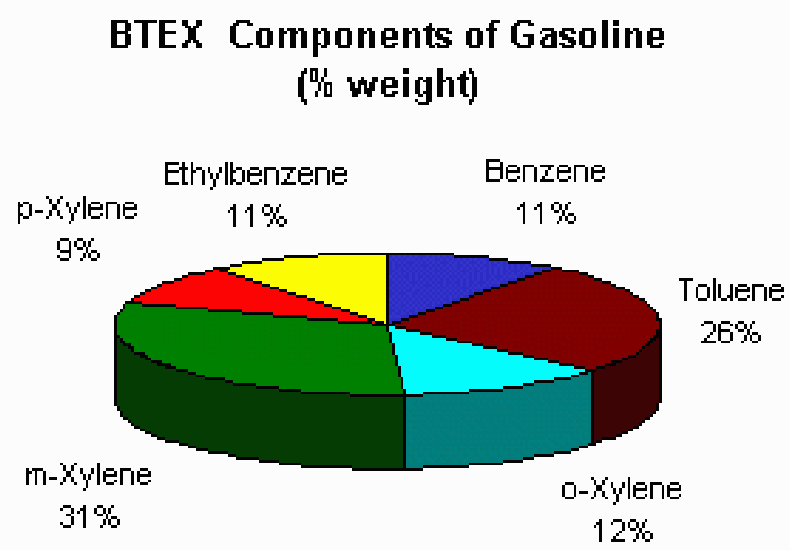
Partially biodegraded diesel



Benzene, toluene, ethylbenzene, xylenes (BTEX)

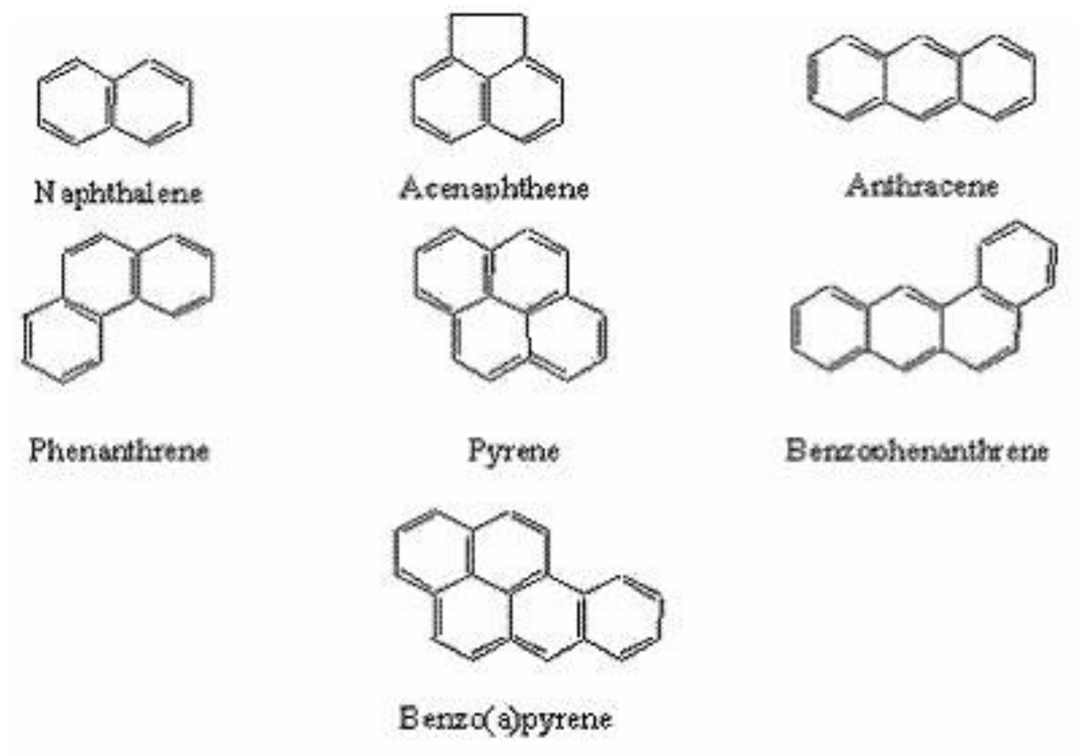
- BTEX components are more water soluble than all other components of crude oil
- BTEX components are toxic and benzene is carcinogenic





Polyaromatic hydrocarbons (PAHs)

Polyaromatic hydrocarbons are aromatic organic compounds composed of two or more benzene rings:



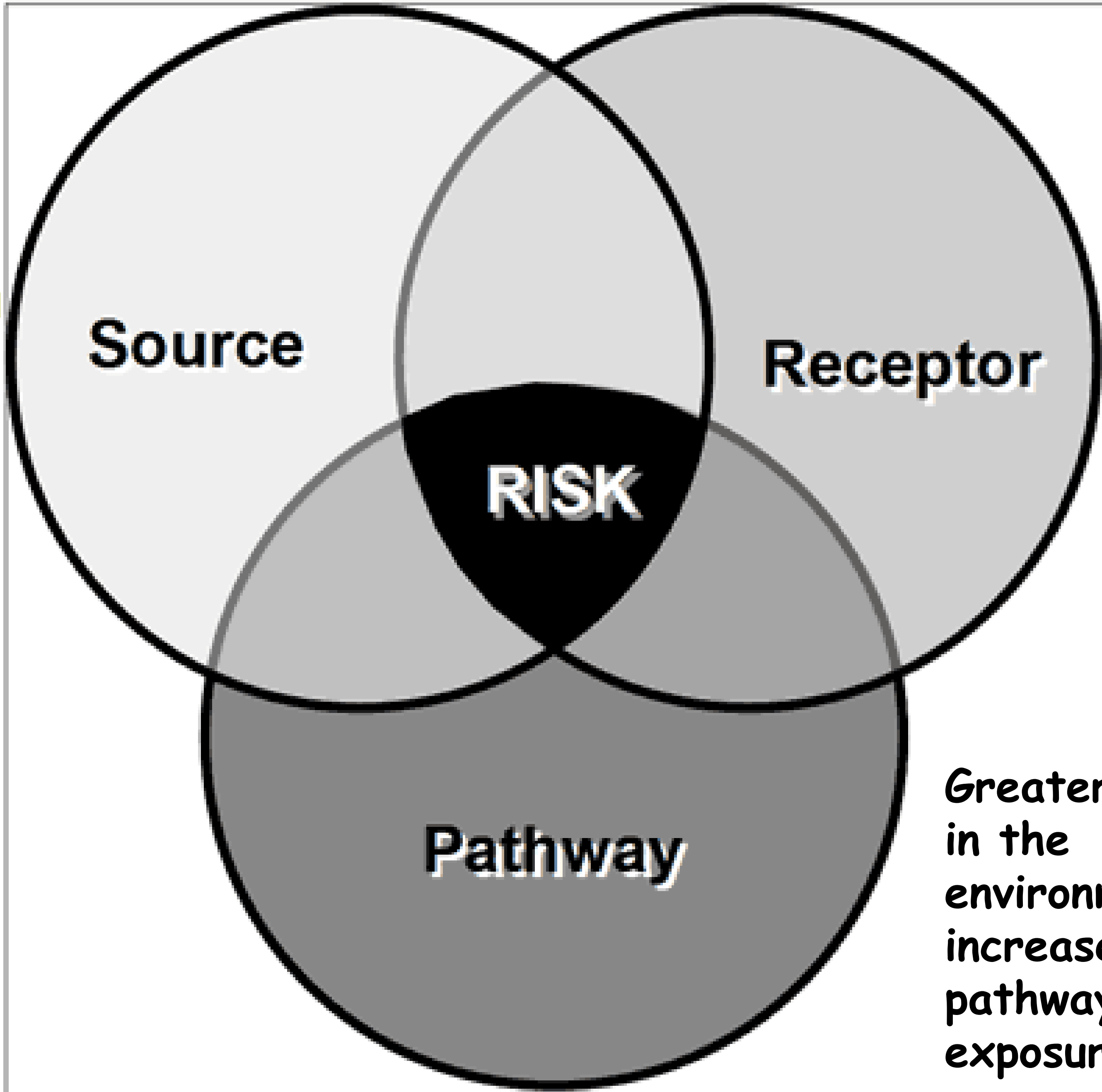
Polyaromatic hydrocarbons (PAHs)

- PAHs are common constituents of crude oils and coal
- Many of the larger-ring compounds are carcinogenic
- Common properties of PAHs
  - high viscosities
  - low aqueous solubilities
  - low vapor pressures
  - a strong affinity for adsorbing to soil matrices
  - specific gravities greater than water
- These unique physical and chemical characteristics result in the relative immobility of PAHs in the subsurface.

Table 2. PAH content of 60 crude oils. All data are reported in mg/kg oil.

PAH	Minimum	Maximum	Mean	Detection Frequency
Naphthalene	1.2	3700	422.9	60
Acenaphthene	nd	58	13.9	48
Anthracene	nd	17	3.4	24
Phenanthrene	nd	916	176.7	59
Fluorene	1.4	380	73.6	60
Benz[a]anthracene	nd	38	5.5	40
Fluoranthene	nd	26	3.9	24
Chrysene	4	120	28.5	60
Pyrene	nd	82	15.5	58
Dibenz[a,h]anthracene	nd	9.2	1.0	28
Benzo[a]pyrene	nd	7.7	2.0	45
Benzo[b]fluoranthene	nd	14	3.9	60
Benzo[k]fluoranthene	nd	7	0.46	56
Indeno[1,2,3-cd]pyrene	nd	1.7	0.06	4
Benzo[ghi]perylene	nd	9.6	1.53	38

PAHs in bold font have been shown to be carcinogenic in laboratory animals. (Ref. 9)
nd = below detection limits.



Greater mobility in the environment increases pathways for exposure and risk

Bioremediation, it's all about microbes eating hydrocarbons

- Hydrocarbons have been present in nature for millions of years
- Microorganisms which use hydrocarbons for food are widely distributed in nature in: soil, seawater, surface water, groundwater

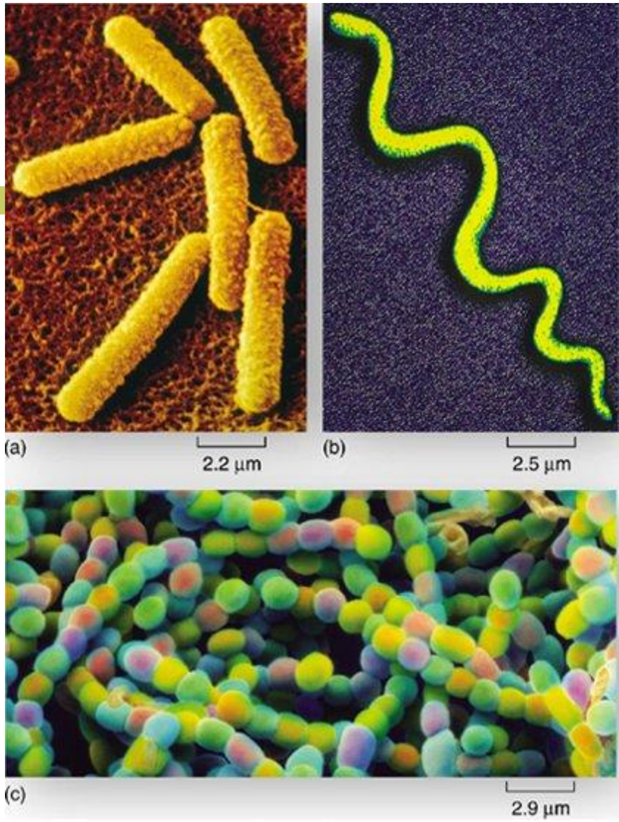
Microorganisms + hydrocarbons →

more microorganisms +

carbon dioxide (CO_2) +

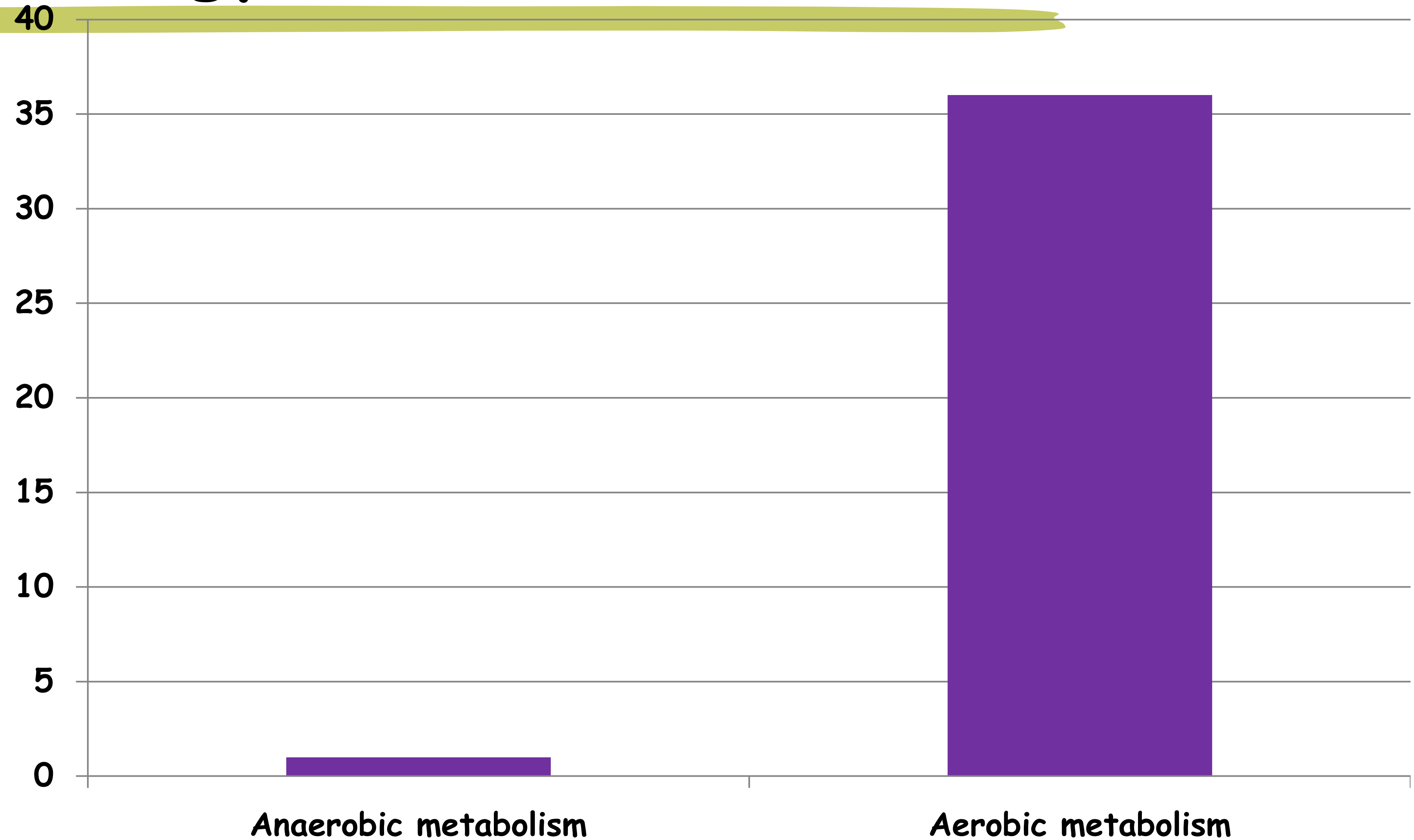
water (H_2O)

What kinds of microorganisms eat hydrocarbons?



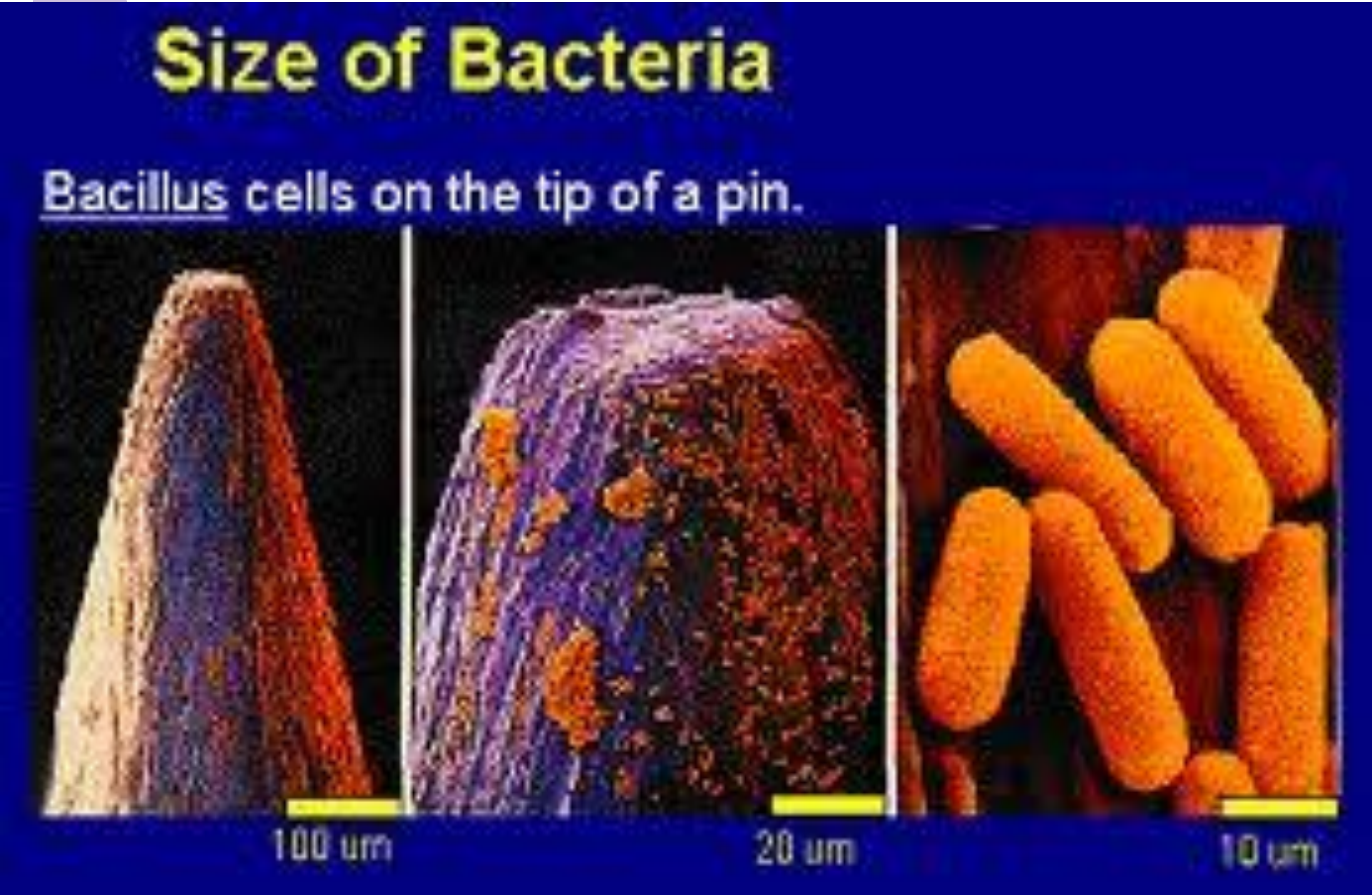
- Mostly bacteria
  - Bacteria
    - Found everywhere
    - Growth by cell division
    - Some are motile
    - 1 – 10 microns in size
      (a micron is 1 millionth of a meter)
    - Some are
      - aerobic – oxygen required
      - obligate anaerobic – oxygen toxic
      - facultative – can survive and grow in presence or absence of oxygen

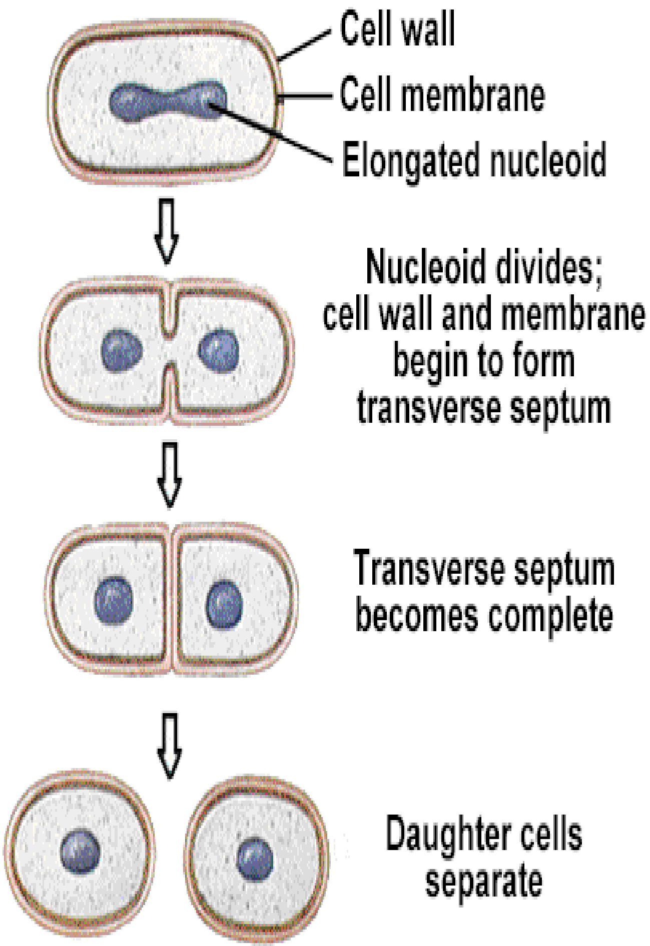
Relative energy production in anaerobic and aerobic metabolism per unit of energy source

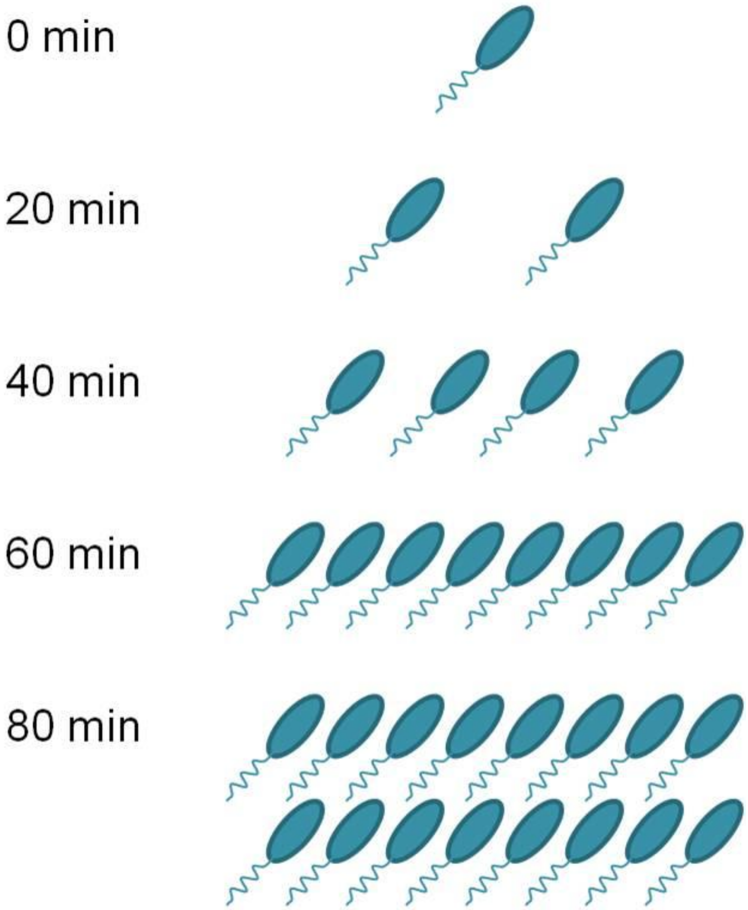


What kinds of microorganisms eat hydrocarbons?

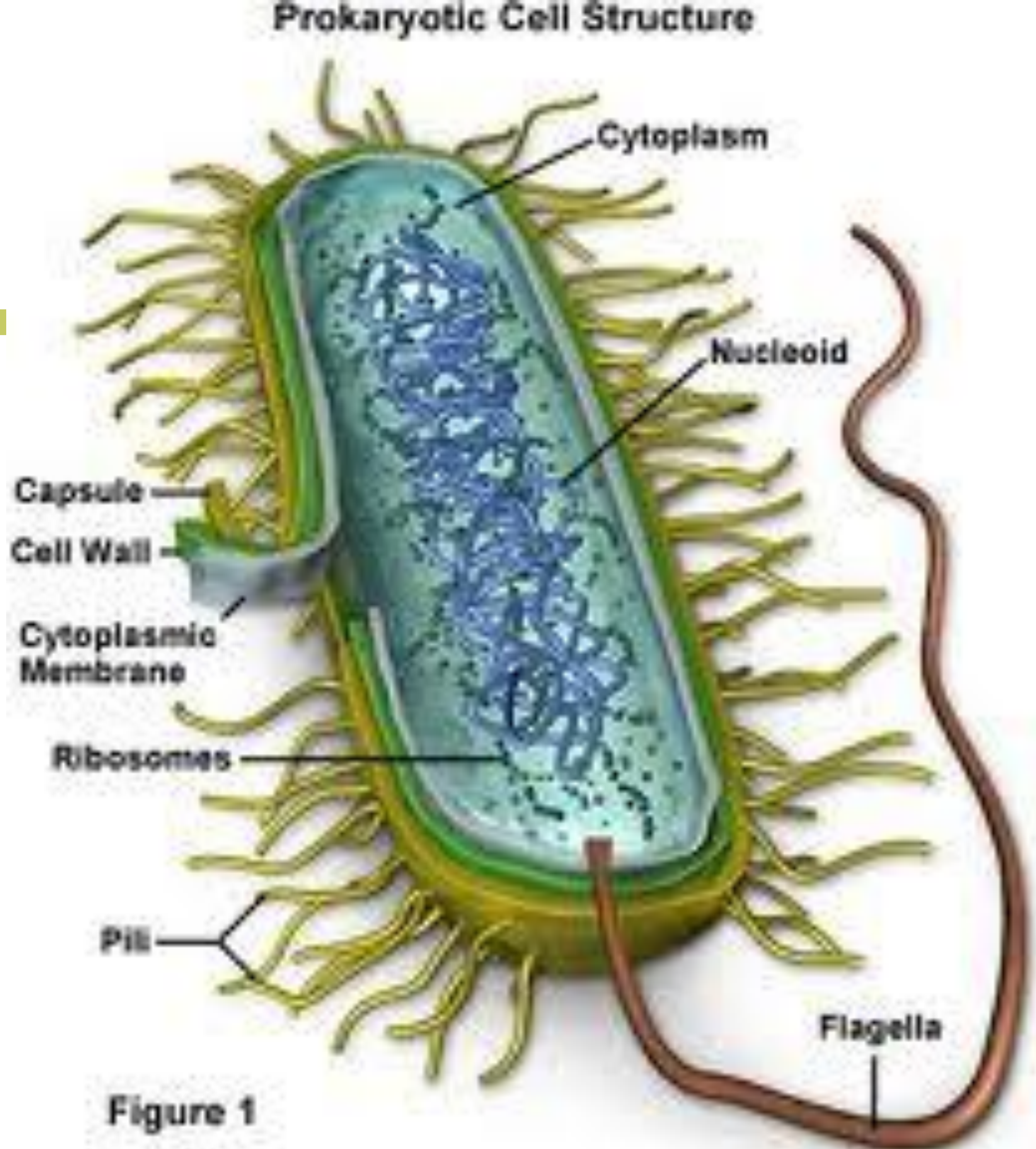
- Aerobic bacteria are the most the most efficient hydrocarbon degraders when treating surface contamination!!


Size of Bacteria
Bacillus cells on the tip of a pin.
100 um
20 um
10 um





10 hours growth = > 1 Billion cells!


Prokaryotic Cell Structure
Cytoplasm
Nucleoid
Capsule
Cell Wall
Cytoplasmic Membrane
Ribosomes
Pili
Flagella
Figure 1

What about fungi?

Why do the microbes eat the hydrocarbons?

*Food for **growth** and reproduction, in other words to make more bugs!*

Growth takes material and energy – both are supplied by the hydrocarbons

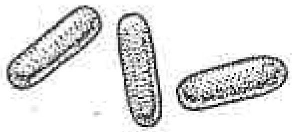
No growth, no hydrocarbon degradation!

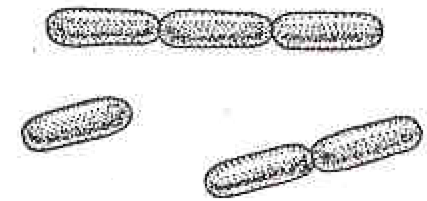
No growth, no hydrocarbon degradation!

So the key to getting the most out of bioremediation is optimizing the *growth* of the microbes

- Getting the microbes together with the hydrocarbon
- Making sure the microbes have enough of the right nutrients
- Getting oxygen to the microbes
- Optimizing environmental conditions (to the extent we can)
- Moisture! Moisture! Moisture!

When you do all of this you are doing bioremediation!





+ hydrocarbons
+ nutrients
+ water
+ air + warm temperatures

+ carbon dioxide and water

+ nutrients
+ water
+ air + warm temperatures

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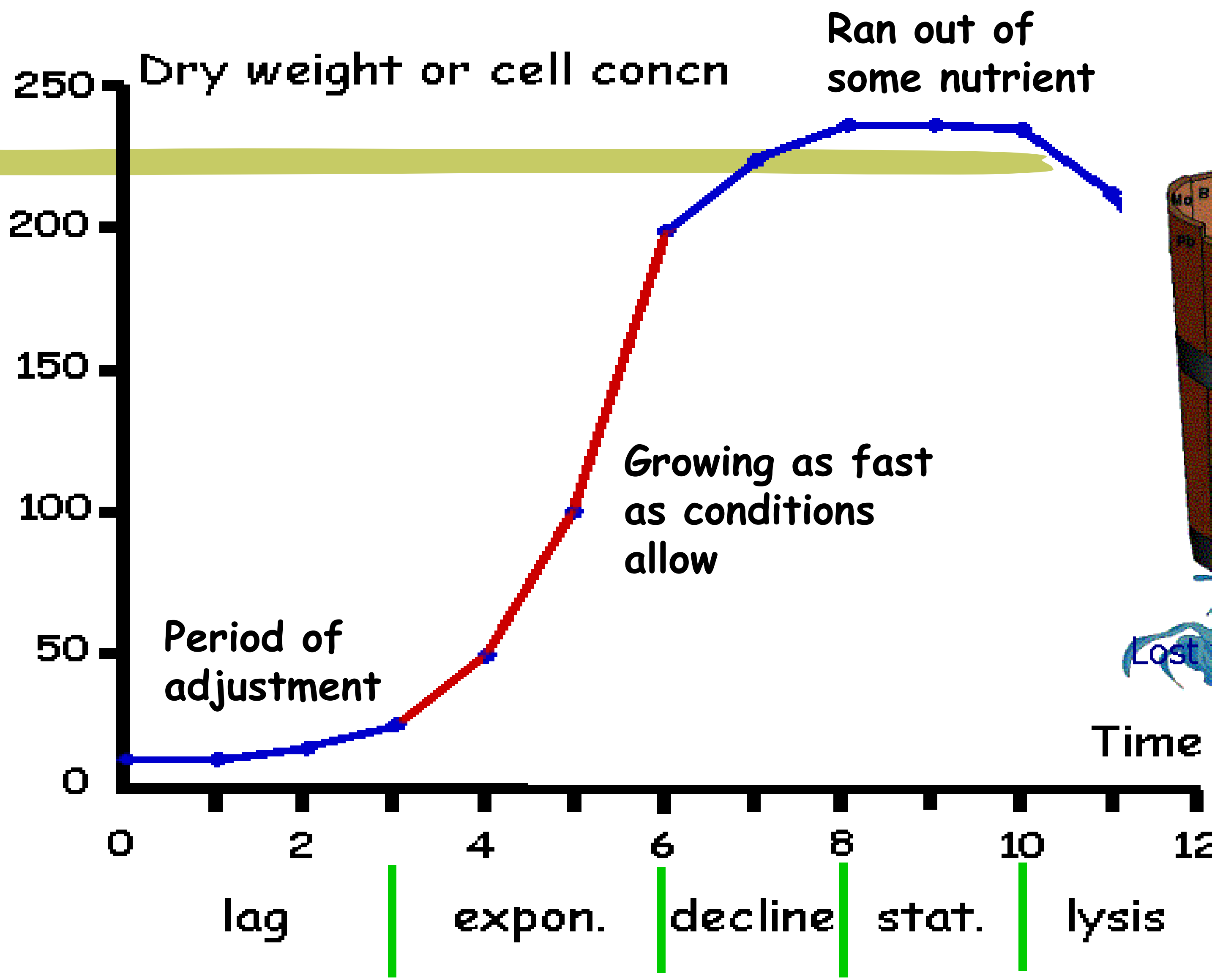
Elemental composition of a microorganism

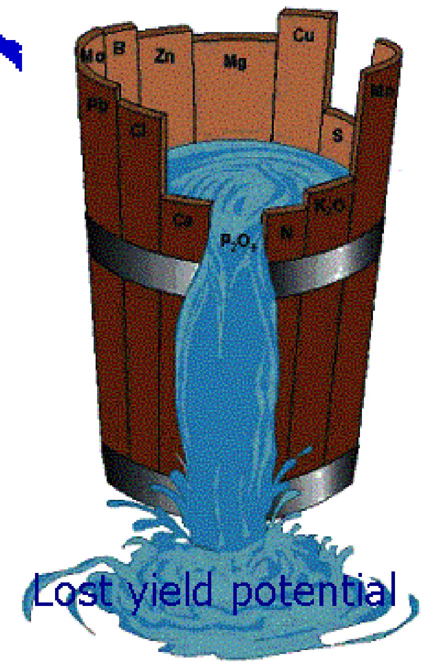
Element	% of dry weight
Carbon	50
Oxygen	20
Nitrogen (N)	**14**
Hydrogen	8
Phosphorus (P)	**3**
Sulfur	1
Potassium	1
Magnesium	0.5
Calcium	0.5
Iron	0.5
Misc Inorganic	1.5

Notice that there is a big requirement for N and P!

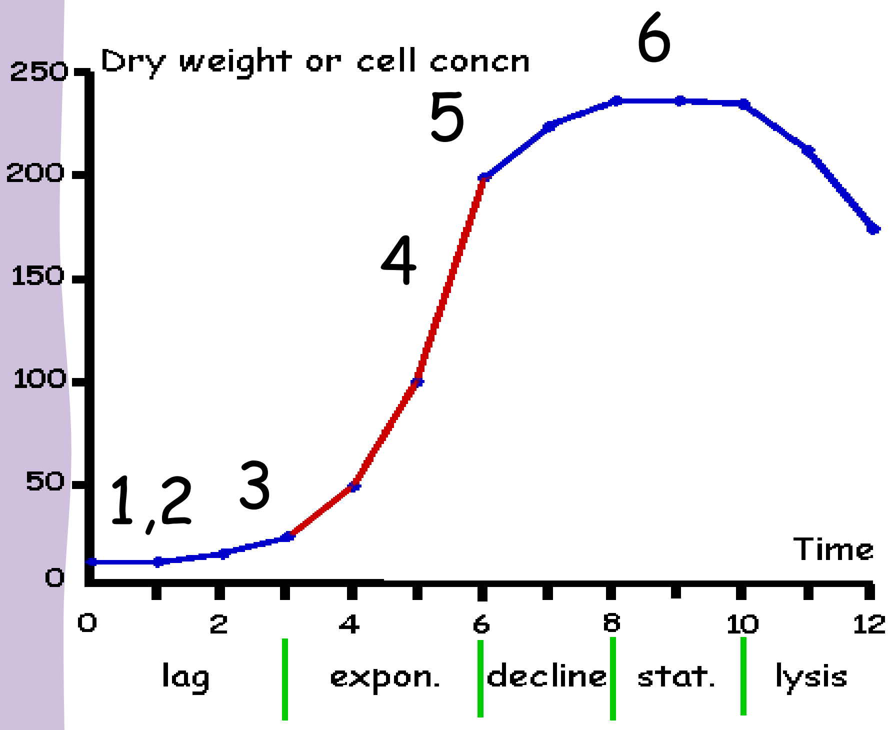
You are what you eat, so bugs that eat hydrocarbons must also eat lots of N and P in order to grow

Batch growth of microorganisms



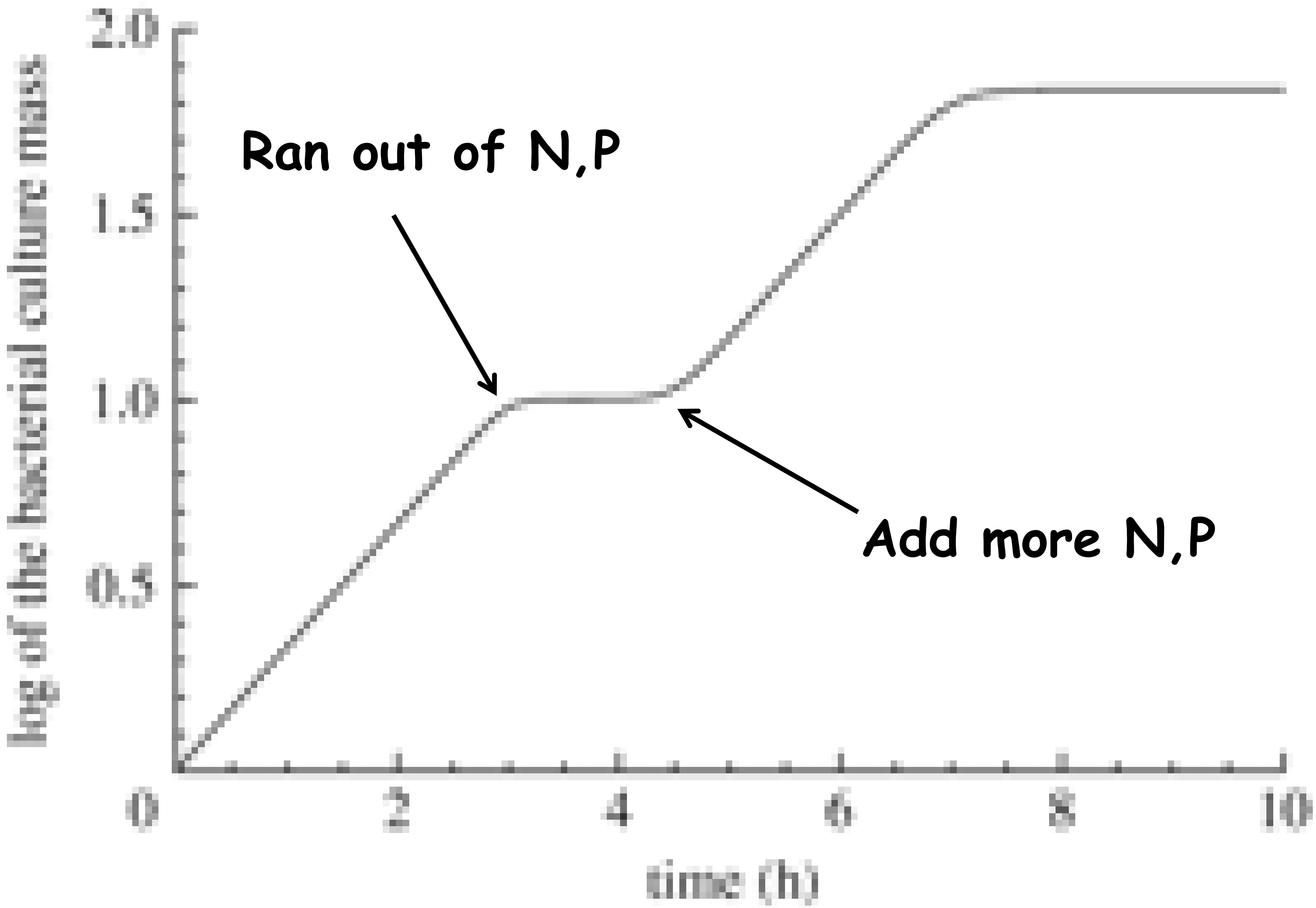


Let's relate this specifically to the bioremediation of a crude oil spill



1. The spill takes place
2. At this point we take care of the nutritional needs of the bugs, make sure the bugs come into contact with the hydrocarbon, supply air and moisture
3. Hydrocarbon degrading bacteria begin to grow
4. Bacteria are growing as fast as environmental and nutritional conditions permit
5. Growth slows significantly because bugs are running out of something – hopefully hydrocarbons
6. If nutritional limitations are not relieved hydrocarbon degraders begin to die off

Replenishing a limiting nutrient



The key to getting the most out of bioremediation is optimizing the *growth* of the microbes

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- Moisture! Moisture! Moisture!

Factors affecting the growth rates of microorganisms - Oxygen

T (°C)	Solubility of oxygen (mg/L) from air
0	14.6
20	9.1
25	8.2
40	6.4
50	5.5

The rate of transfer of a gas into water is proportional to the solubility of that gas in water

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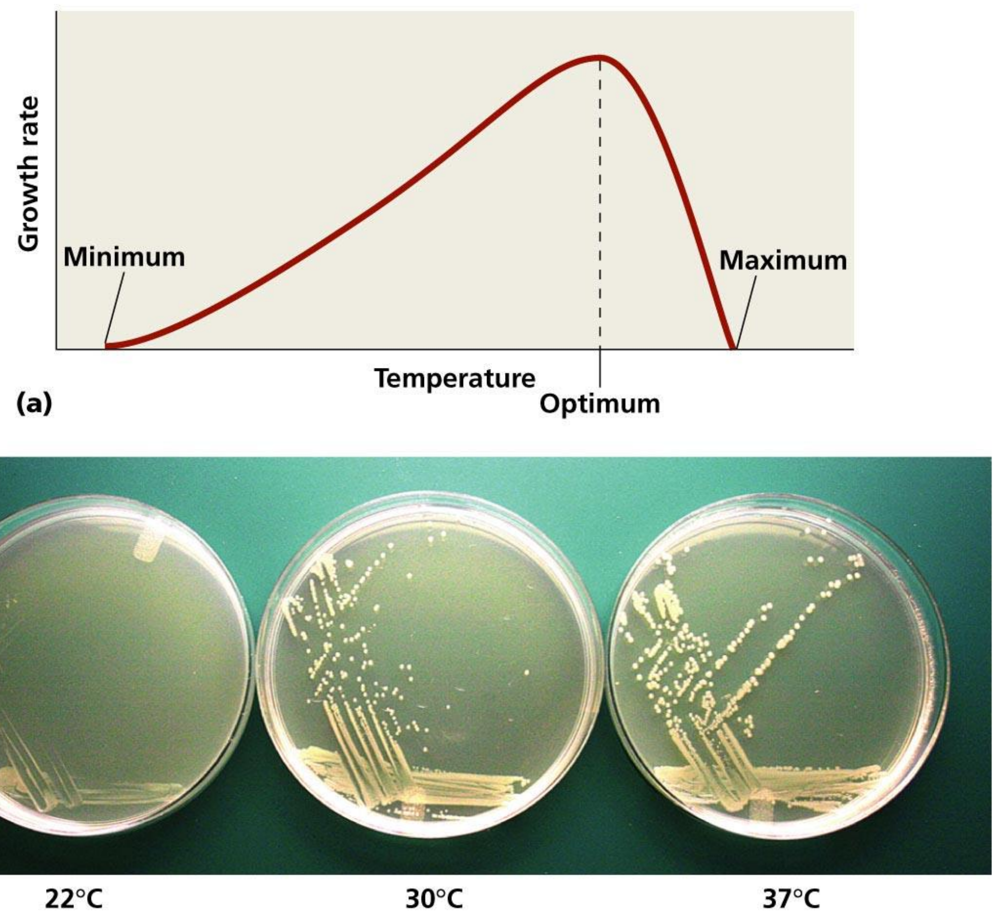
Factors affecting the growth rates of microorganisms Temperature

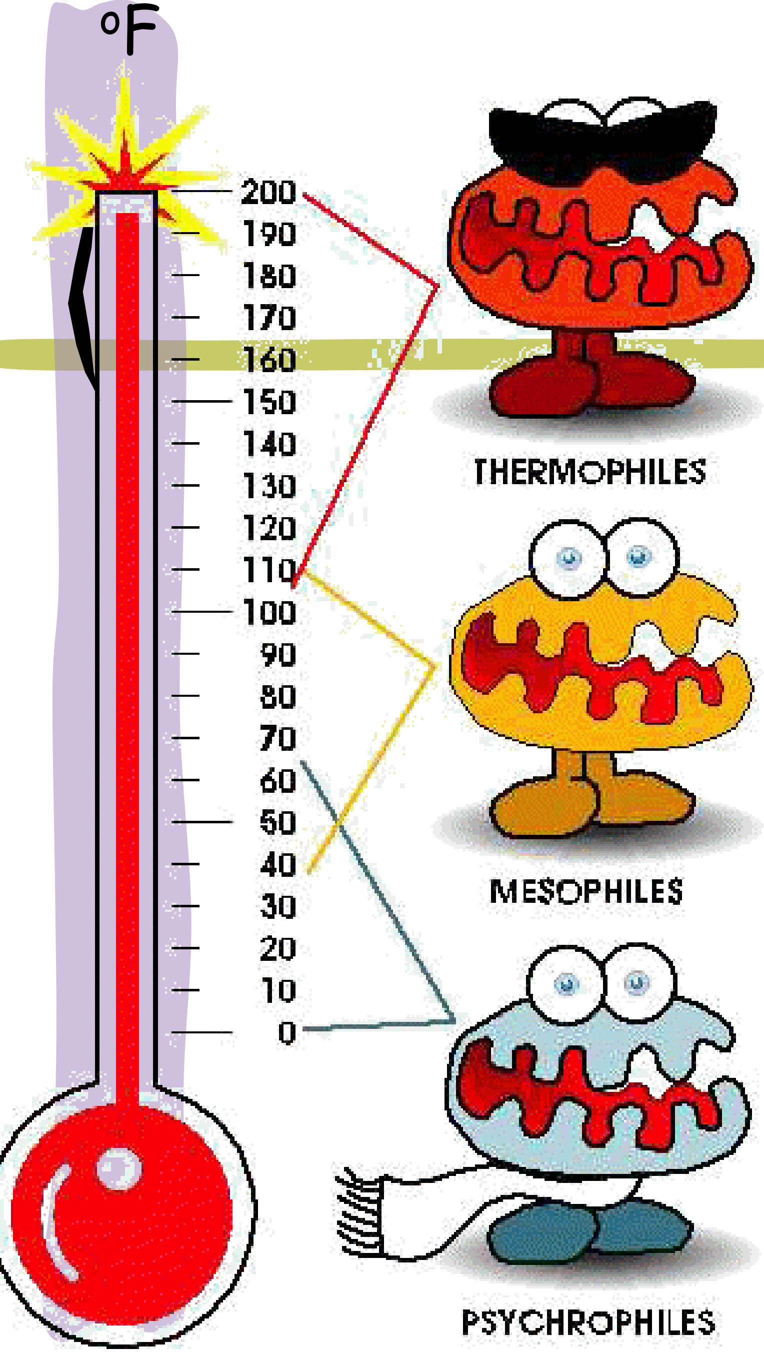


Enzymes (catalytic proteins) like many biological polymers can be very sensitive to temperature

Heat

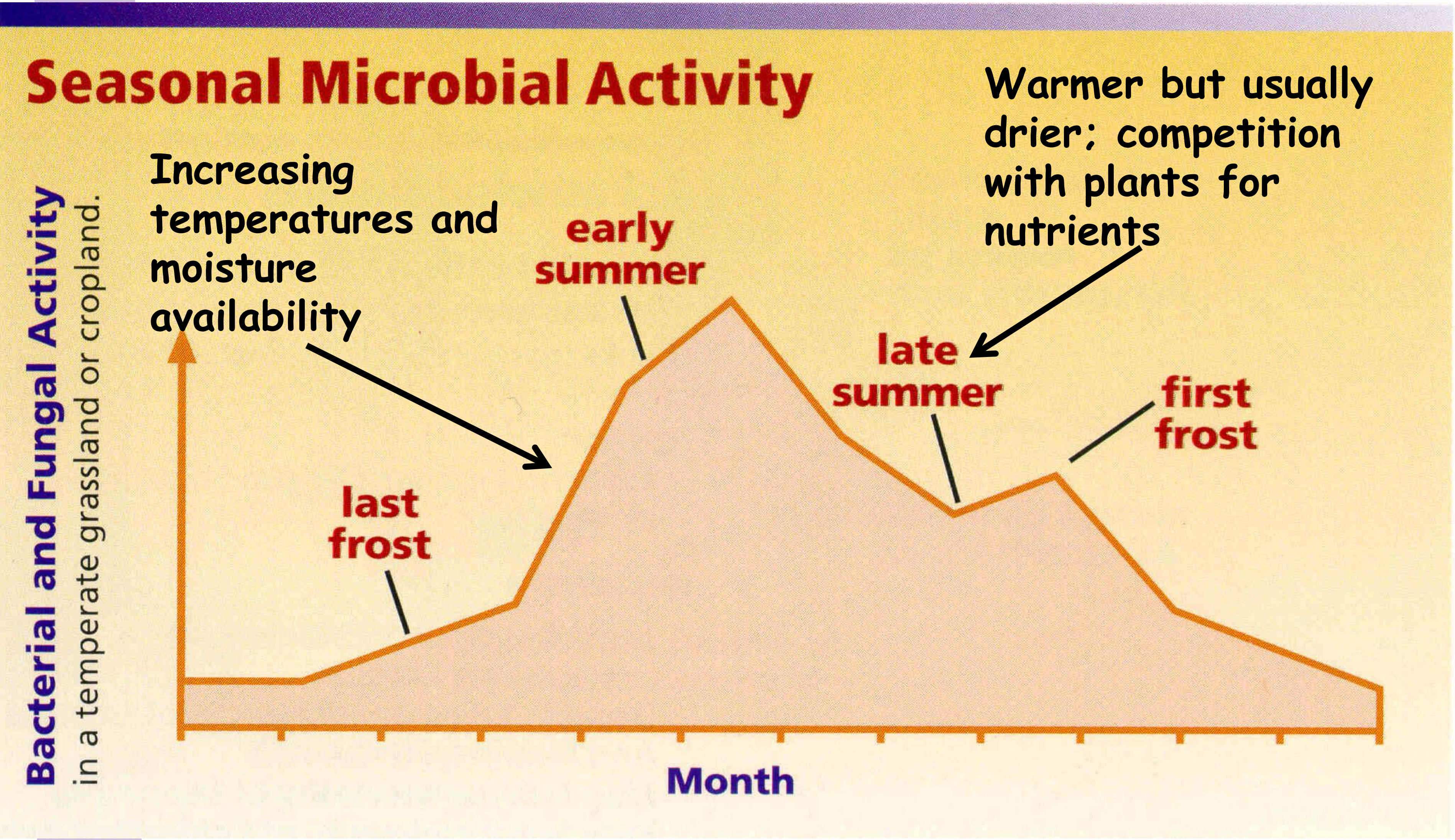
Functional

Not functional



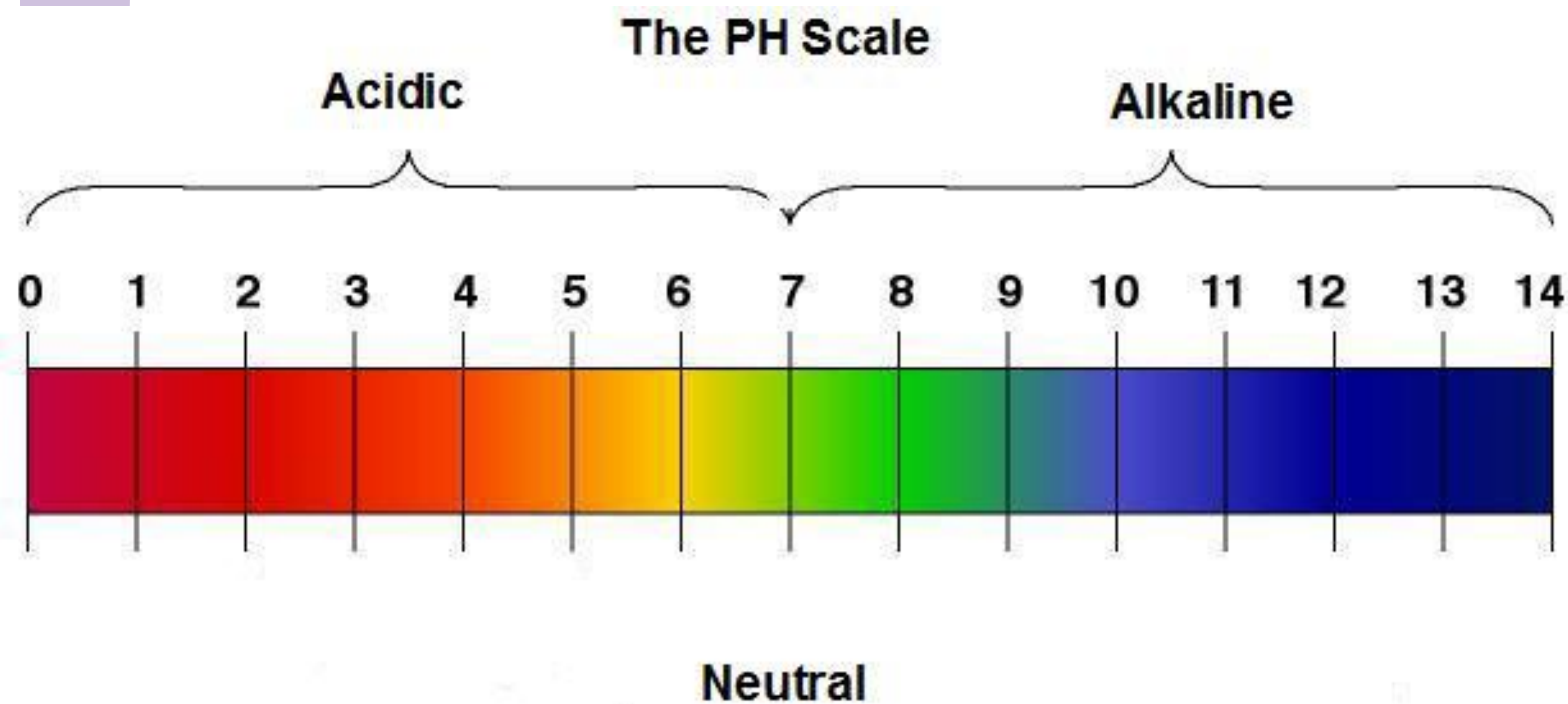
Fastest growers

Slowest growers

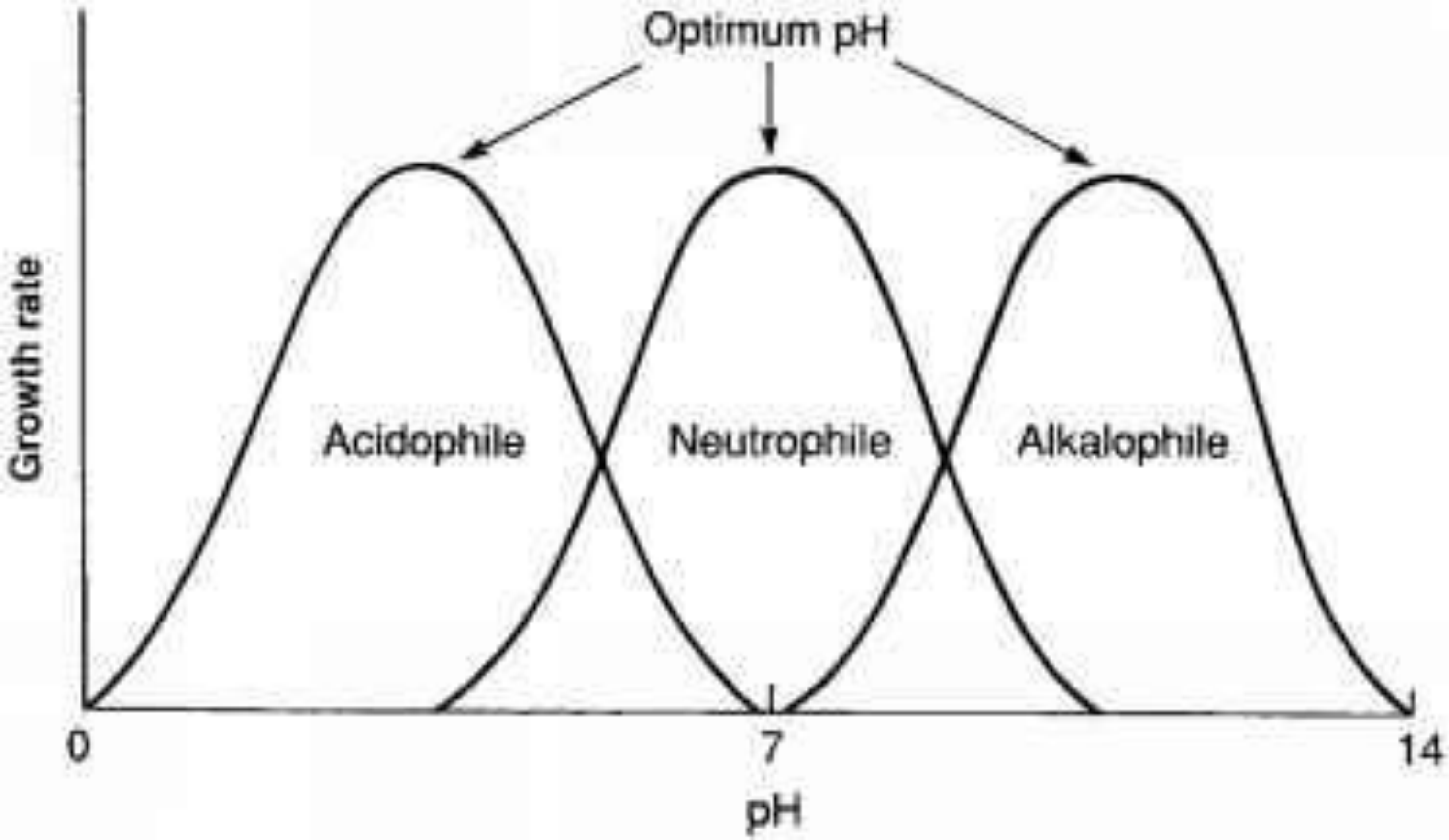


Seasonal Microbial Activity
Warmer but usually drier; competition with plants for nutrients
Increasing temperatures and moisture availability
early summer
late summer
first frost
last frost
Bacterial and Fungal Activity
in a temperate grassland or cropland.
Month

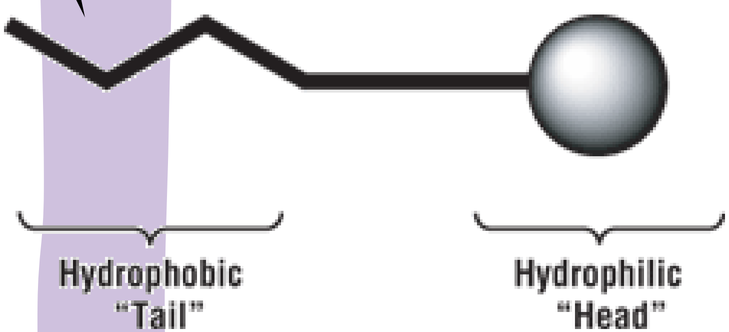
The pH scale

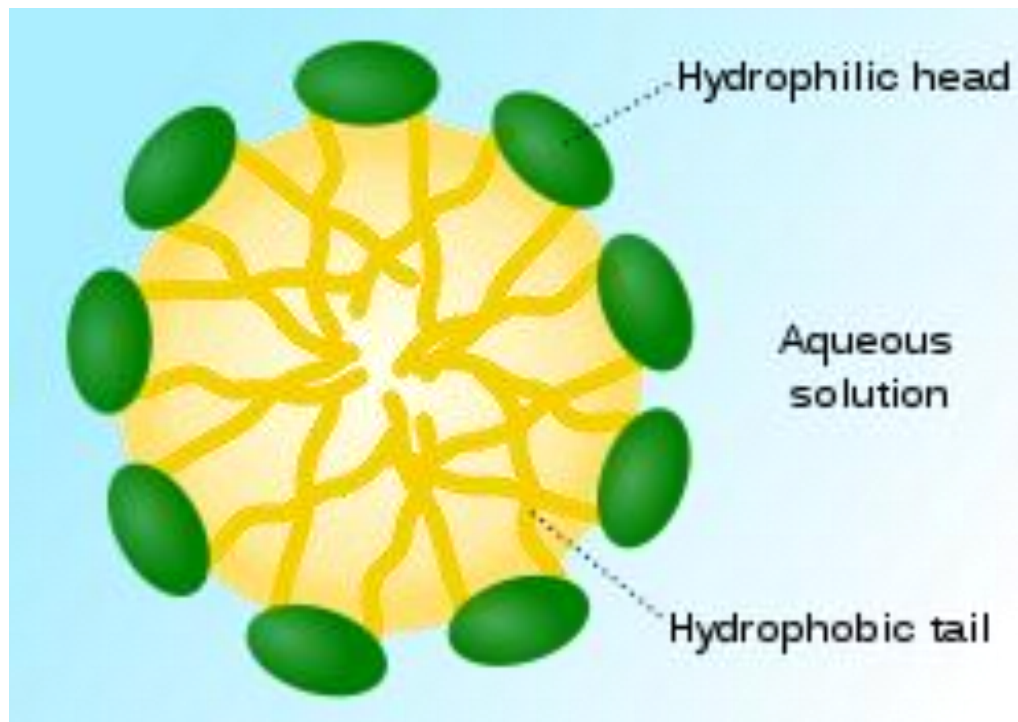

The PH Scale
Acidic
Alkaline
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Neutral

Factors affecting the growth rates of microorganisms - pH

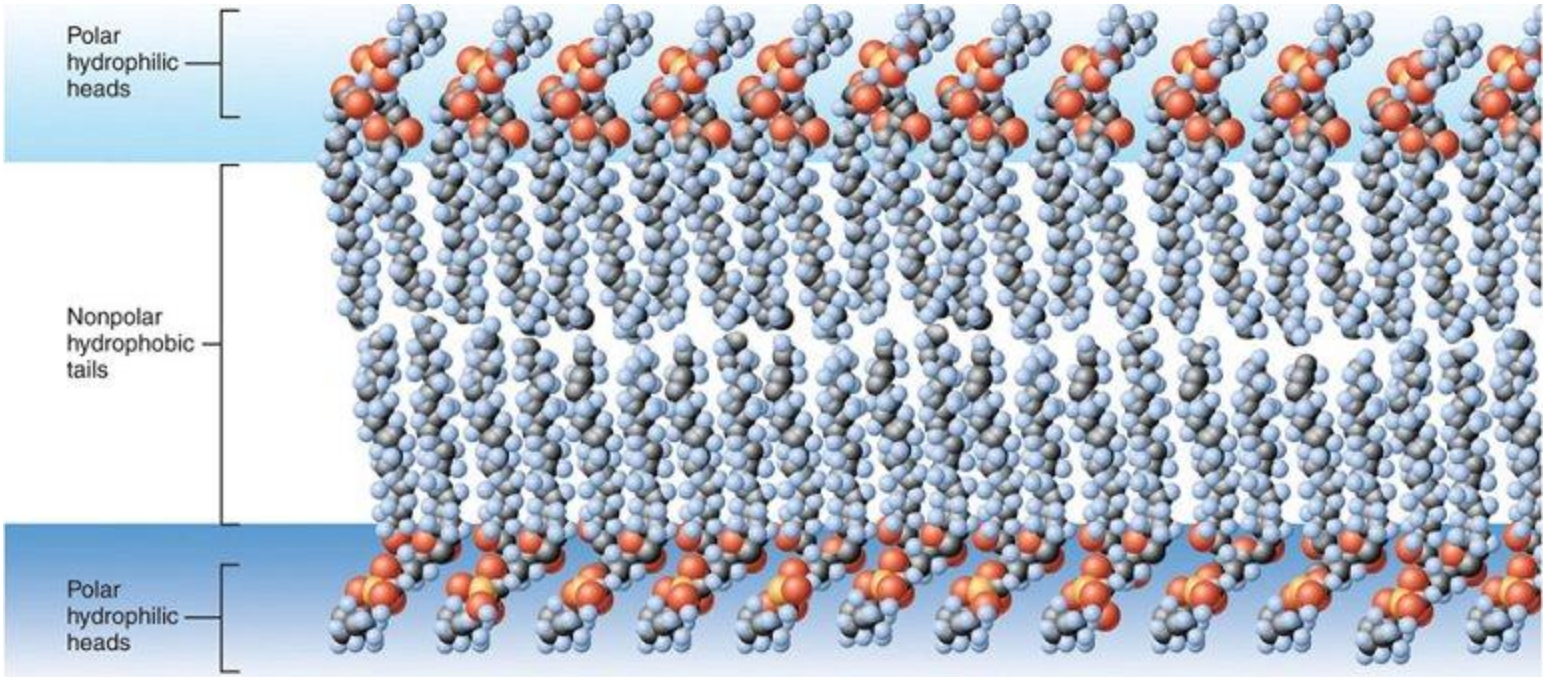


Other factors affecting growth rates: Surfactants and detergents

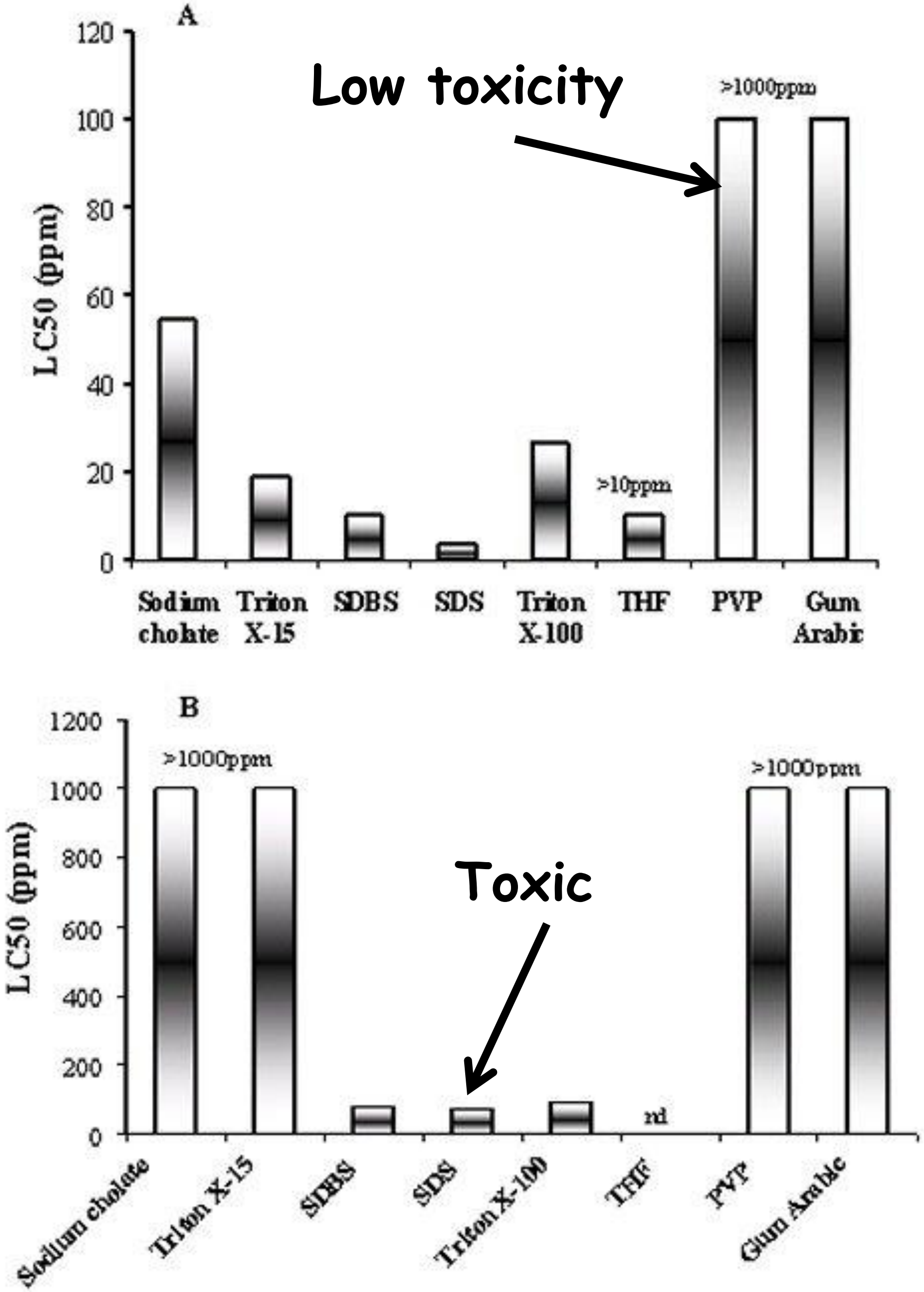




Cell membranes can be disrupted by detergents and surfactants



Surfactants and detergents can make membranes leaky



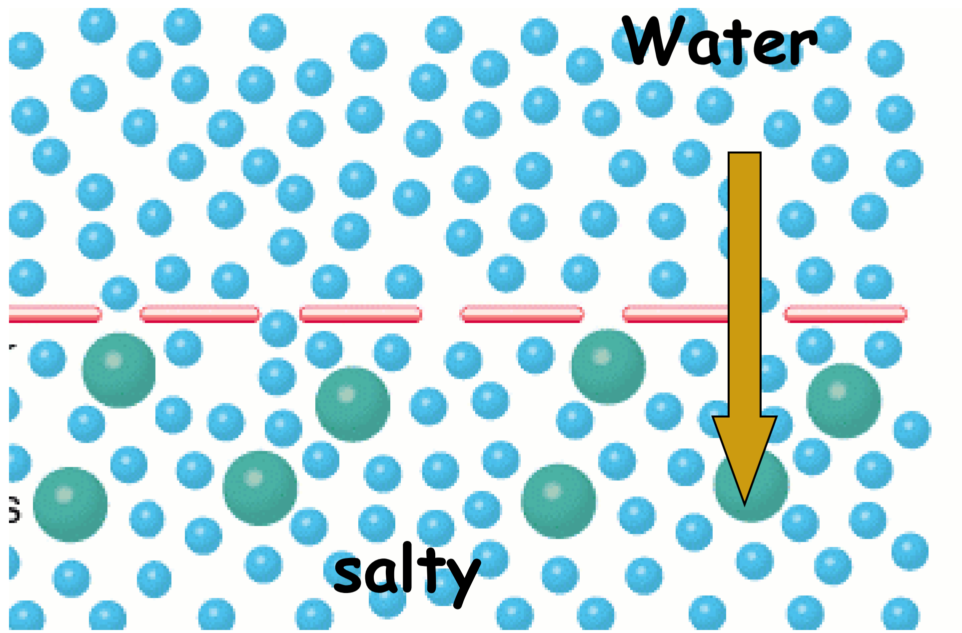
Response of two potential hydrocarbon degraders to various surfactants.
LC50 is the surfactant concentration that is lethal to 50% of the bacteria exposed to the surfactant. ppm = mg/kg

Other factors affecting growth rates: Salinity

Inside cell

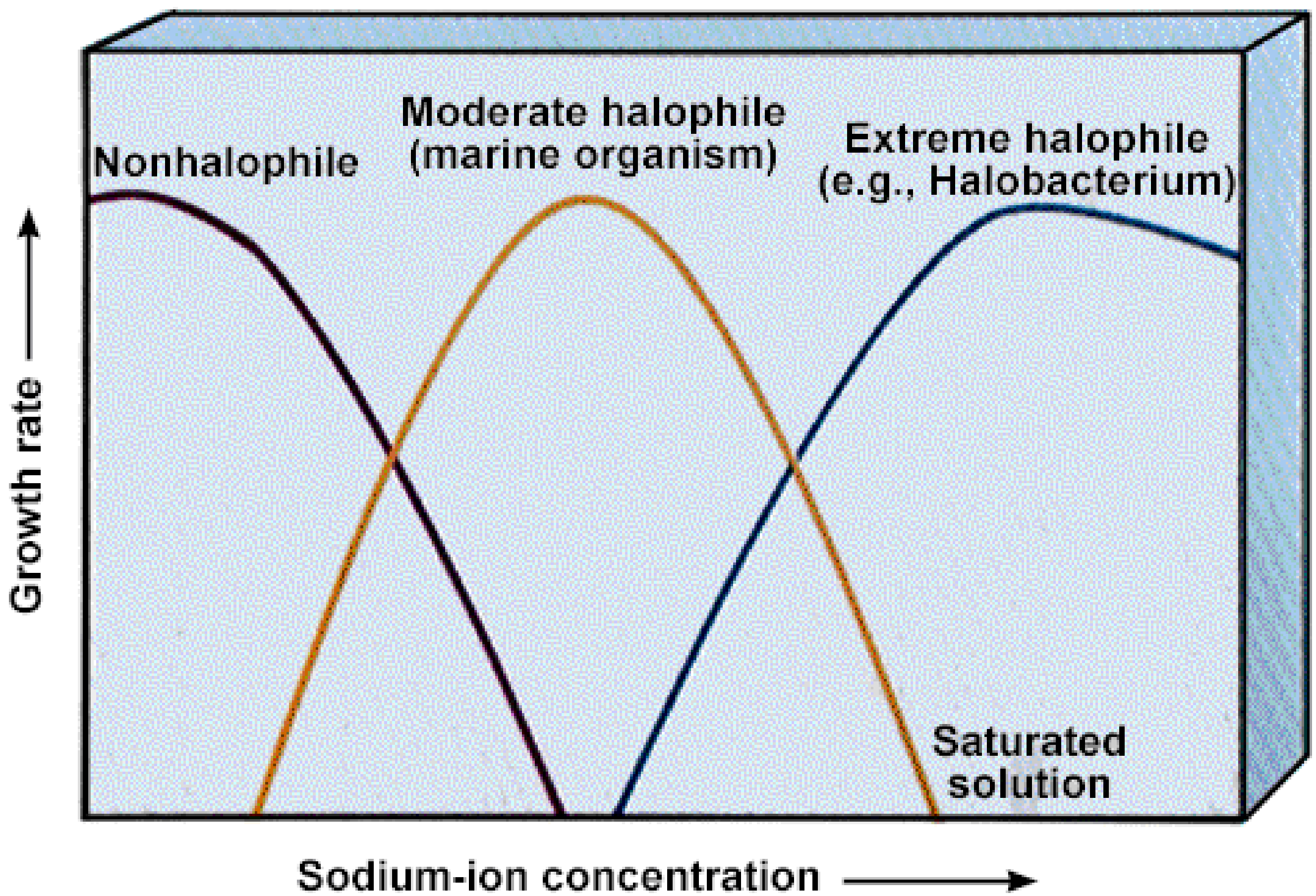
Cell membrane

Outside cell



High salinity makes it difficult for the bugs to keep water in the cell. Dehydrated cells don't work right!

Some bacteria are tolerant or even require high salt concentrations



Other factors affecting growth rates: Toxic chemicals

Organic compounds toxic to targeted higher organisms may also be toxic to microorganisms such as

- herbicides
- pesticides
- rodenticides

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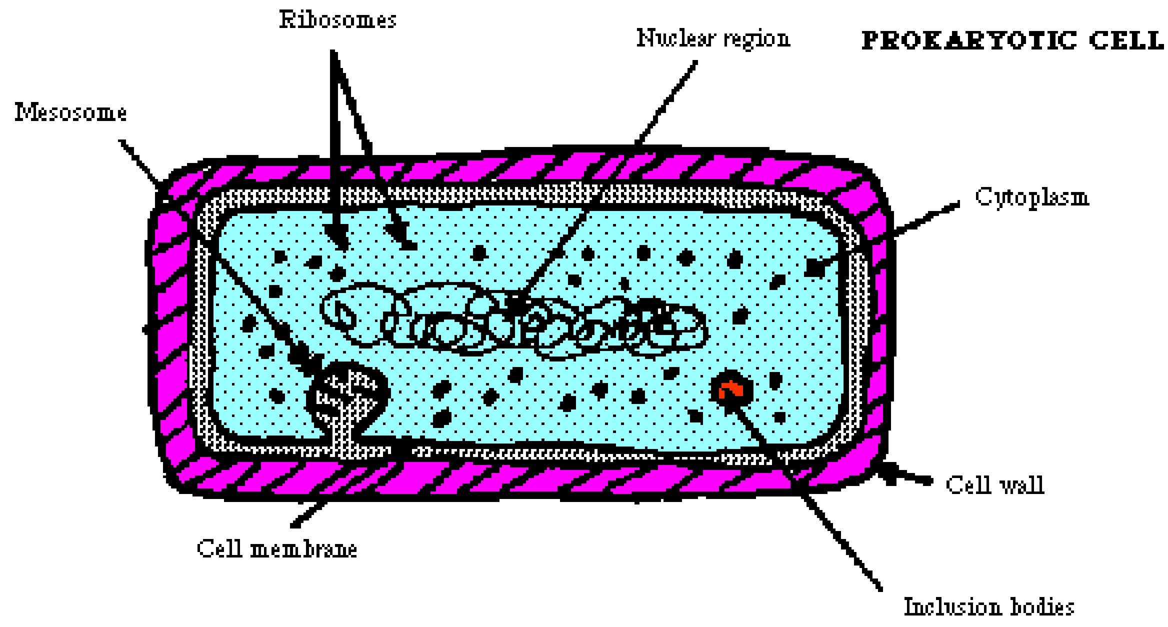
- Getting oxygen to the microbes
- Optimizing environmental conditions (to the extent we can)
- **Moisture! Moisture! Moisture!**

Other factors affecting growth rates: Water

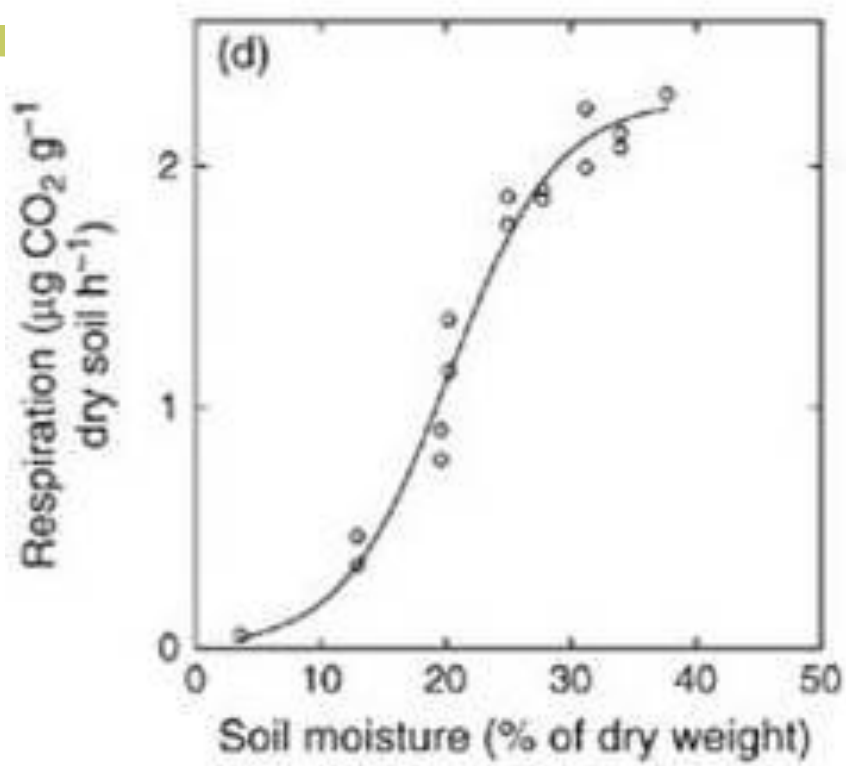
- Water – Water is the universal solvent for cellular biochemicals, growth substrates, nutrients, and O_2

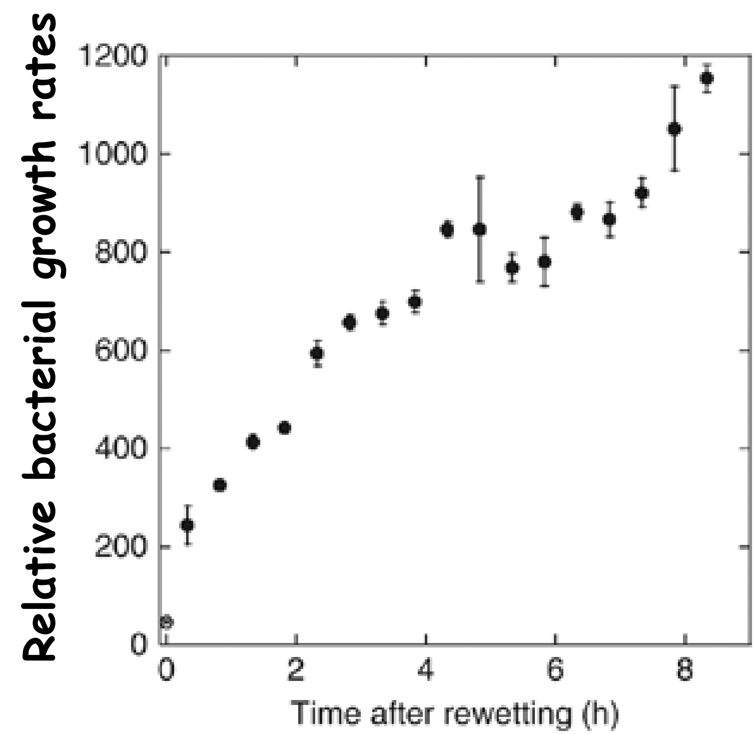


Other factors affecting growth rates: Water

- Lack of sufficient moisture has a tremendous effect on activity of soil microbes
- Nothing works right or moves around in the cell as needed without enough water

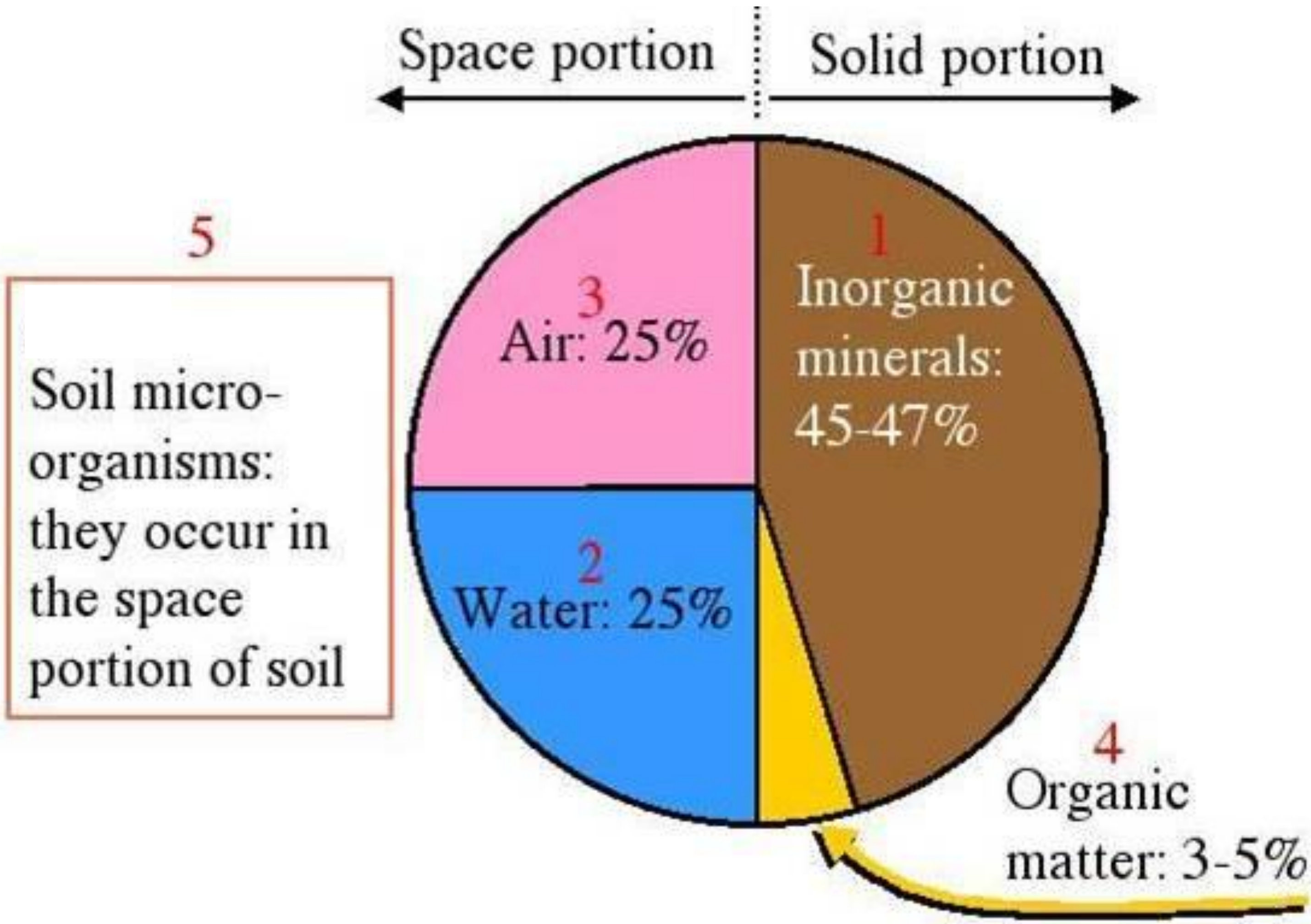
Effect of soil moisture on growth of bacteria in soil



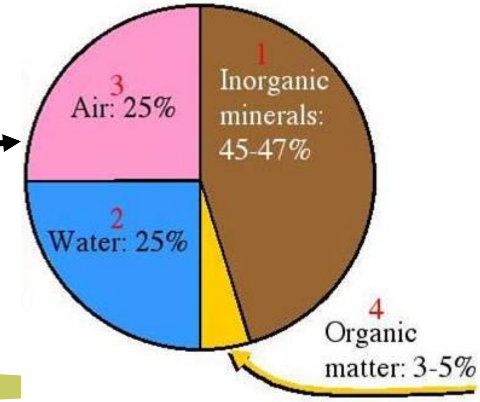


Soil as habitat

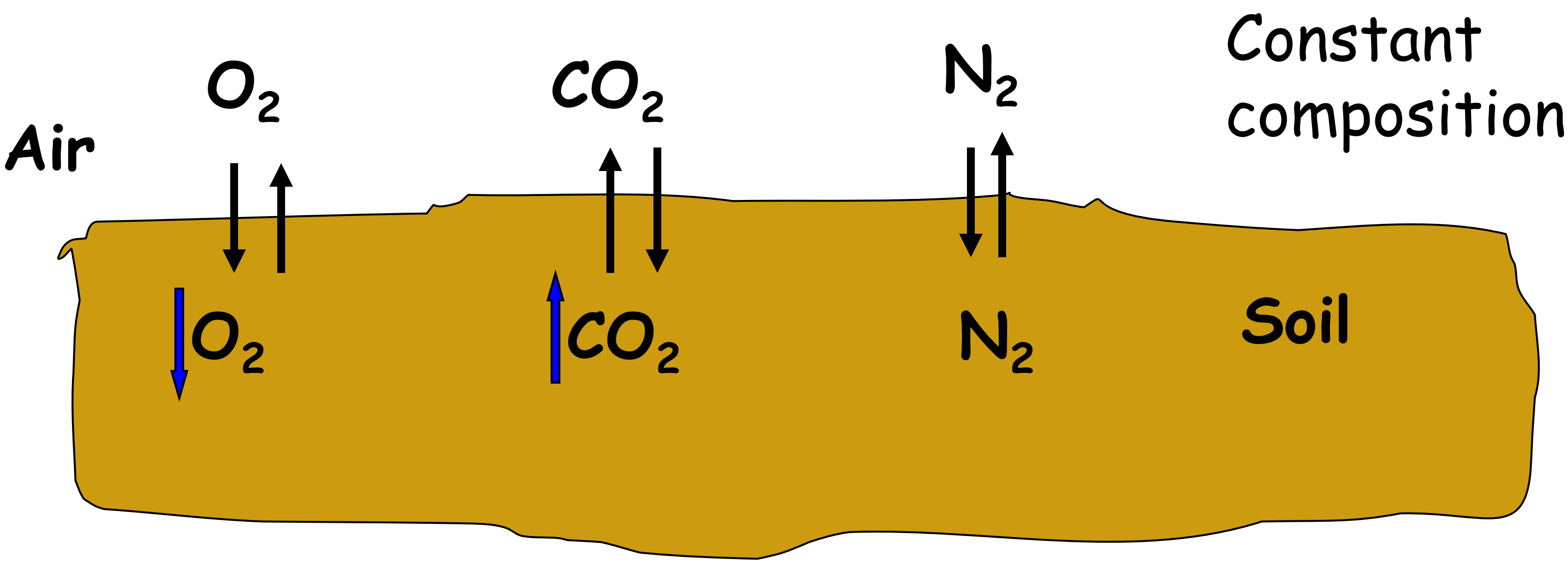
Volume composition of a loam surface soil



Soil gas

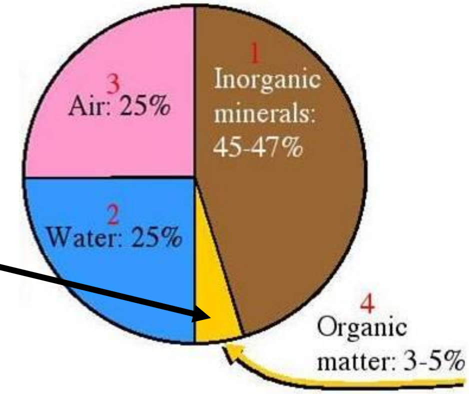


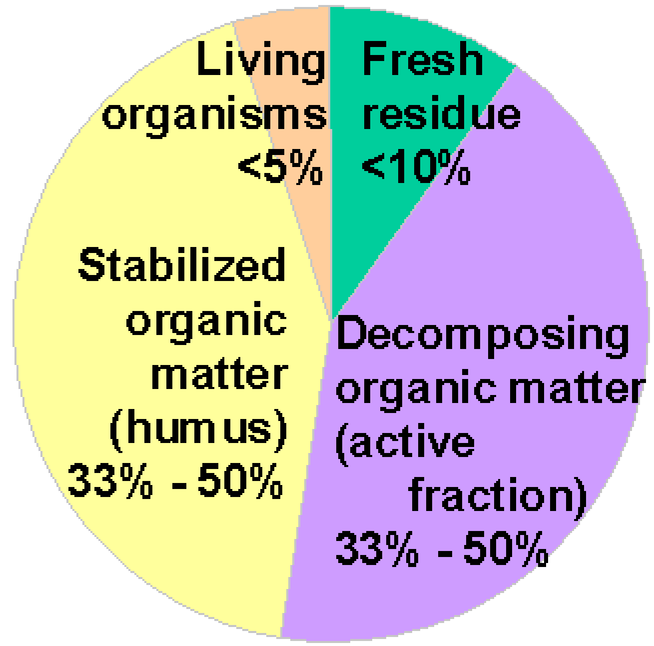
- Soil gas components come from the atmosphere and from biological activity in the soil

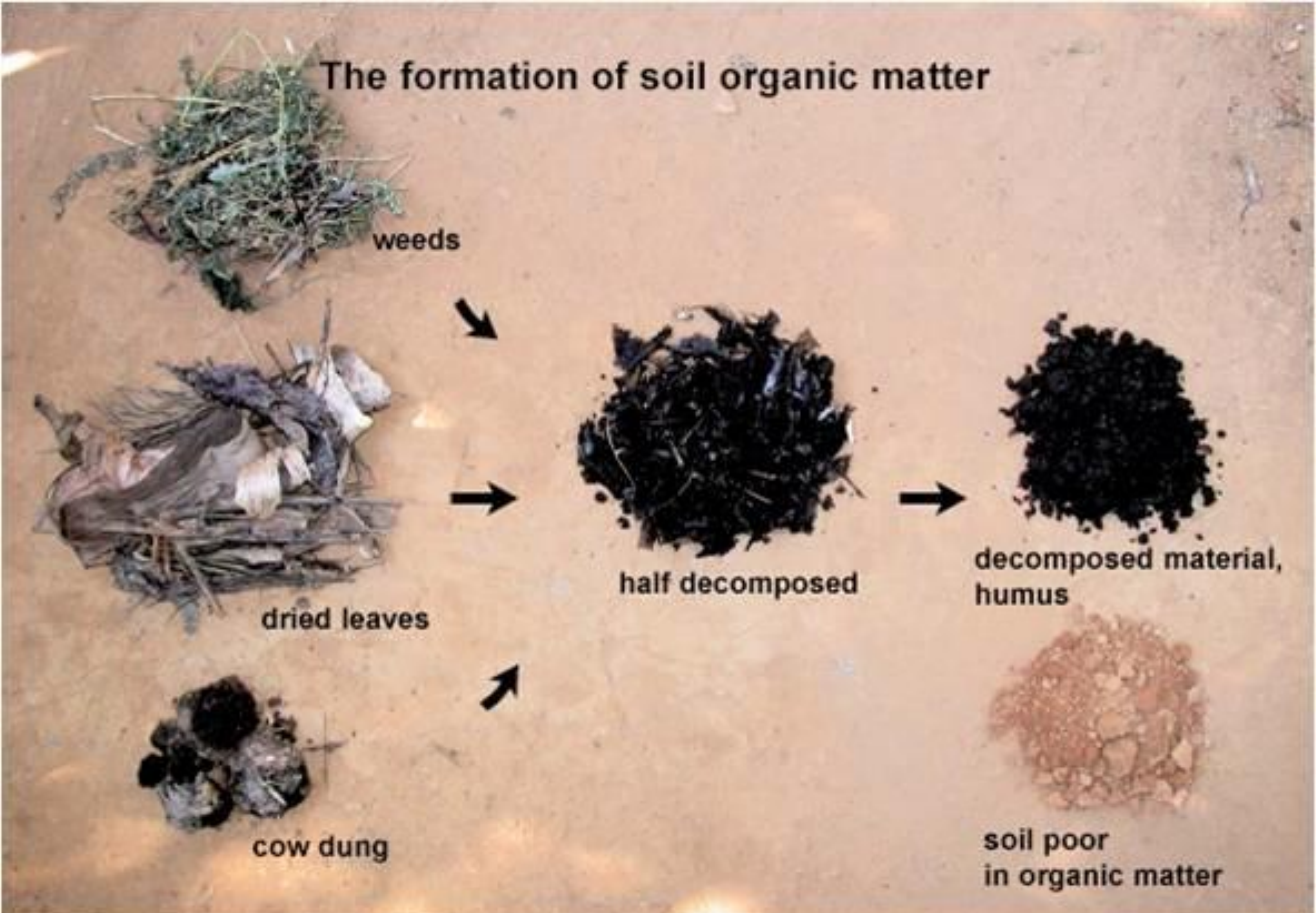


Composition changes in soil gas due to microbial activity indicated by blue arrows

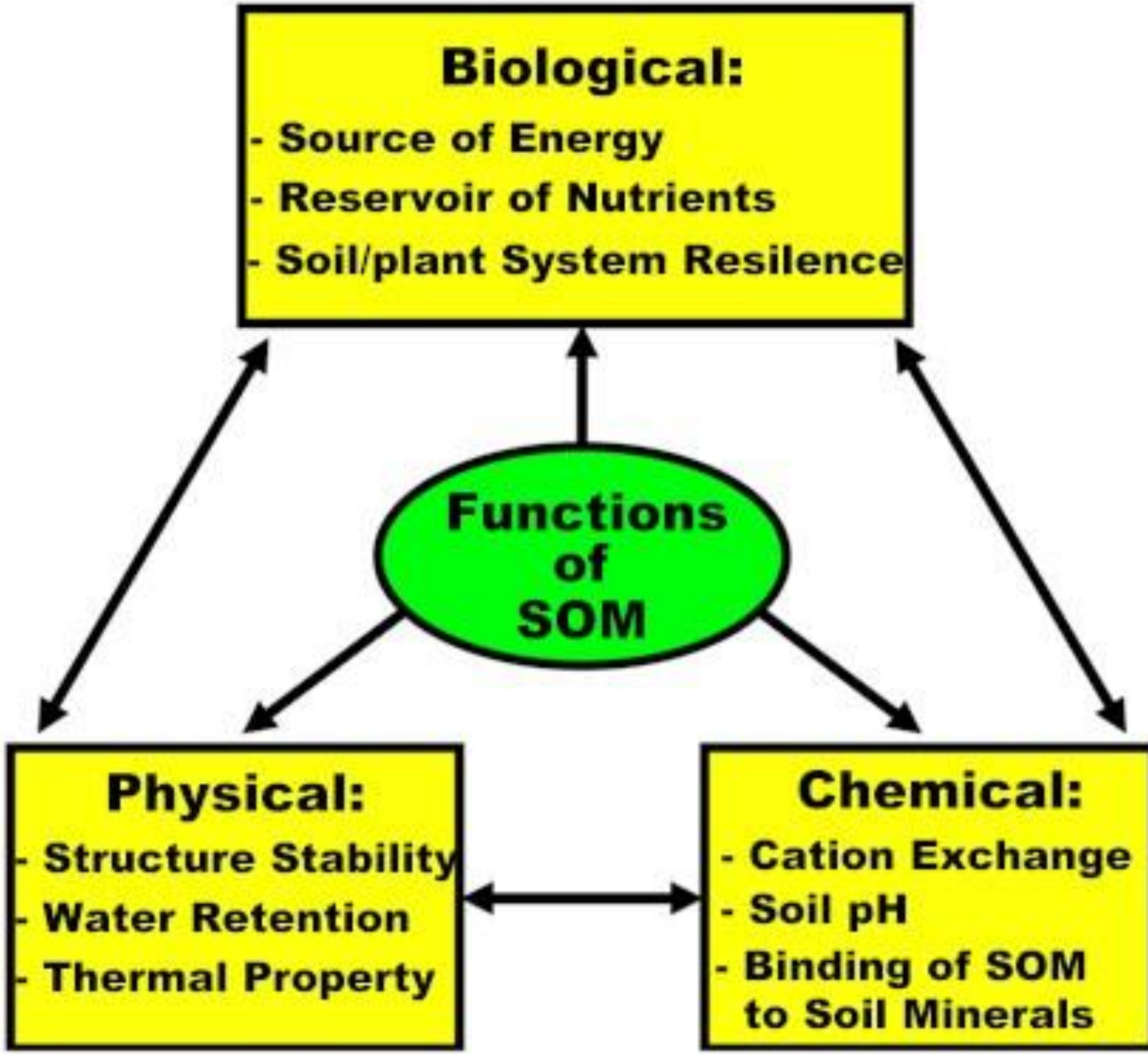
Soil organic matter



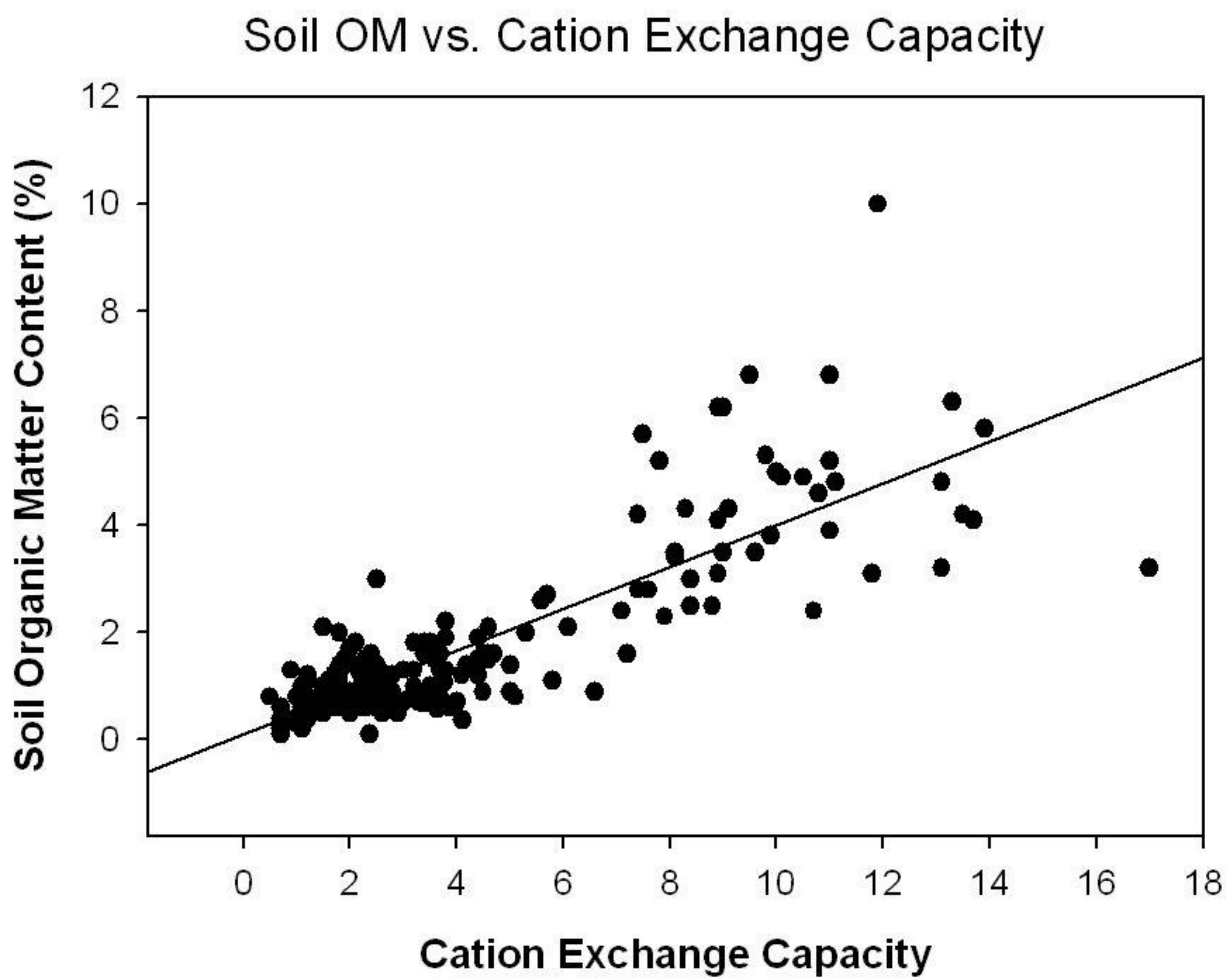


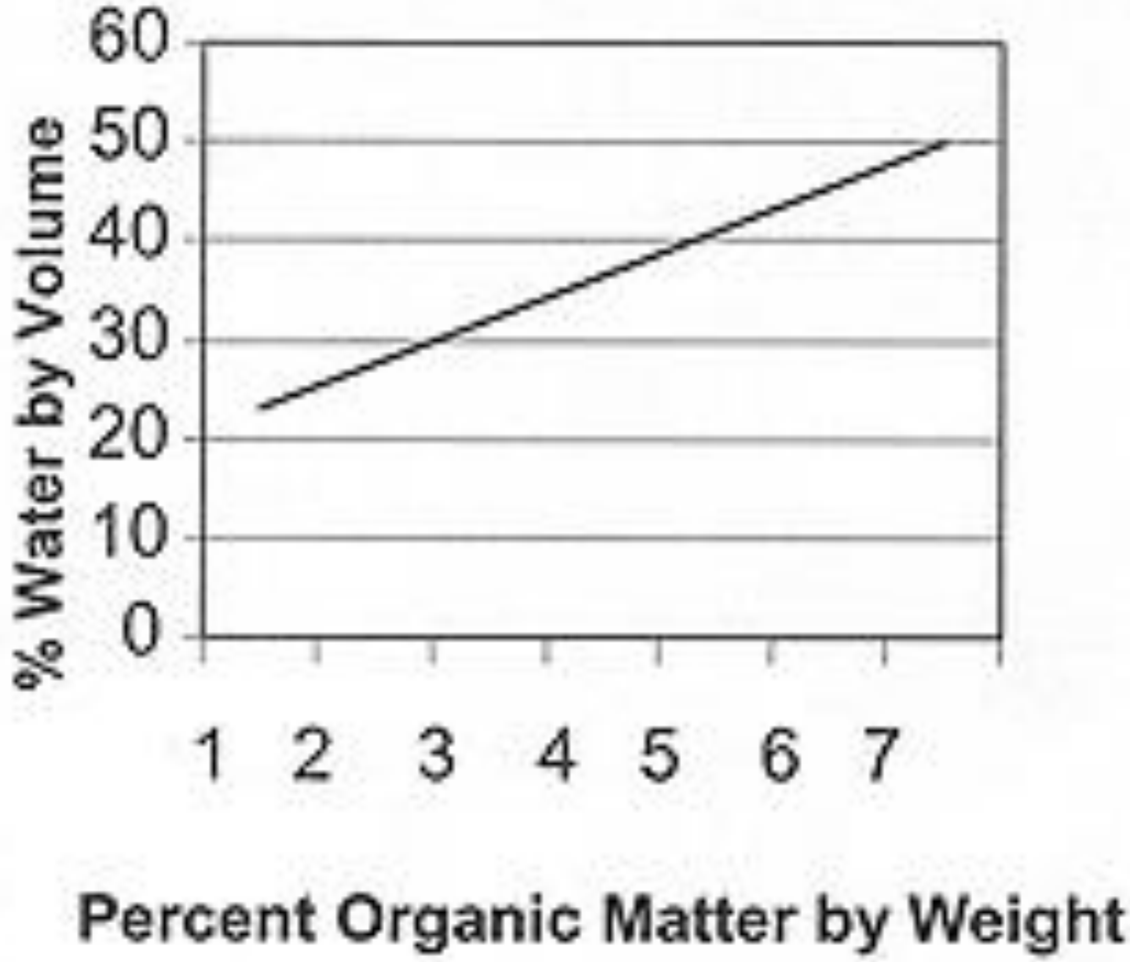


The formation of soil organic matter
weeds
dried leaves
cow dung
half decomposed
decomposed material,
humus
soil poor
in organic matter


Biological:
- Source of Energy
- Reservoir of Nutrients
- Soil/plant System Resilence
Functions of SOM
Physical:
- Structure Stability
- Water Retention
- Thermal Property
Chemical:
- Cation Exchange
- Soil pH
- Binding of SOM to Soil Minerals

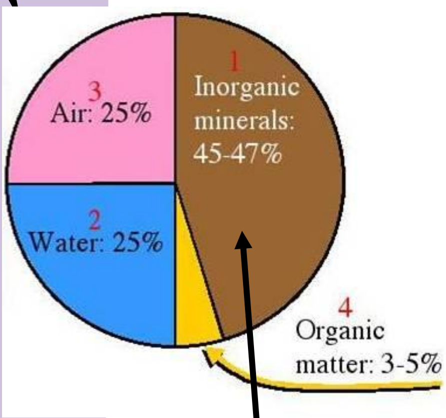
Cation binding sites hold key plant nutrients in the soil



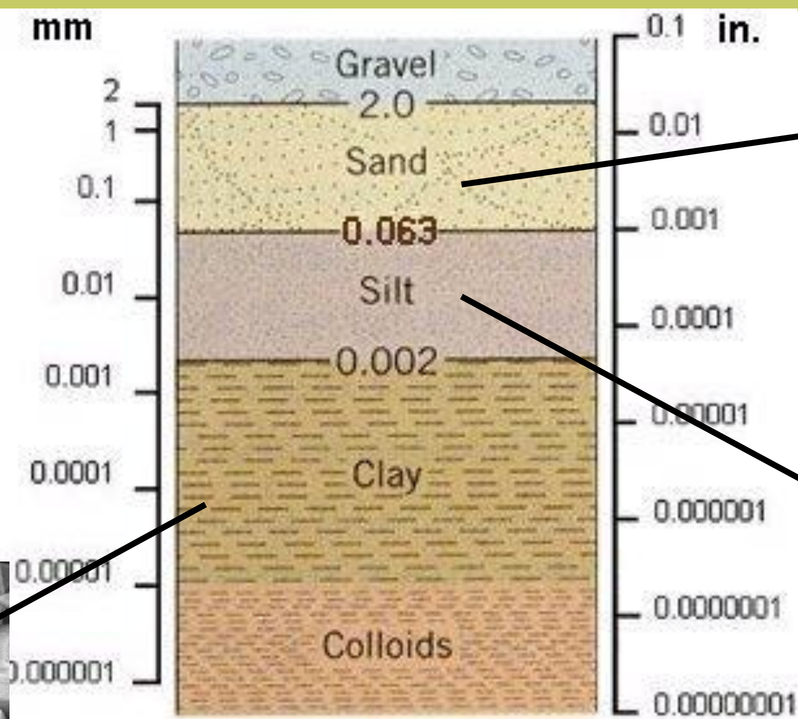


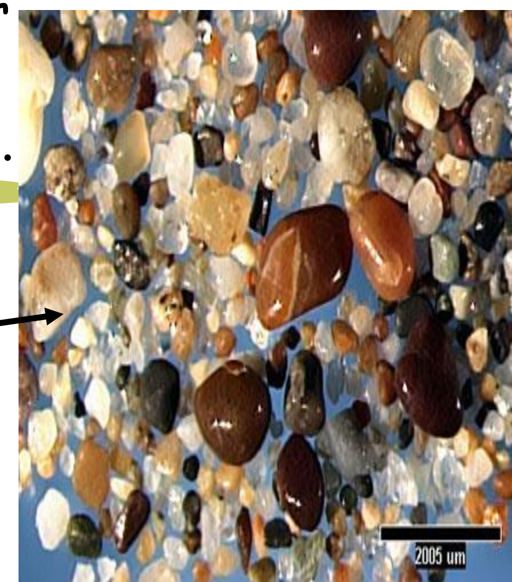
For every 1% of soil organic matter, the soil can hold 16,000 gallons of plant-available water per acre of soil down to one foot deep

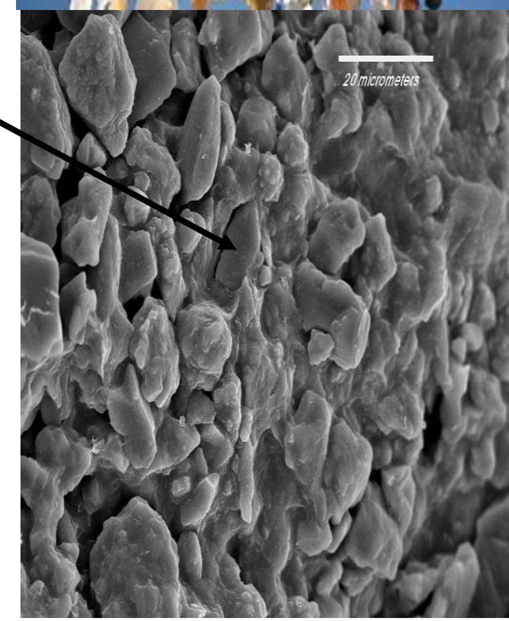
Soil texture: If the texture is too coarse or sandy it will be unable to hold water. Inability to hold water causes a loss of soil organic matter and an inability to support growth. If the soil is too fine it will impede water flow and infiltration.

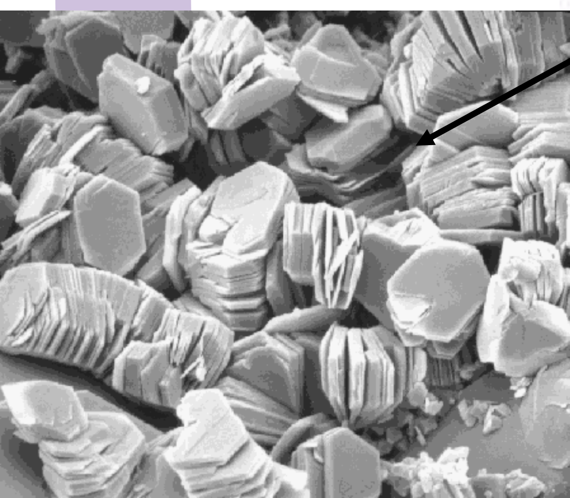


Mineral matter

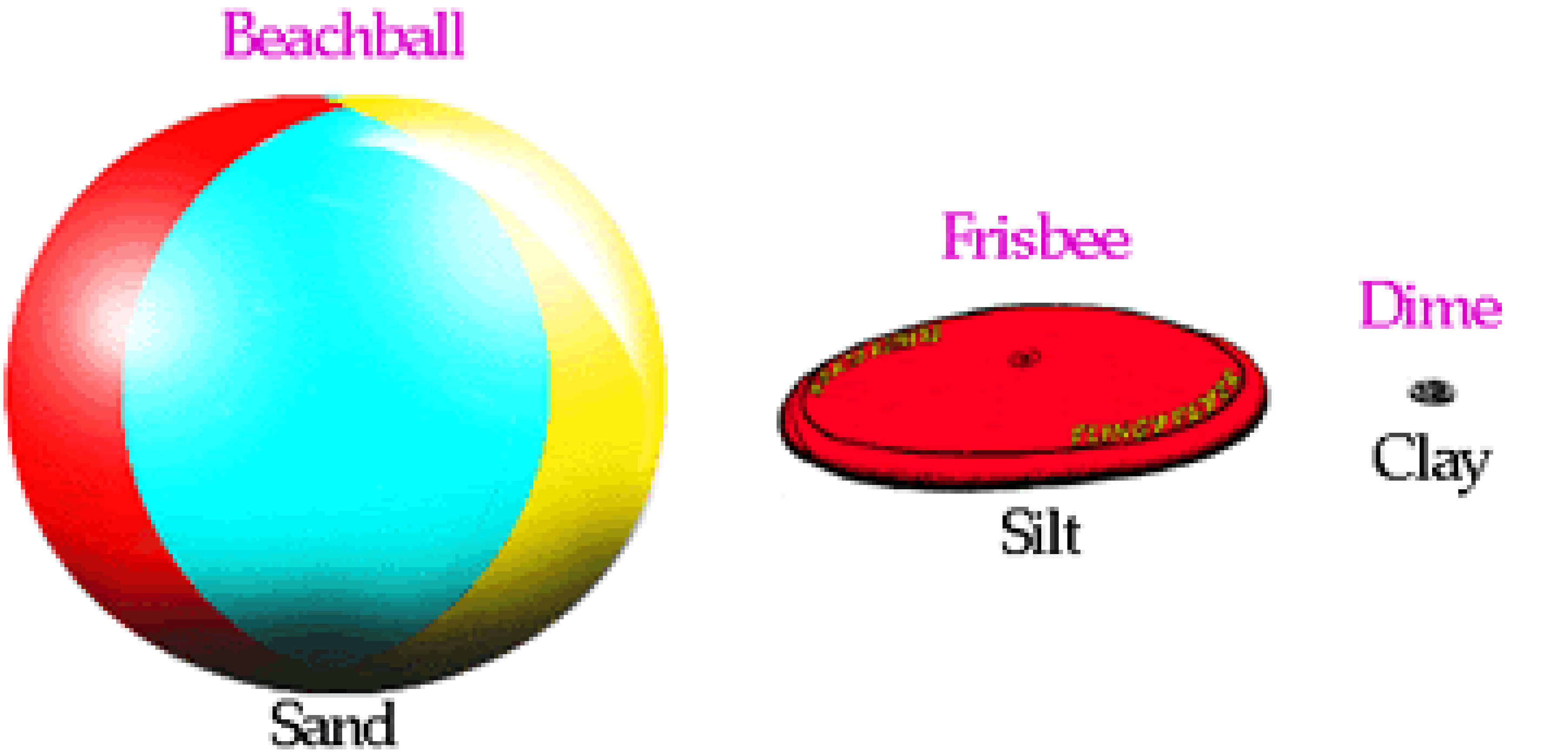








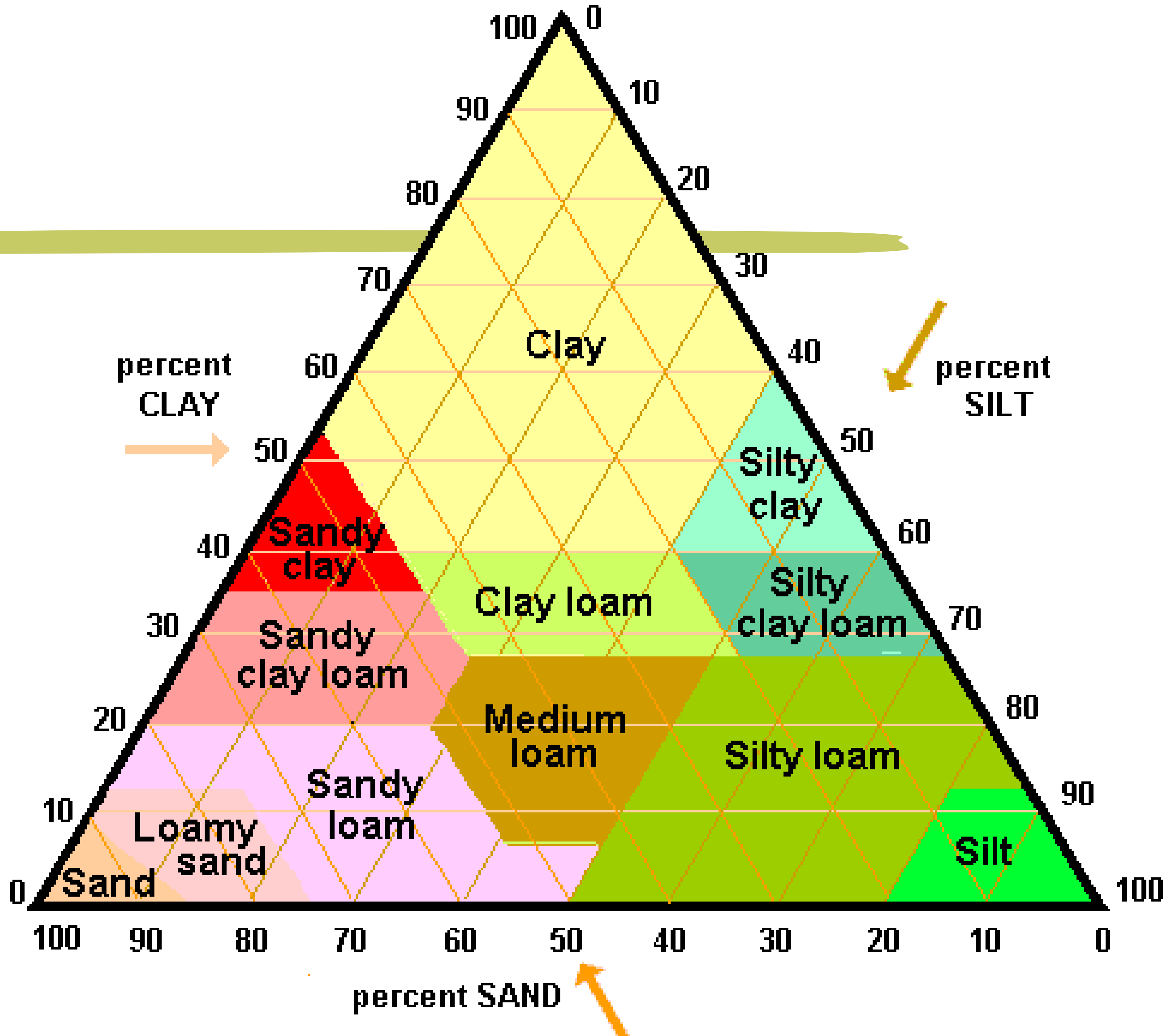
USDA Standard Relative Particle Size



Sand (2.00 - 0.05 mm)

Silt (0.05 mm - 0.002 mm)

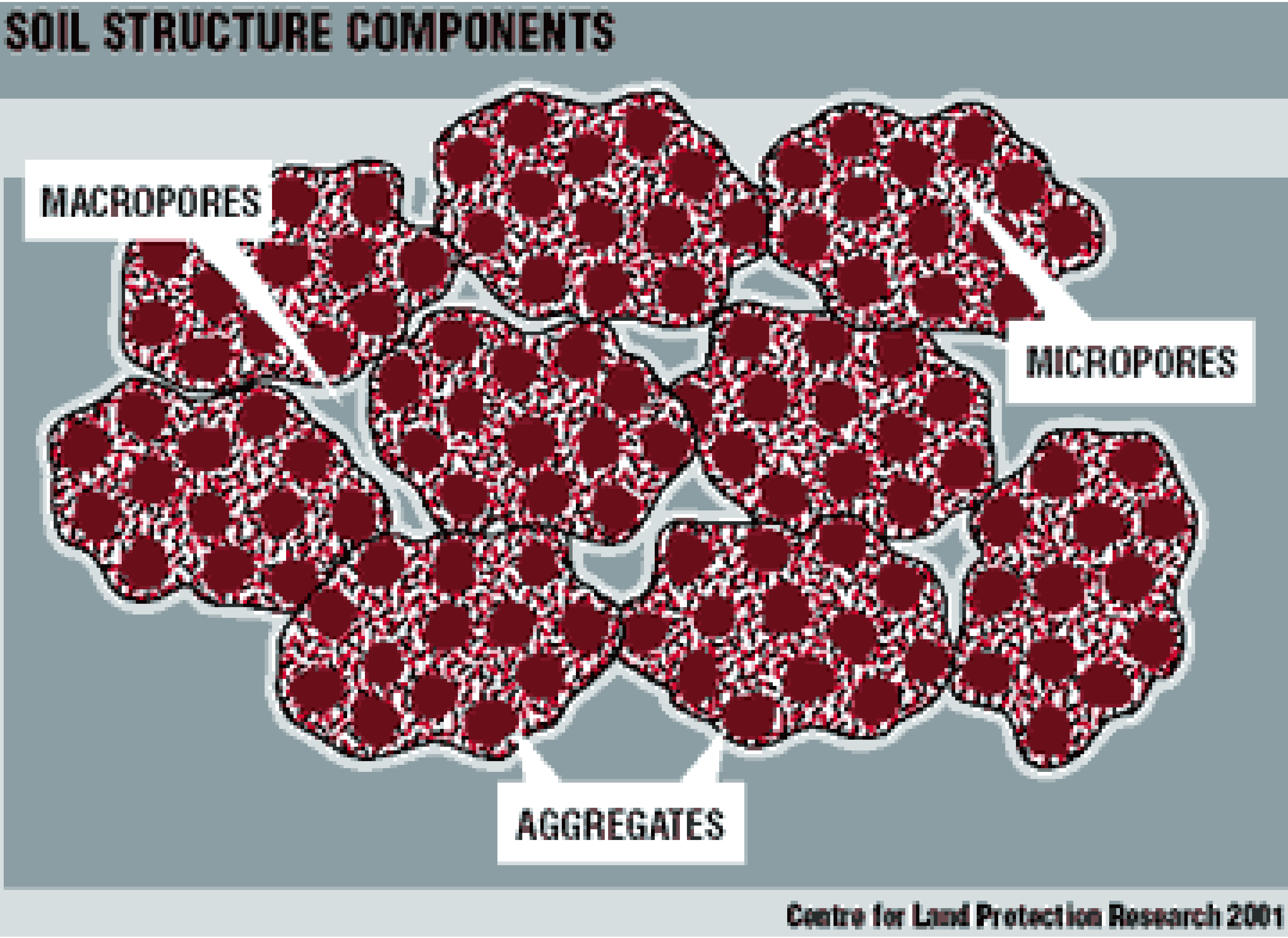
Clay (< 0.002 mm)



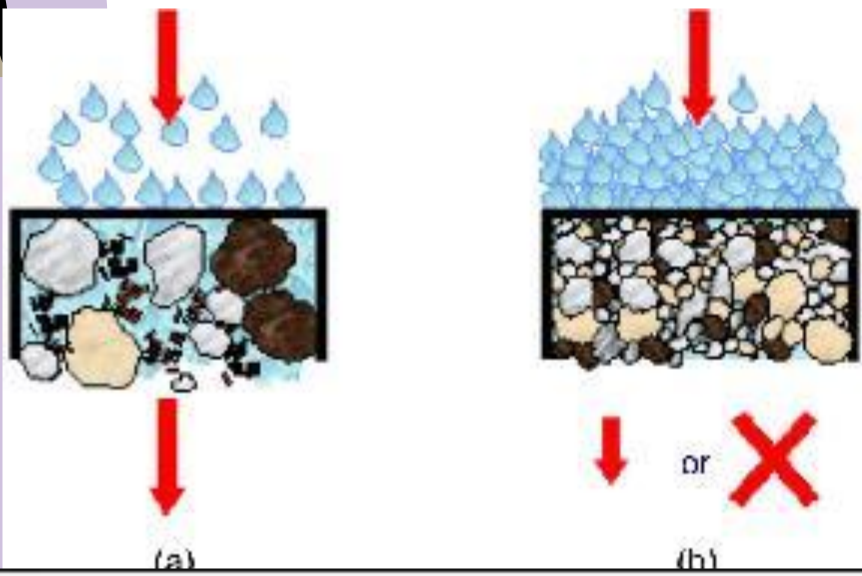
Clay
Silty clay
Sandy clay
Clay loam
Silty clay loam
Sandy clay loam
Medium loam
Silty loam
Sandy loam
Loamy sand
Sand
Silt
percent CLAY
percent SILT
percent SAND
100
90
80
70
60
50
40
30
20
10
0

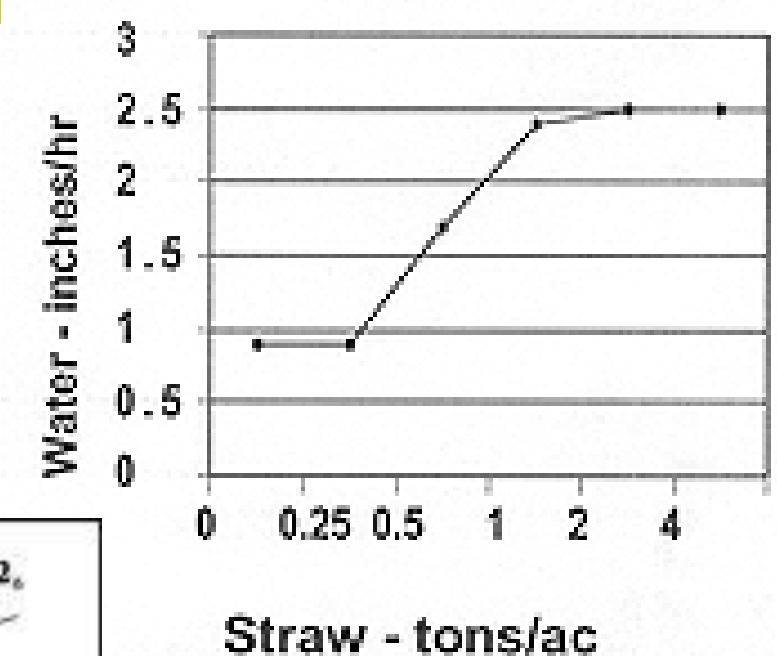
Soil structure

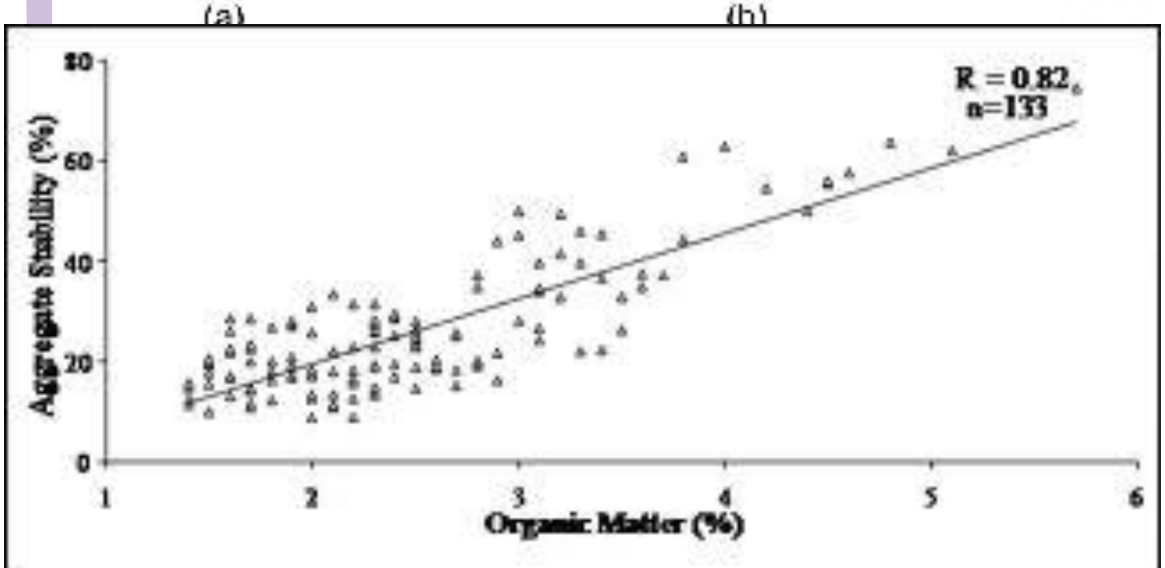
- Soil **structure**: the aggregation or sticking together of soil particles
- Aggregation results from:
  - Humic substances (soil organic matter)
  - Plant roots
  - Microorganisms
- Aggregates maintain a loose, open, granular condition in the soil which favorably impacts:
  - Movements of gases
  - Movement of water and water retention
  - Root penetration
  - Microbial growth


SOIL STRUCTURE COMPONENTS
MACROPORES
MICROPORES
AGGREGATES
Centre for Land Protection Research 2001

A well aggregated soil has good permeability to water and air







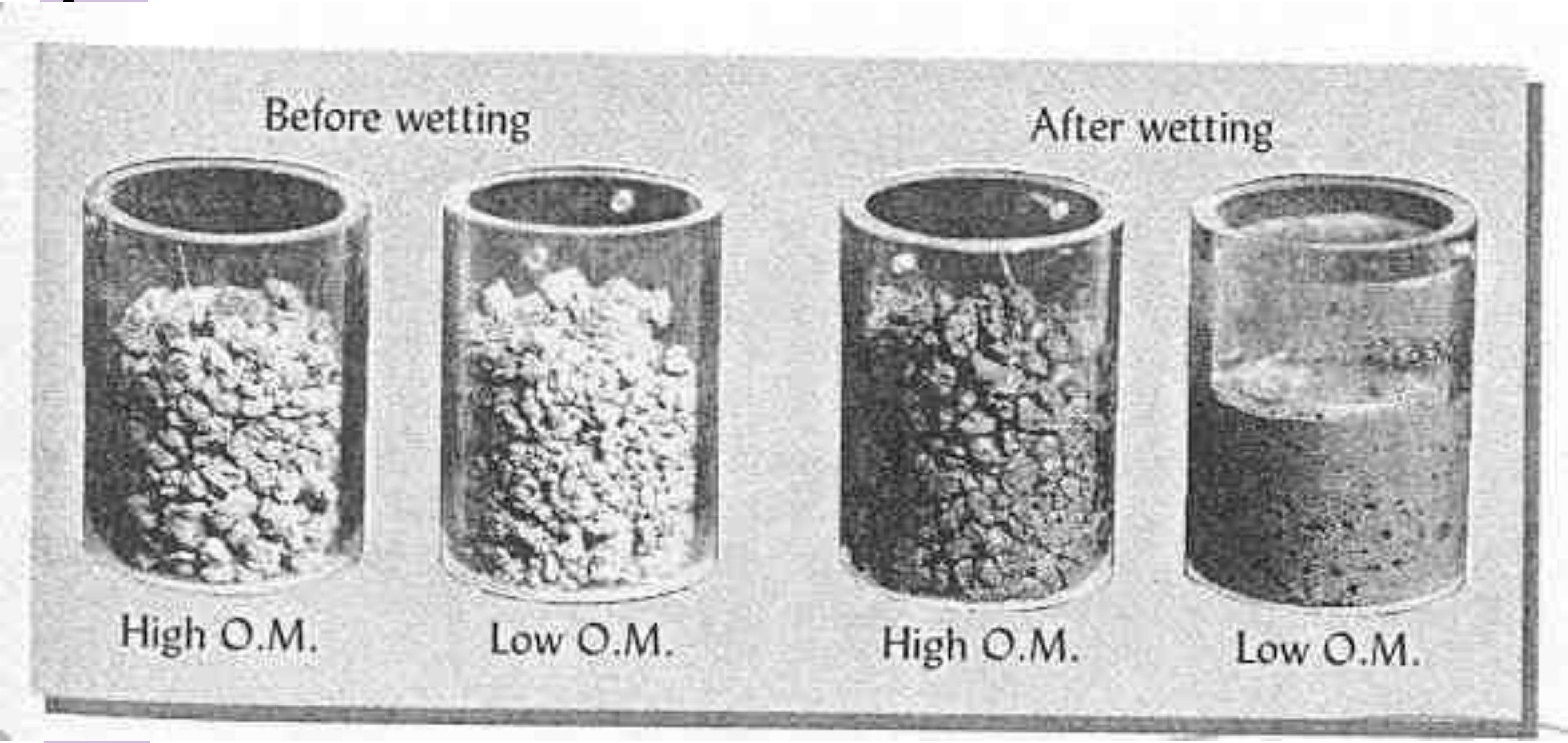
Soil organic matter and aggregate stability

High organic matter

Low organic matter

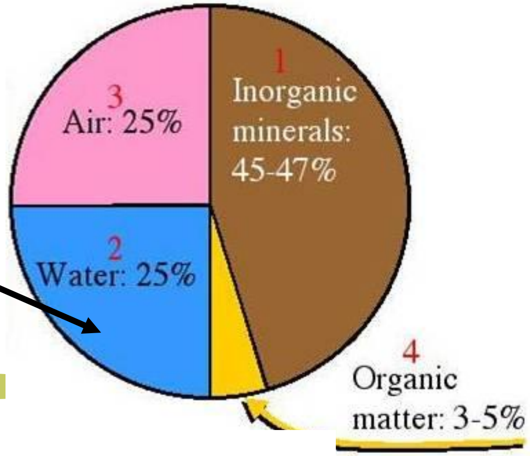
When the soil organic matter concentration is low soil readily disperses in water

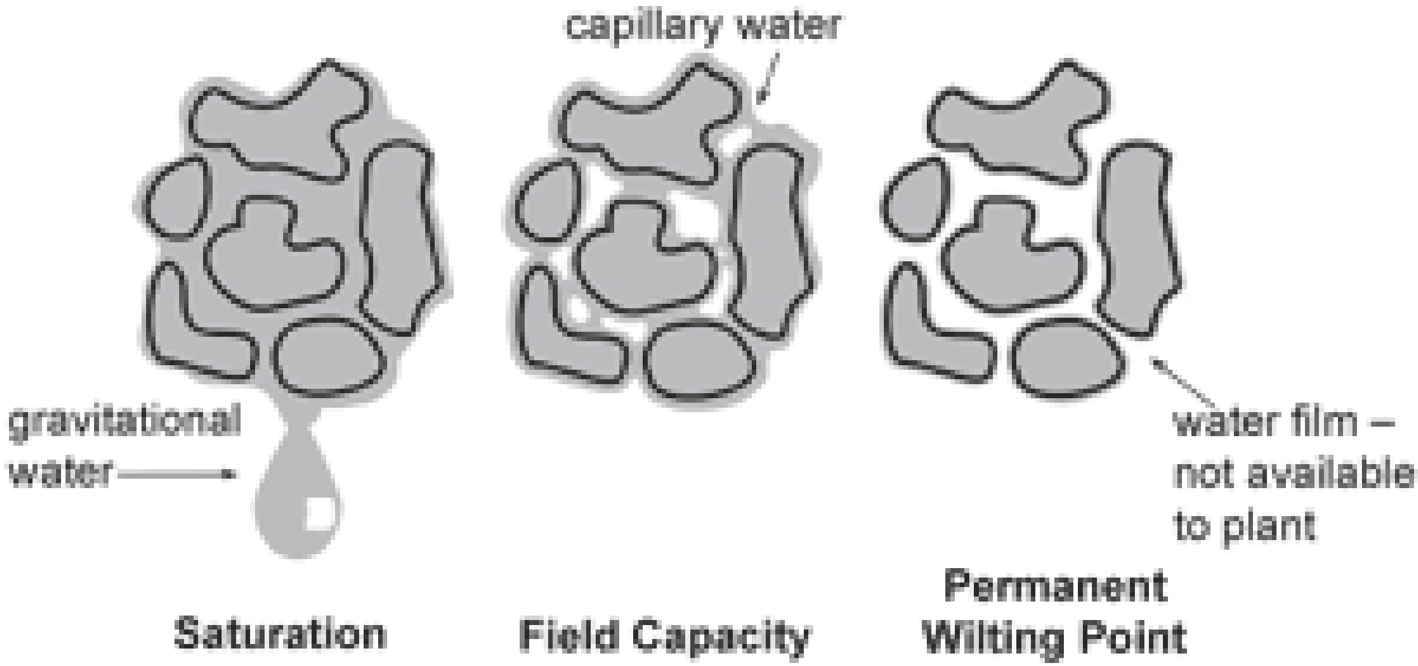
Soil organic matter and aggregate stability



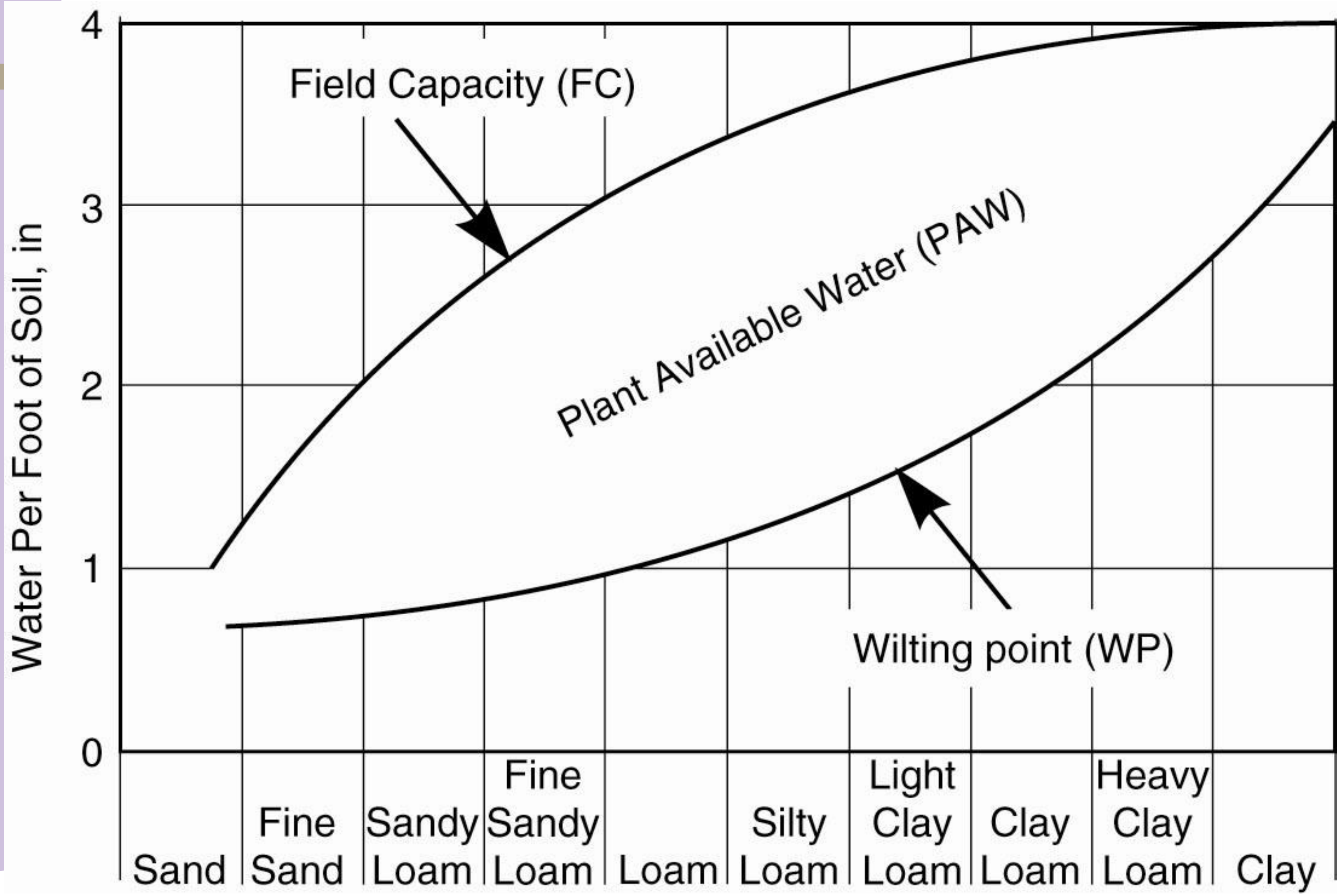
OM = soil organic matter

Soil and water





Plant available water and texture

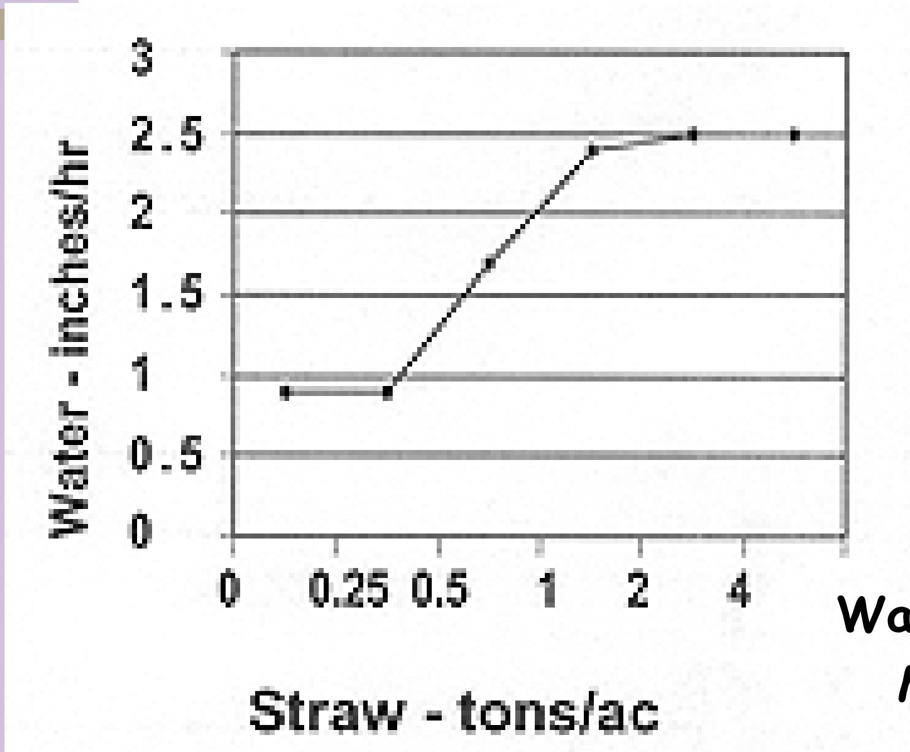


Movement of water in soil: gravity and capillary suction

- **Infiltration** is the movement of water from the soil surface **downwards** into the soil profile.
  - Water moves by **gravity** into the open pore spaces in the soil then is drawn deeper into the soil by **suction**.
  - The **more pore spaces** and the **less existing moisture** in the soil, **the higher the infiltration rate will be**.
  - Soil texture and soil structure, to a large degree, determine what the infiltration rate will be.
  - Good infiltration rates also mean good penetration of air and moisture into the soil

Infiltration rate	Interpretation
Less than ½ inches per hour	Poor drainage
½ inch – 1 inch per hour	Moderate drainage
More that 1 inch per hour	Good drainage

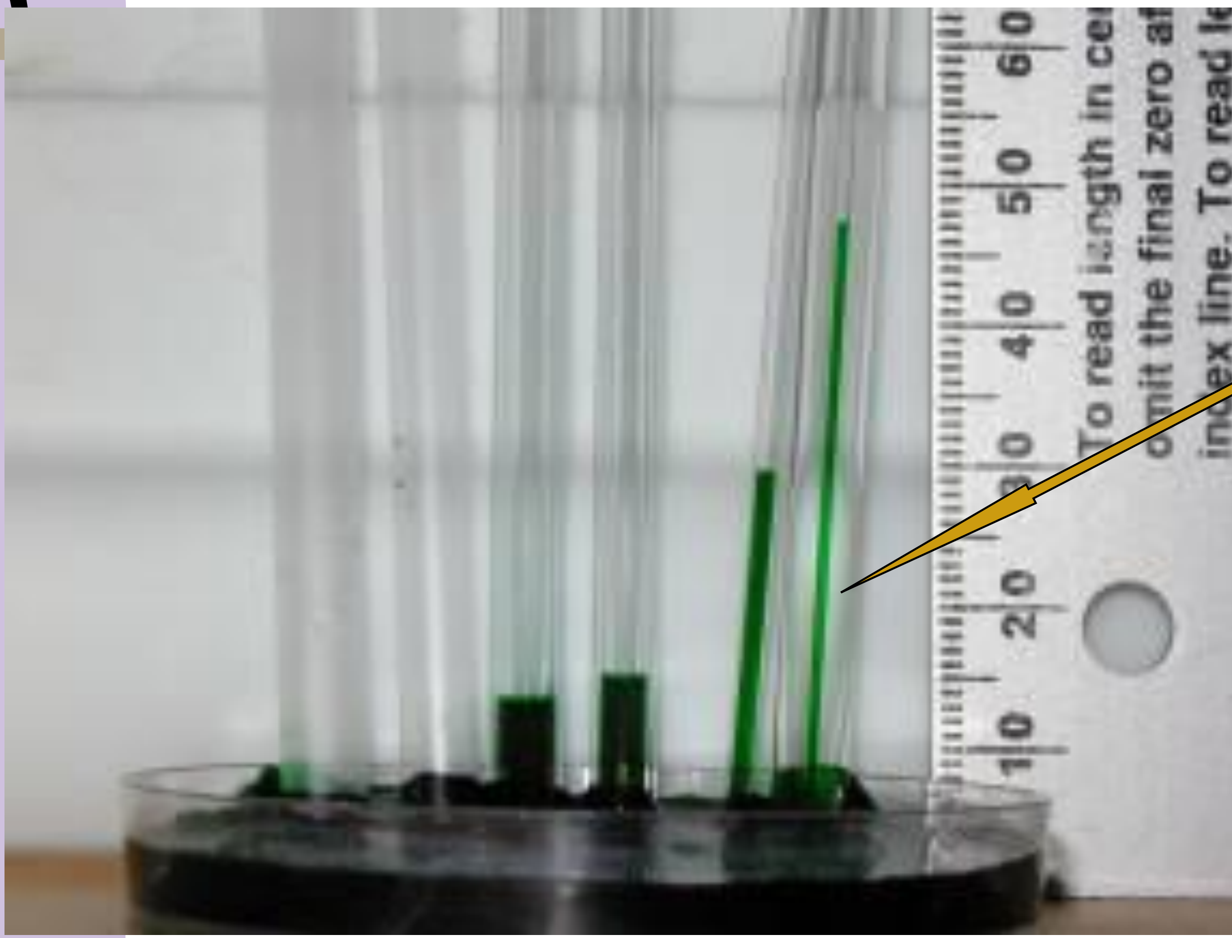
Effect of organic matter on infiltration rate



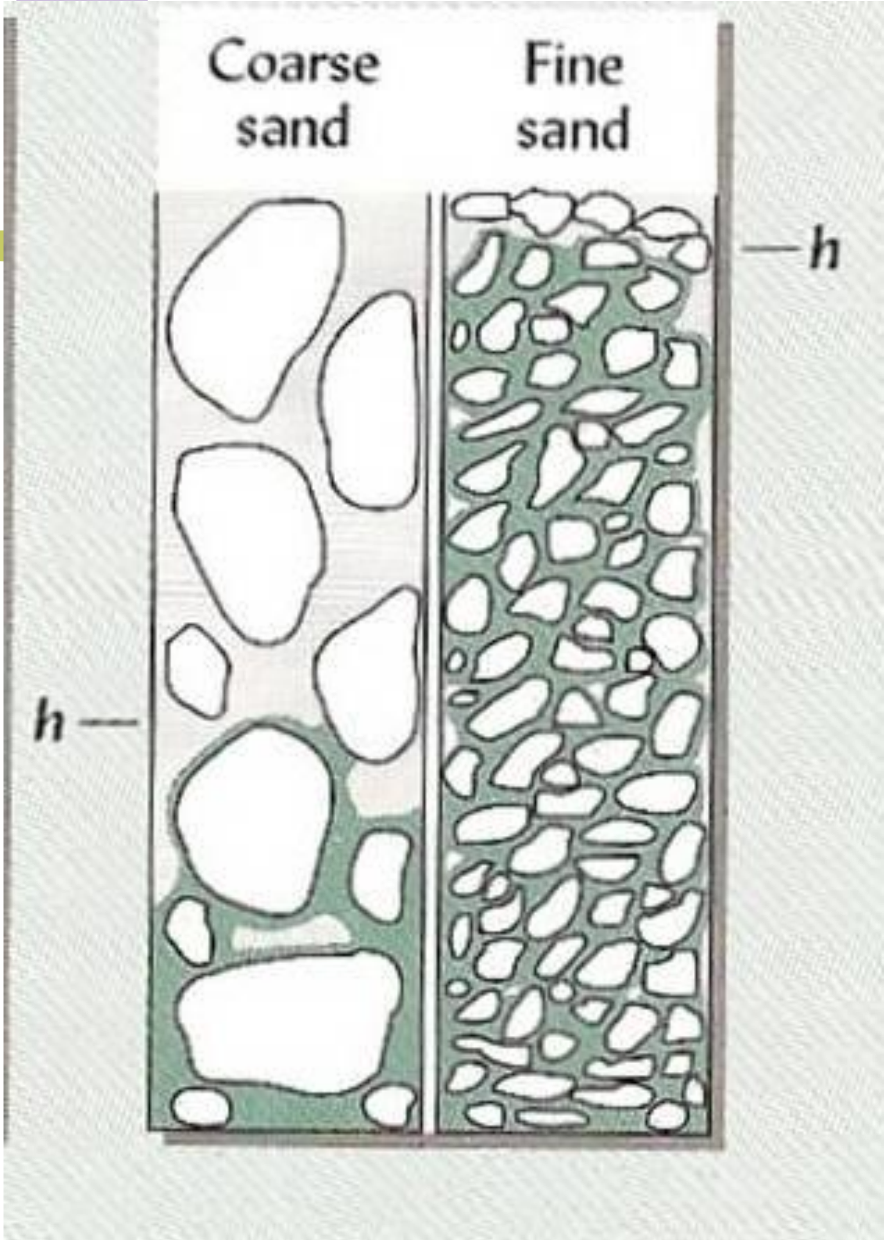
Water entry into the soil after 1 hour

Manure Rate (Tons/acre)	Inches of water
0	1.2
8	1.9
16	2.7

Capillary suction

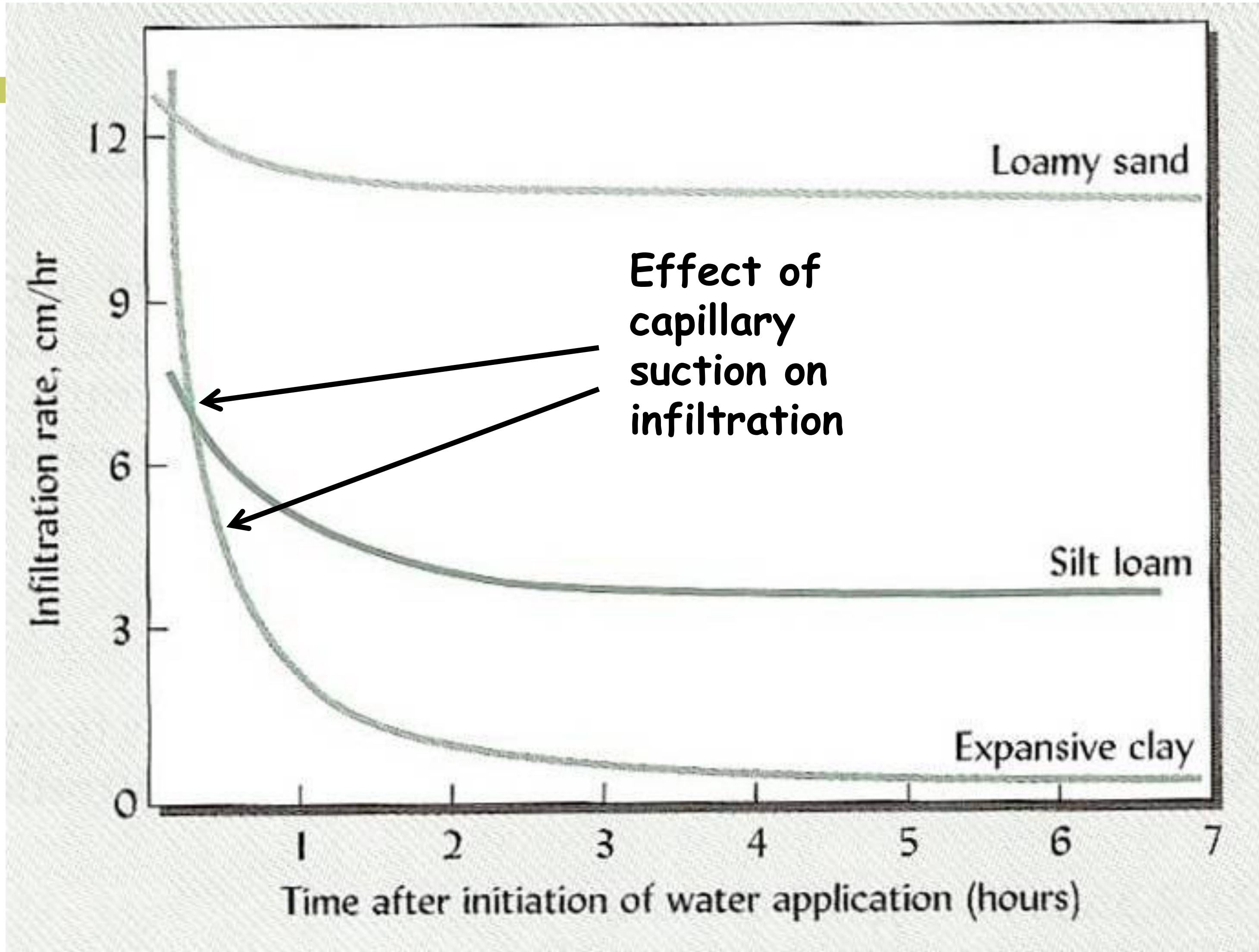


Greatest capillary rise is seen in smallest diameter capillary



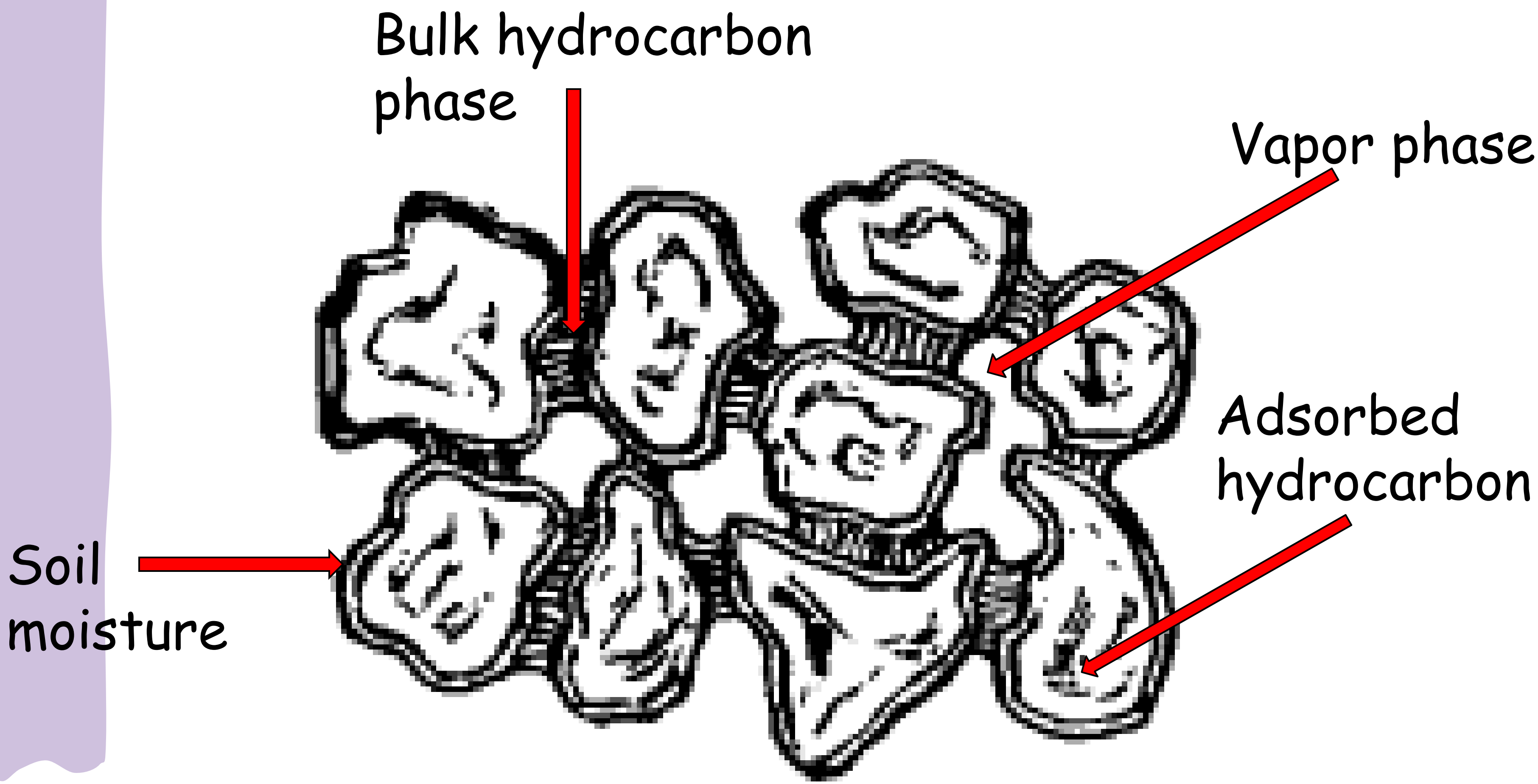
The height of capillary rise is proportional to 1/d where d = diameter of soil pores

Movement of water in soil: gravity and capillary suction



The fate of hydrocarbons in the environment

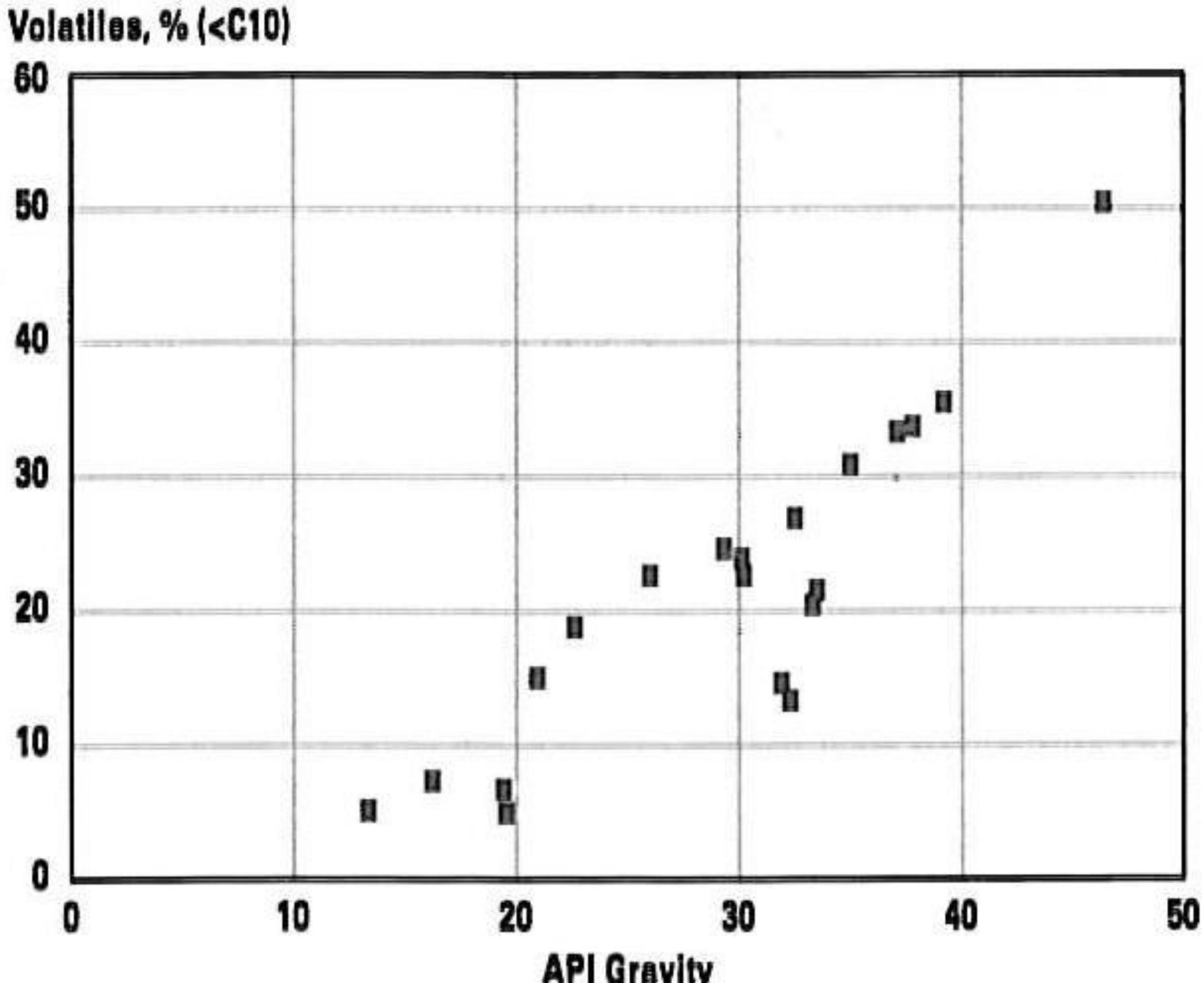
Hydrocarbon distribution in unsaturated contaminated soil



Weathering of petroleum hydrocarbons

- Intrinsic biodegradation
  - Biodegradation that occurs naturally without our help
    - Usually limited to n-alkanes
    - Typically stopped by depletion of a nutrient like nitrogen and limited contact between hydrocarbon and microbes
- Evaporation
  - Loss of more volatile components
  - Effected by temperature and contact between hydrocarbon and air
- Solubilization
  - Limited to BTEX and some naphthalenes
  - Requires contact with water

Loss of volatiles (<C10) at 40 °C for a range of crude oils

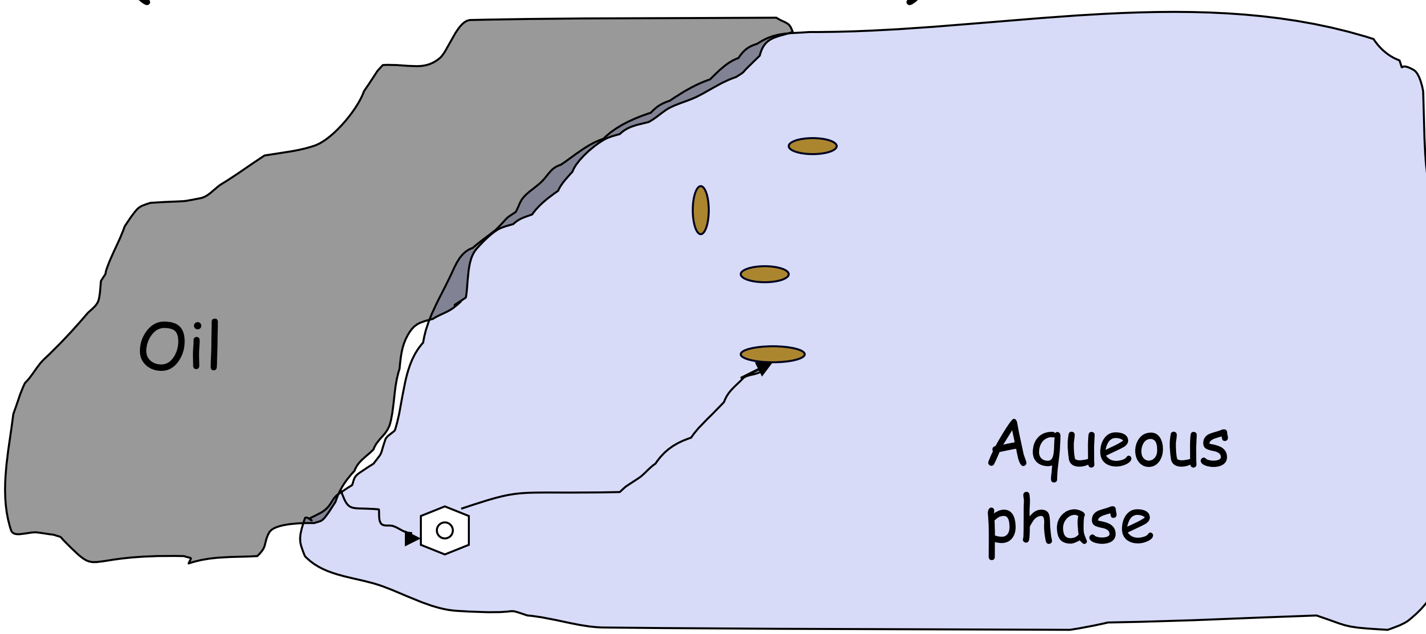


Source: Sara Mc Millen, Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

Bioavailability of petroleum hydrocarbons

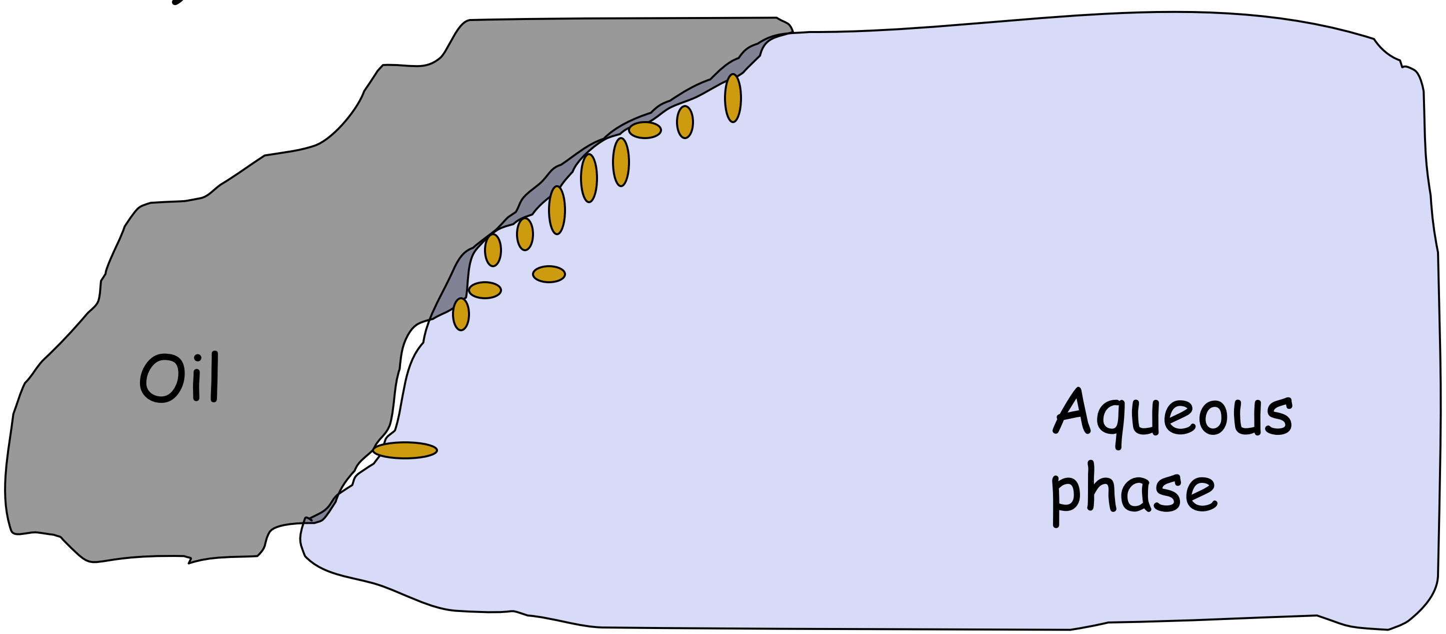
Uptake of hydrocarbons by microorganisms occurs through three mechanisms:

- Solubilization of hydrocarbon in an aqueous phase (in the dissolved state)



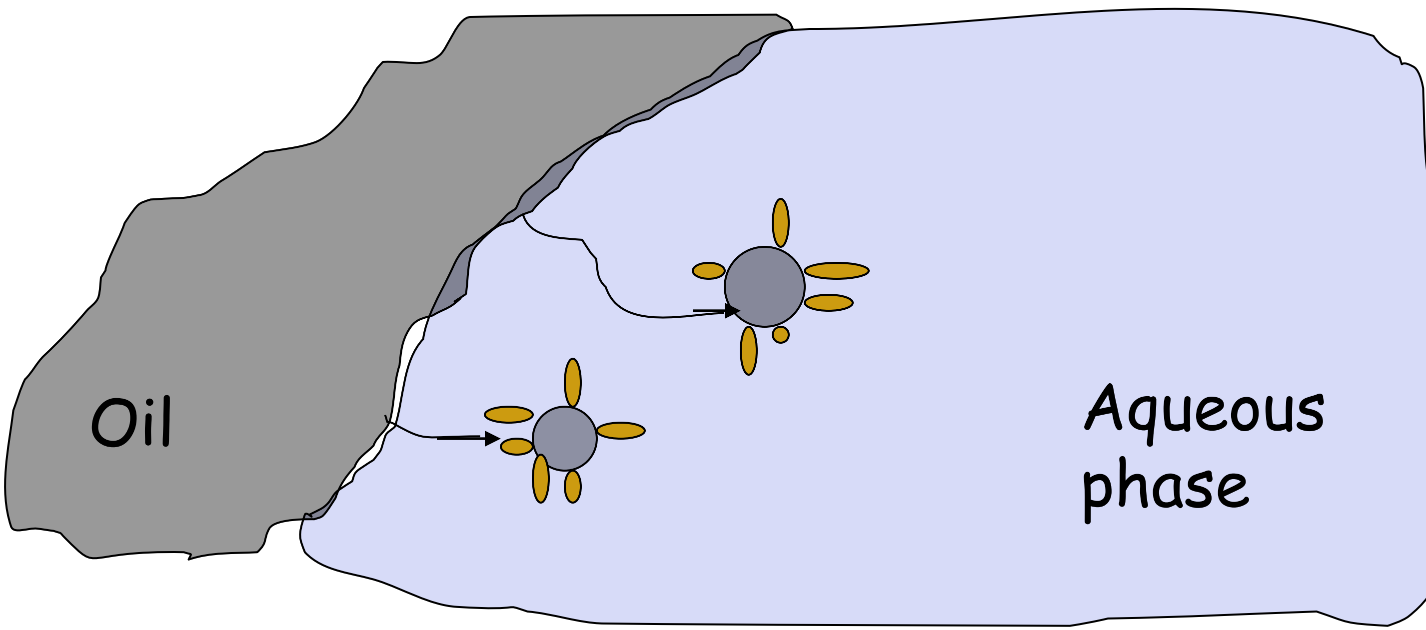
Bioavailability of petroleum hydrocarbons

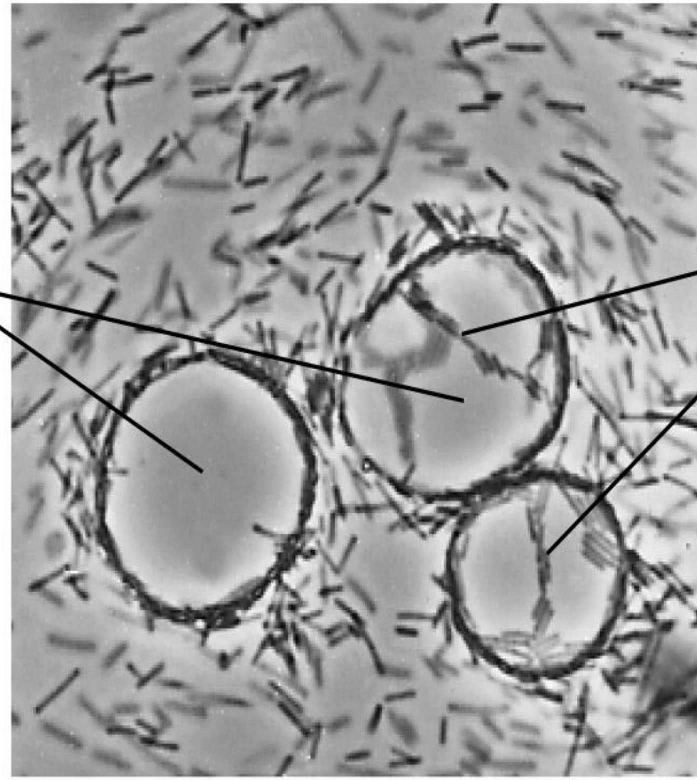
- Direct contact of microorganisms with a bulk liquid hydrocarbon phase (interfacial contact)



Bioavailability of petroleum hydrocarbons

- Emulsification of hydrocarbon followed by direct contact and/or solubilization (some bacteria have the capability of producing bioemulsifying agents)

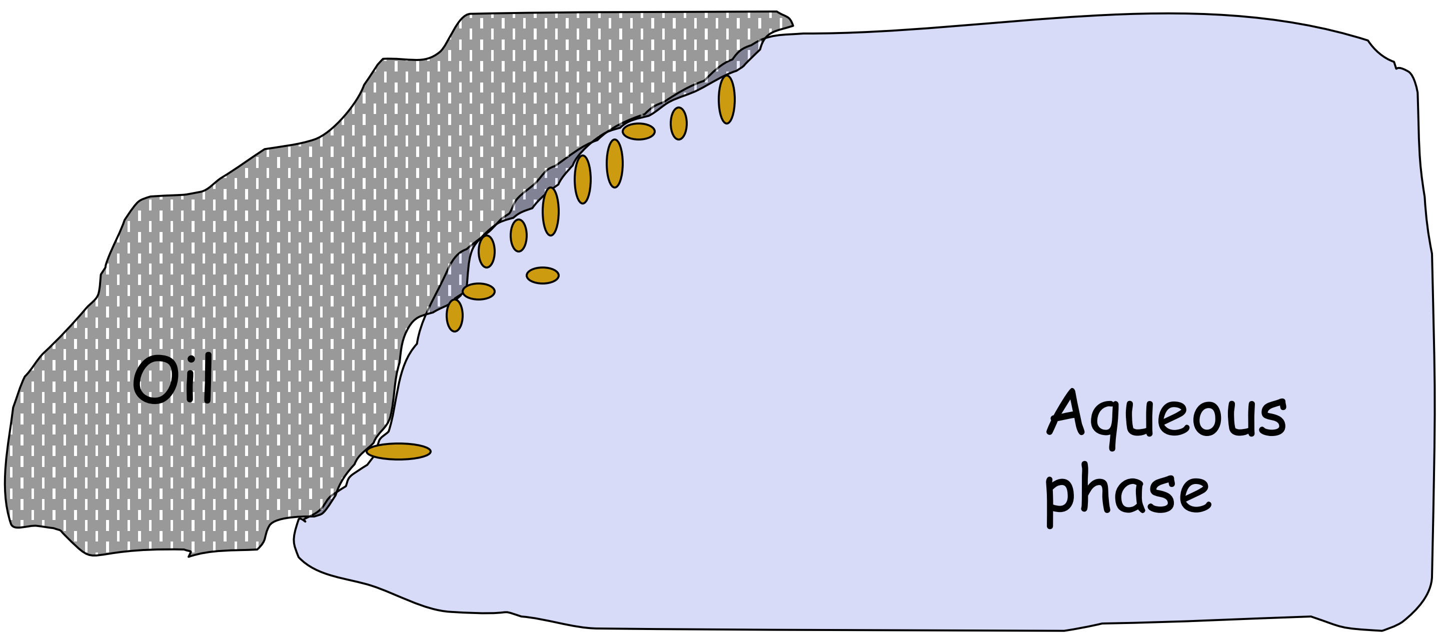




Oil-degrading bacteria congregate at the oil-water interface but not within the oil droplet itself

Bioavailability of petroleum hydrocarbons

- What if the hydrocarbon is a solid phase?
  Same process but much slower!



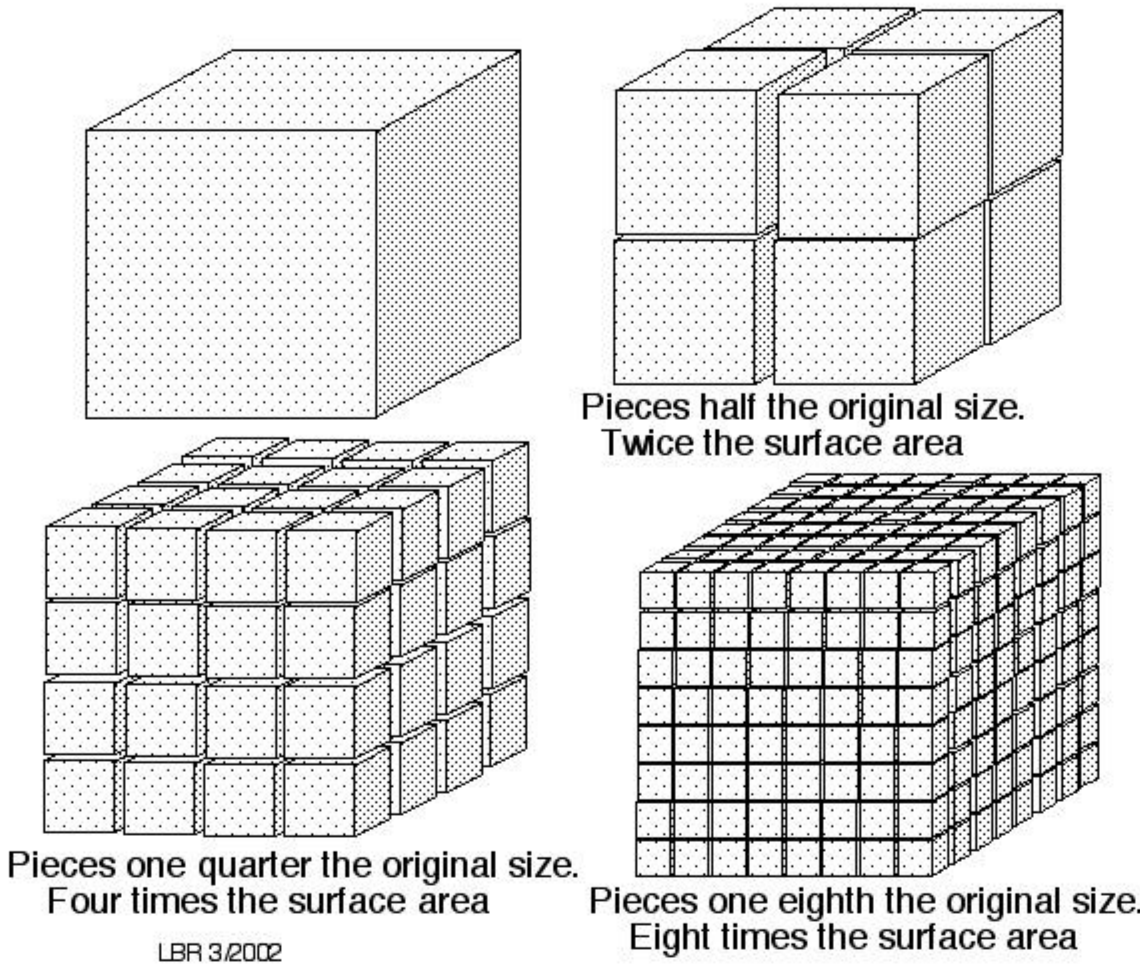
Therefore, biodegradation of hydrocarbons in soil is favored by:

- Greater solubility in water
  - No control
- Increased surface area of a liquid phase
  - This we can influence by dispersing the hydrocarbon in the soil
- Liquid vs. solid phase (temperature and fraction of heavy hydrocarbons play a role)
  - We exert control on this factor by reducing volatilization of light hydrocarbons that serve as solvents for heavier hydrocarbons

The key to getting the most out of bioremediation is optimizing the *growth* of the microbes

- **Getting the microbes together with the hydrocarbon**
- Making sure the microbes have enough of the right nutrients
- Getting oxygen to the microbes
- Optimizing environmental conditions (to the extent we can)
- Moisture! Moisture! Moisture!

Increasing surface area for contact between soil water and hydrocarbon is very important to increasing rates of biodegradation!



Sampling and analysis of petroleum hydrocarbons in soil

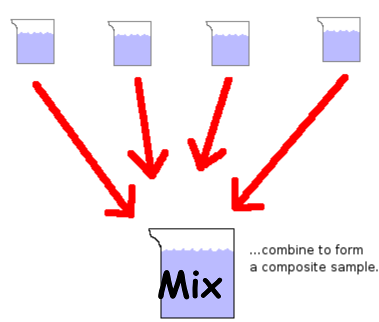
Soil sampling

- Hydrocarbon (and nutrient, salt, etc.) concentrations in soil at oil- (or brine-) impacted sites are highly variable over the area of the spill
- The sampling method is dependent on what you want to know
  - Grab sample – soil sample from a single spot in the impacted site
    - Analysis of this sample tells you only the characteristics of that sample point
    - Usually used to determine the distribution of a contaminant over a site or for screening

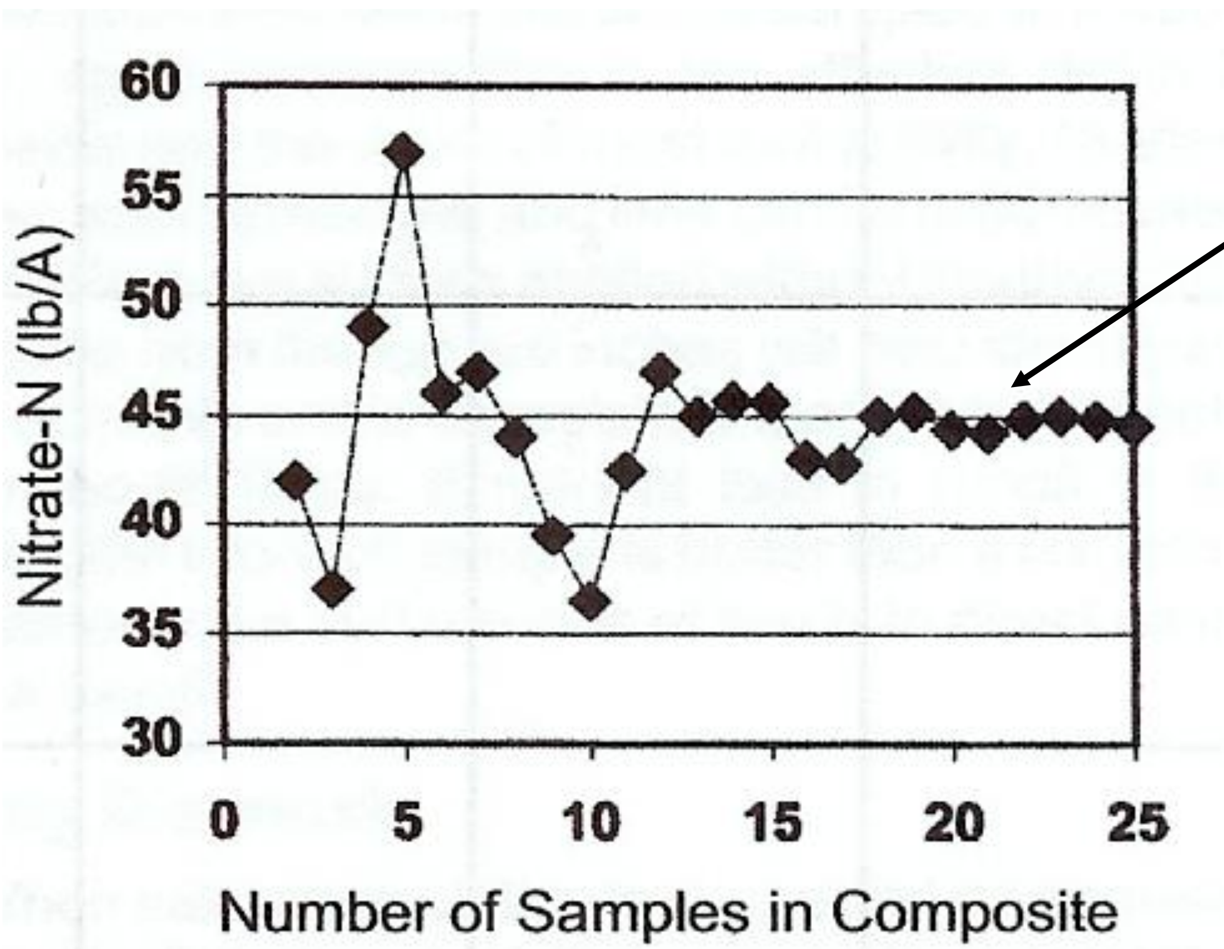
Soil sampling

- Composite sample – composed of a mixture of **equal volumes** of grab samples from several individual spots in the impacted site
  - Analysis of this sample can tell you the average concentration of a contaminant (or nutrient) over the area sampled
  - Usually used to reduce analytical costs

Equal volumes of several grab samples from different locations



How many discrete or grab samples per composite?



20 is the standard

Soil sampling

- Make a sketch of the site
- Note easily recognizable features:
  - Clearly different soil types
  - Different amounts of oil staining
  - Different slopes
  - Very rocky conditions
- Establish a permanent reference point for measurements and add dimensions to your sketch
- Take photos of the site which include your reference point

Soil sampling

- Soil samples for hydrocarbon analysis are generally taken in glass jars with Teflon-lined lids (completely full), stored and shipped at 4 ^{o}C
- There is a maximum holding time before extraction and a maximum holding time before analysis of the extract for the analysis to be considered valid
- Consult with the appropriate regulatory agency for their requirements

See Appendix for special considerations for soil sampling when litigation is possible

Analysis of petroleum hydrocarbons in soil

Total Hydrocarbon Measurements

- Total Petroleum Hydrocarbons (TPH) - Method 418.1
  - "measure of mineral oils only"
  - Summary of method:
    - Soil extracted with Freon 113 to solubilize hydrocarbons
    - Extract treated with silica gel to remove polar organics
  - Absorbance determined in the IR region and compared to standards

Analysis of petroleum hydrocarbons in soil

Total Hydrocarbon Measurements

- Oil & Grease – Method 413.1
  - "measure of biodegradable animal greases and vegetable oil along with the relative non-biodegradable mineral oils"
  - Summary of method: Like TPH without silica gel treatment

Analysis of petroleum hydrocarbons in soil

Replacing 418.1

- EPA 1664
  - n-Hexane extraction, with or without silica gel treatment, followed by gravimetric analysis
  - HEM (hexane extractable material)
  - SGT-HEM (silica gel treated hexane extractable material)

Analysis of petroleum hydrocarbons in soil

Replacing 418.1

- TNRCC Method 1005
  - n-Pentane extraction followed by GC with FID detector
  - Separates TPH into three fractions:
    - nC_6 to nC_{12}
    - $>nC_{12}$ to nC_{28}
    - $>nC_{28}$ to nC_{35}
  - TPH reported as sum of the three fractions
  - Newer versions separate into aromatic and aliphatic before GC

Analysis of petroleum hydrocarbons in soil

EPA 8015B

- Gasoline range organics (GRO) and diesel range organics (DRO)
  - Solvent extraction followed by GC/FID
  - GROs: C_6 to C_{10} (bp range 60°C – 170°C)
  - DROs: C_{10} to C_{28} (bp range 170°C to 430°C)

Analysis of petroleum hydrocarbons in soil

Measurement of Specific Target Compounds

- Use gas chromatography (GC) with very sensitive and selective detectors:
  - MS-mass spectrometer (GC/MS refers to a gas chromatograph coupled with a mass spectrometer)
  - PID-photoionization detector (highly selective for aromatics over aliphatics)
  - FID-flame ionization detector (nearly universal response to hydrocarbons, no selectivity)

Analysis of petroleum hydrocarbons in soil – in-house methods

siteLAB

Petroleum Hydrocarbon Solutions

According to the EPA "The UVF-3100A exhibited the following desirable characteristics of a field TPH measurement device:

- Good accuracy
- Good precision
- High-sample throughput
- Low measurement costs
- Ease of use"

Analysis of petroleum hydrocarbons in soil – field methods

One of several commercially available field kits for hydrocarbons in soil; sensitive to moisture

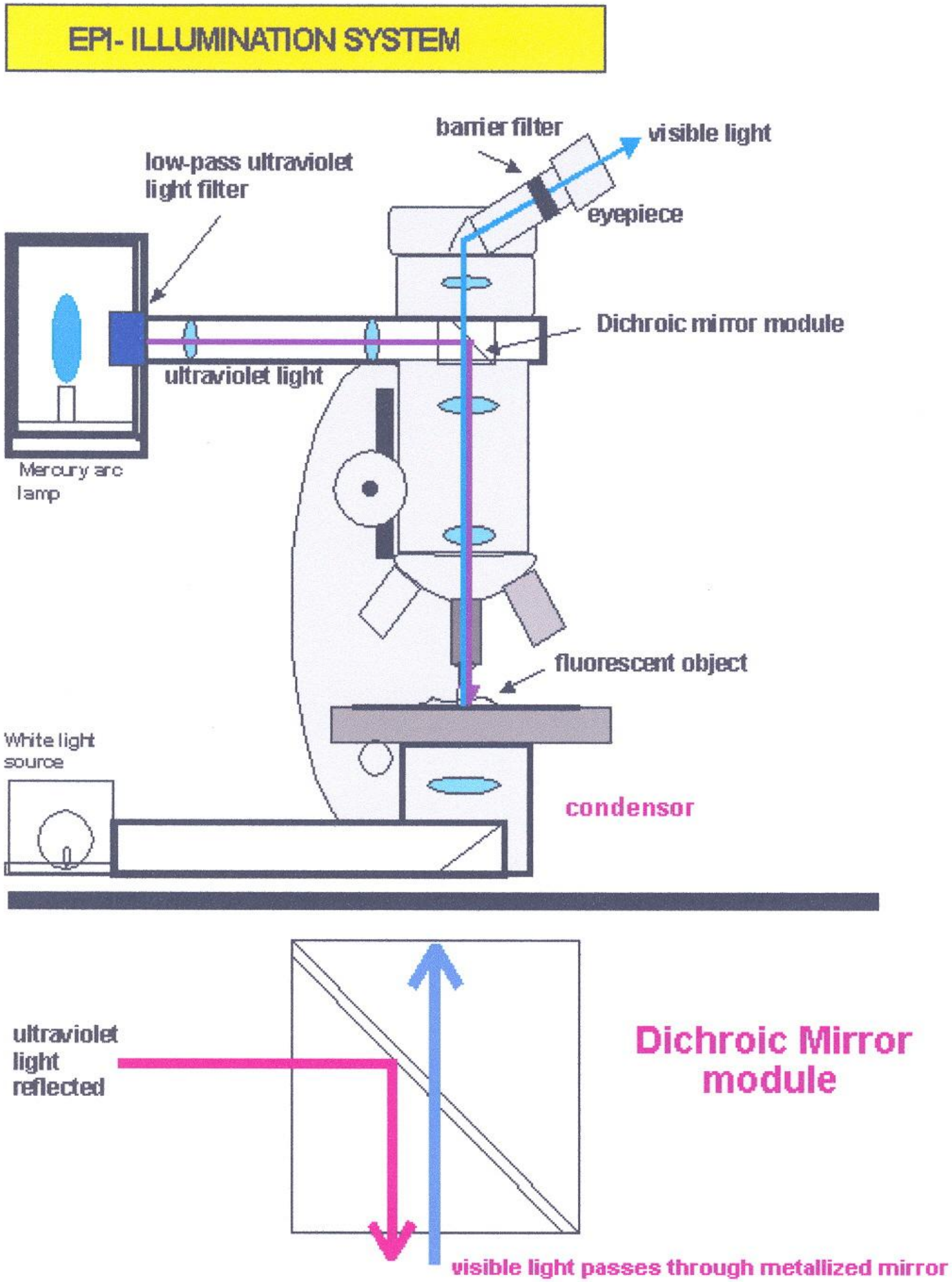
Assessing microbial activity in soils

It is sometimes necessary to provide evidence that the disappearance of hydrocarbons in soil during treatment is actually due to biodegradation. The most straight forward way of doing this is to demonstrate an increase in microbial activity in the soil.

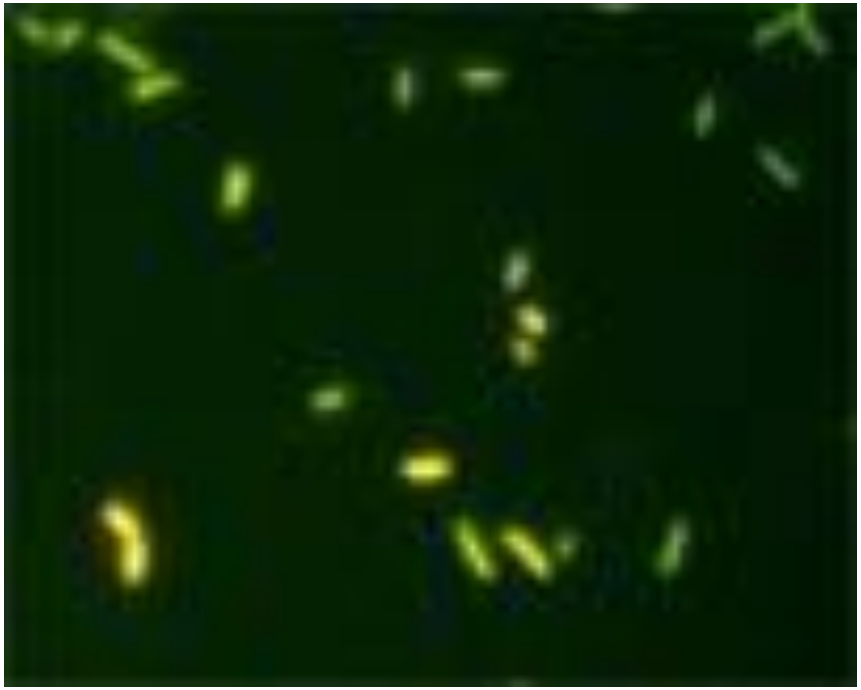
- Counts of microorganisms
  - Direct count for bacteria
  - Viable plate count for bacteria
- Soil gas analysis

Direct count for bacteria

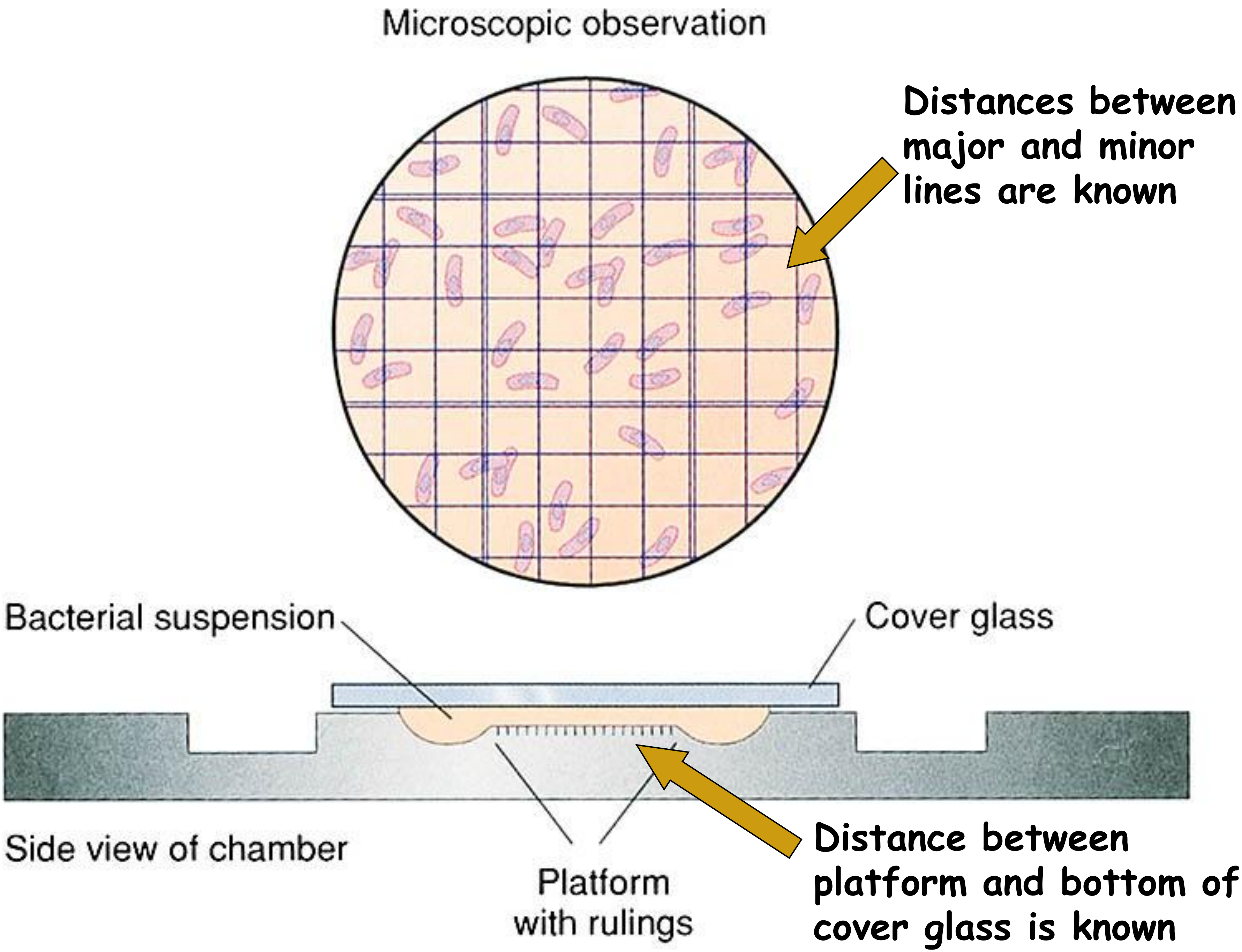
- Soil is extracted with a 0.1% sodium pyrophosphate (10 g of soil and 95 mL extractant and treated with a dye (such as acridine orange) which binds cells and fluoresces under UV light
- Suspensions are examined using a fluorescent microscope and cells counted directly



Fluorescence microscope



Fluorescent bacteria

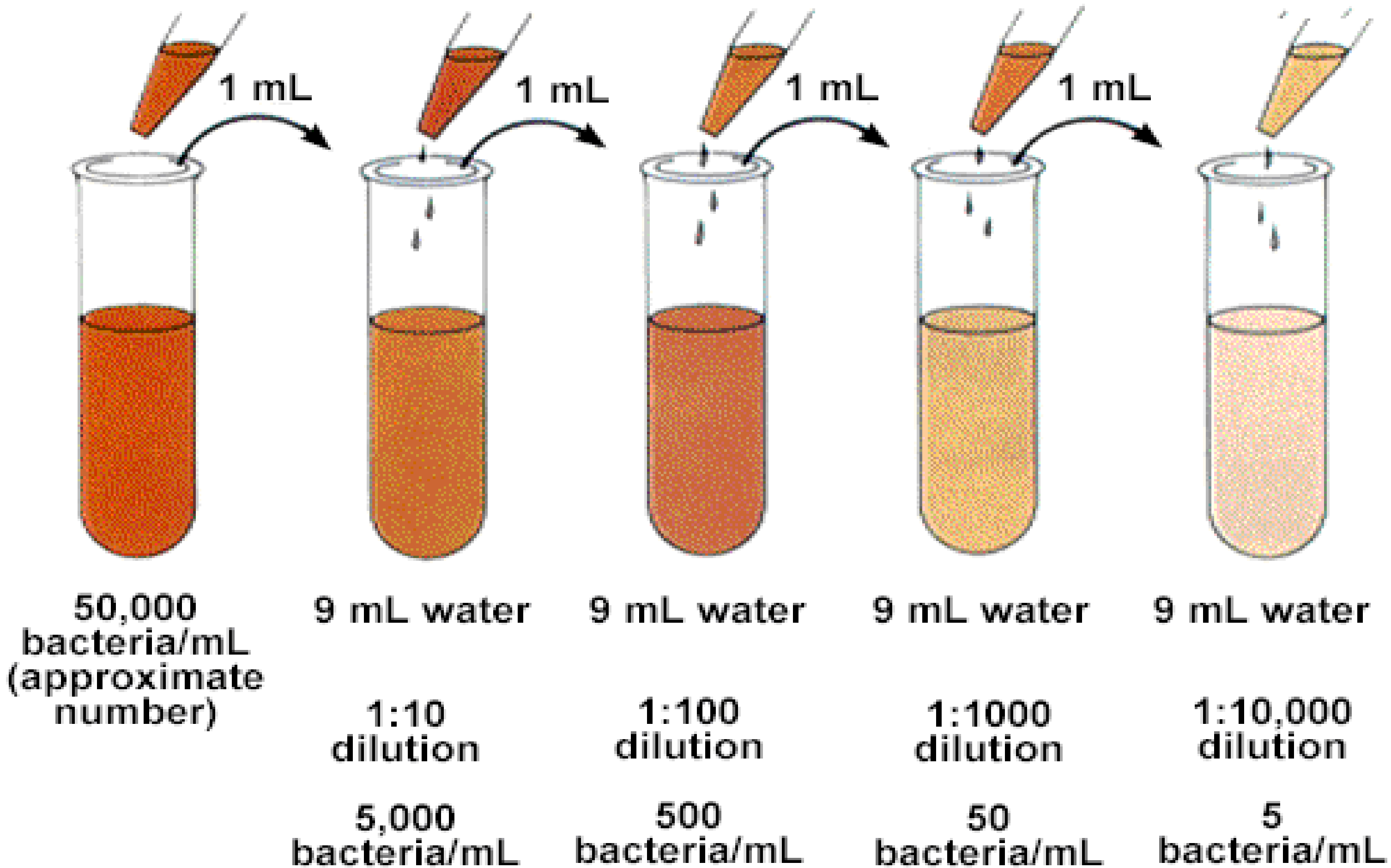

Microscopic observation
Distances between major and minor lines are known
Bacterial suspension
Cover glass
Side view of chamber
Platform with rulings
Distance between platform and bottom of cover glass is known

See the Appendix for a sample direct count calculation

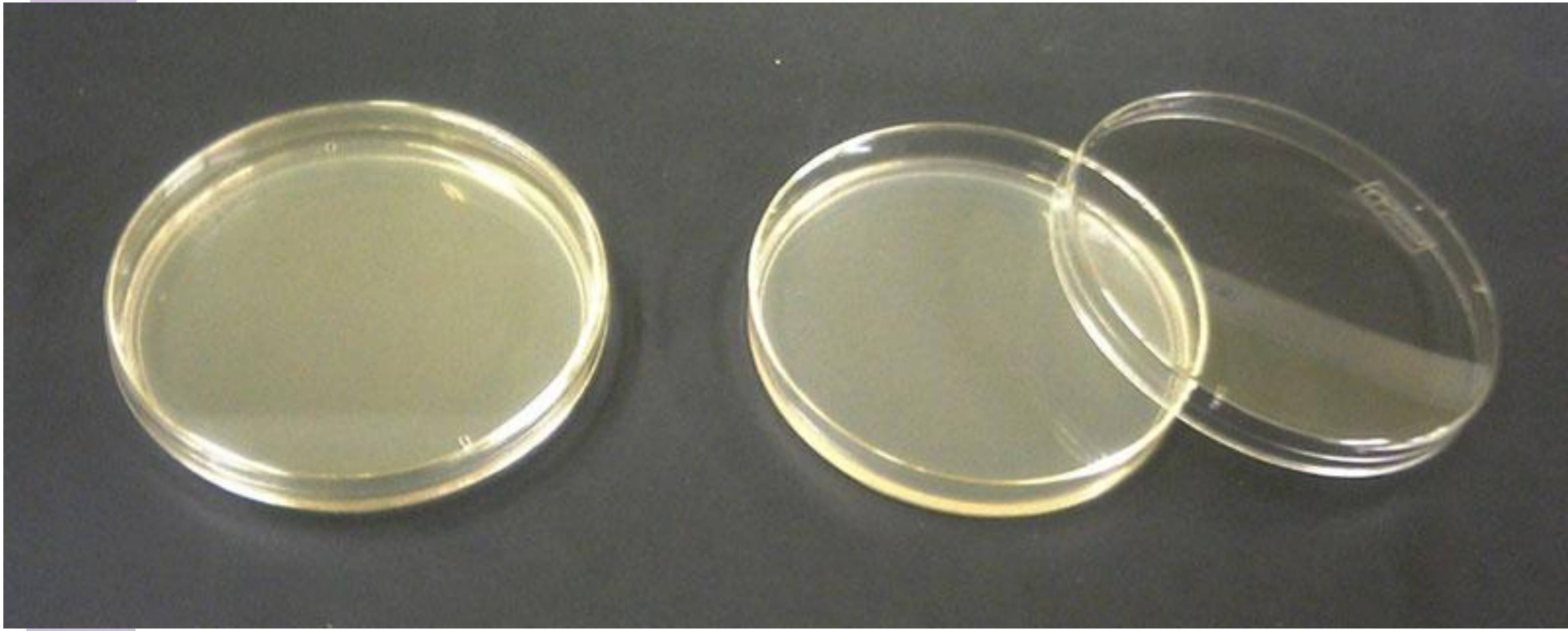
Viable plate count for bacteria

- Soil is extracted with a 0.1% sodium pyrophosphate (10 g of soil and 95 mL extractant) and serially diluted in a phosphate buffer
- Dilutions are spread (0.1 mL) on an agar medium containing all of the nutrient requirements of the bacteria to be counted
- Plates are incubated at a suitable temperature (25-37 ^{o}C) for several days, and colony-forming units (cfu) counted. It is assumed that each cfu resulted from the deposition of one viable bacterial cell on the medium.

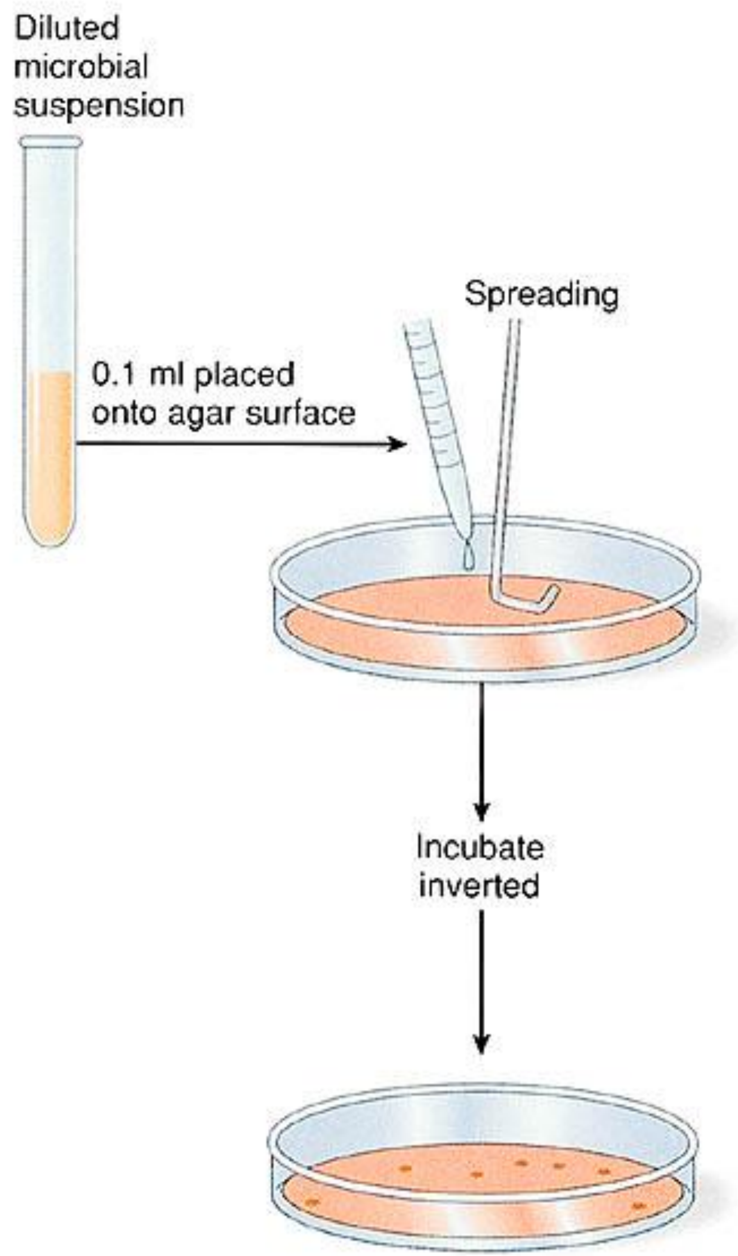
Serial dilution of soil extract



Agar plates for viable plate counts



0.1 mL of each dilution is spread with a sterile glass rod onto one or more agar plates and the plates allowed to incubate for colonies to form.



b.

Development of visible colonies on agar plates

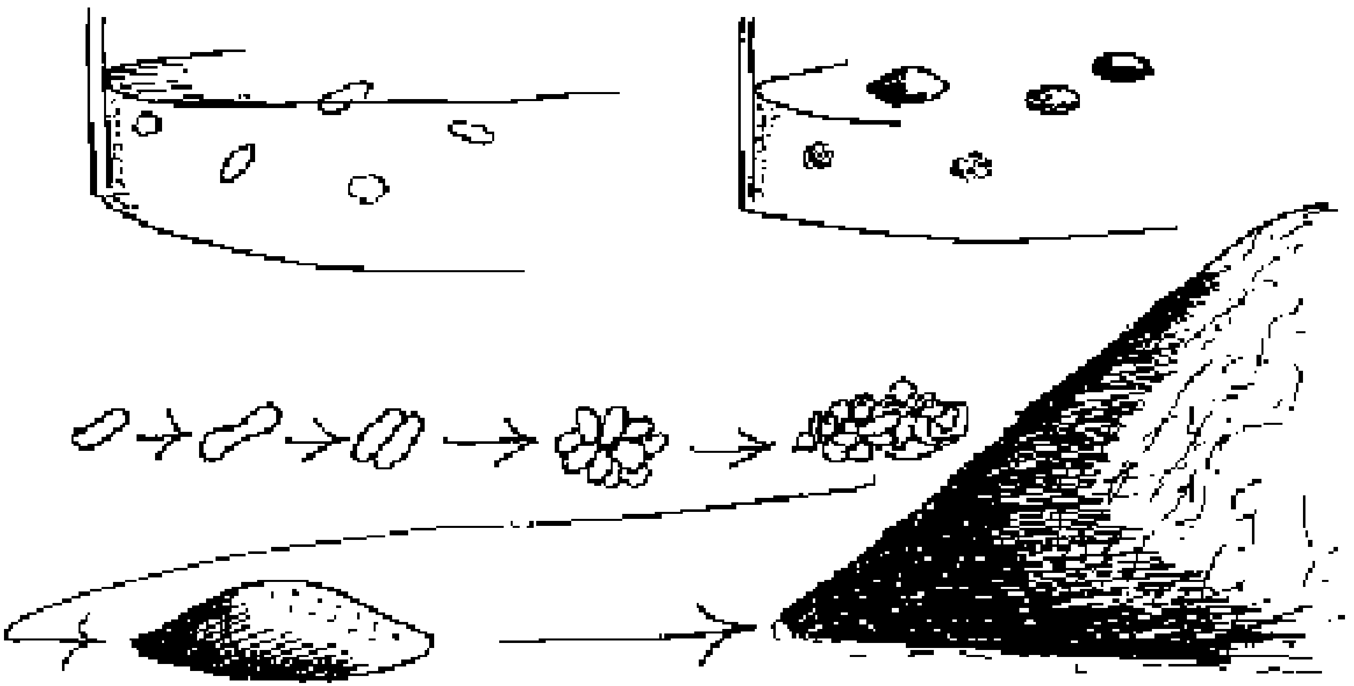
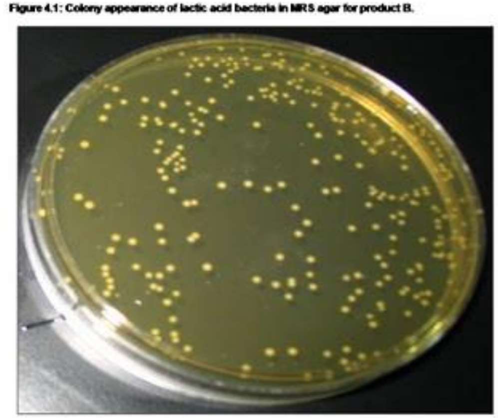
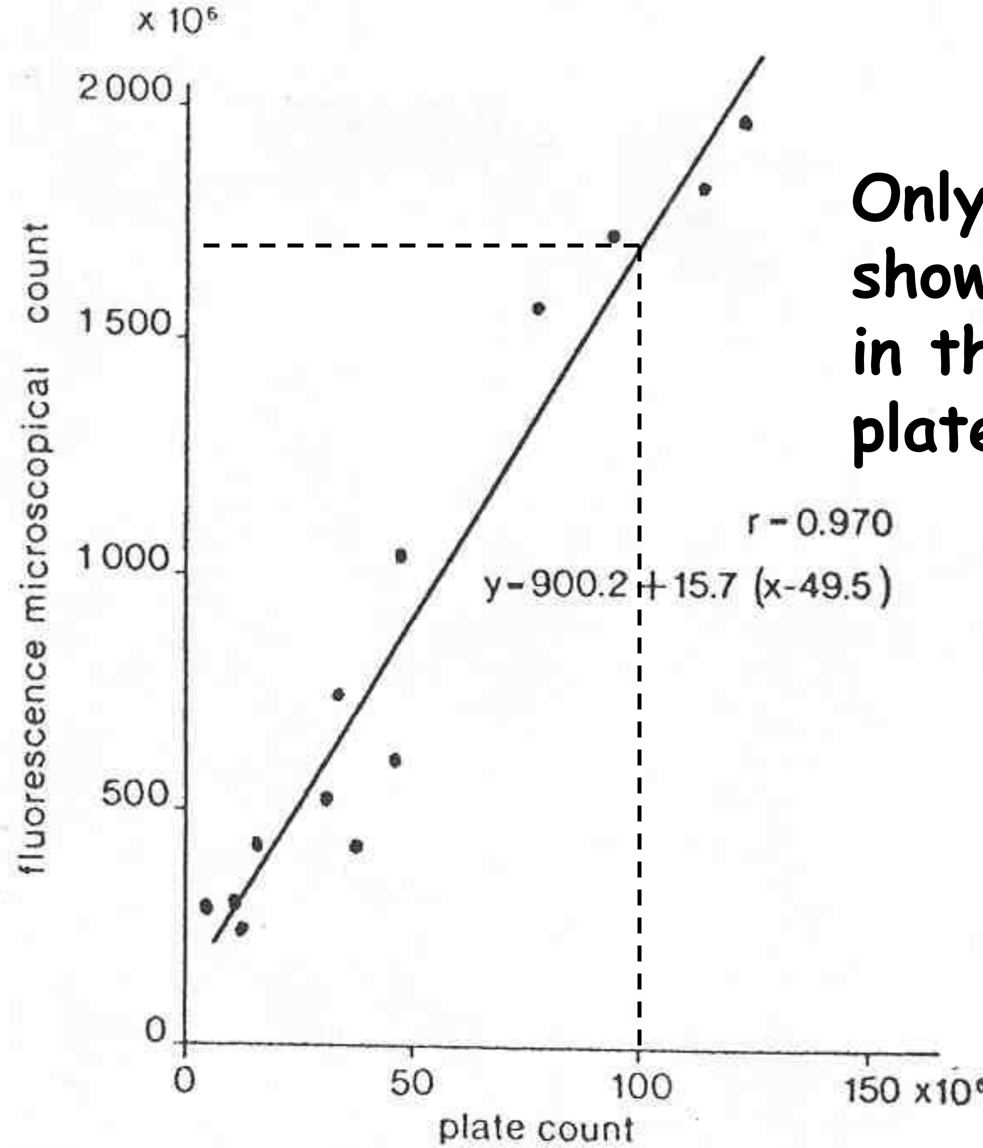


Figure 4.1: Colony appearance of lactic acid bacteria in MRS agar for product B.





Only 6% showed up in the plate count

Correlation between direct microbial counts by fluorescence microscopy and viable plate counts for soil samples.

What use is a viable plate count if it doesn't count everything?

Because the viable plate method counts only **culturable bacteria**, the principal utility of this method in bioremediation is in following ***changes*** in microbial populations.

- An increasing viable plate count indicates stimulation and increasing numbers of bacteria
- A decreasing viable plate count indicates decreasing numbers of bacteria

Vendor speak

- "Two weeks after applying our product to the landfarm the plate count was 6.5×10^8 per gram of soil"
- What does this mean to you?
  - Nothing! – what was the plate count before adding the product?
  - These are not particularly high numbers anyway

Soil gas analysis

- Samples of soil gas withdrawn from the subsurface and analyzed for:
  - Carbon dioxide (CO_2)
  - Oxygen (O_2)

Interpreting soil gas analysis

- Hydrocarbon degradation consumes O_2
  - O_2 concentrations in soil gas below atmospheric concentrations (21%) are indicative of active aerobic microbial activity
- Hydrocarbon degradation produces CO_2
  - CO_2 concentrations in soil gas above atmospheric concentrations (0.035%) are indicative of active aerobic microbial activity
    - Baseline microbial activity in soil can elevate CO_2 concentrations to as much as 1%. Always check background and take measurements at beginning of bioremediation project

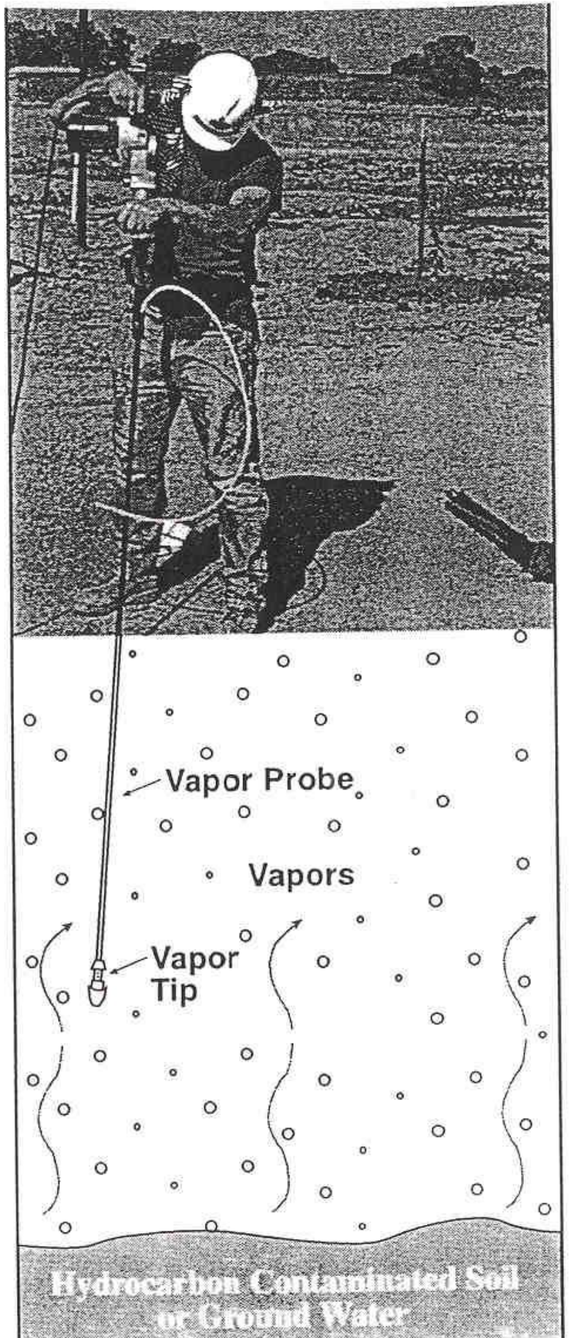
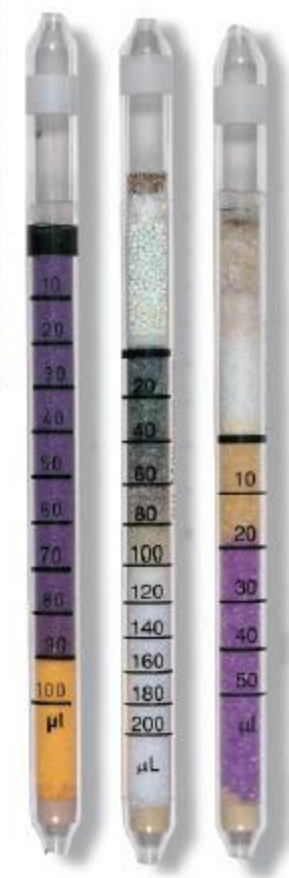
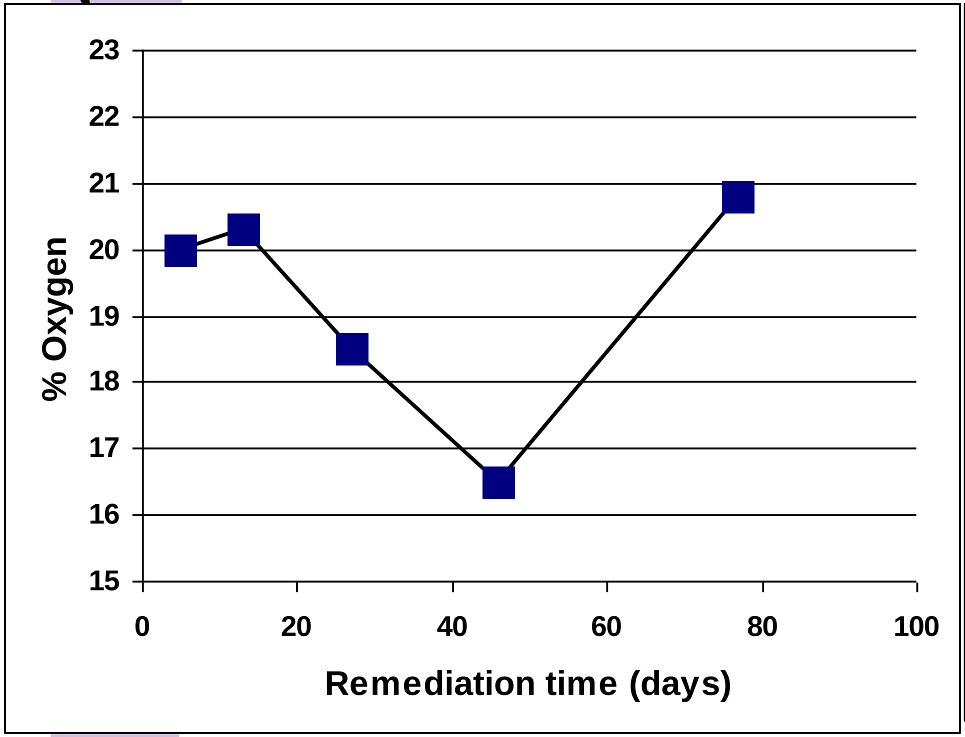


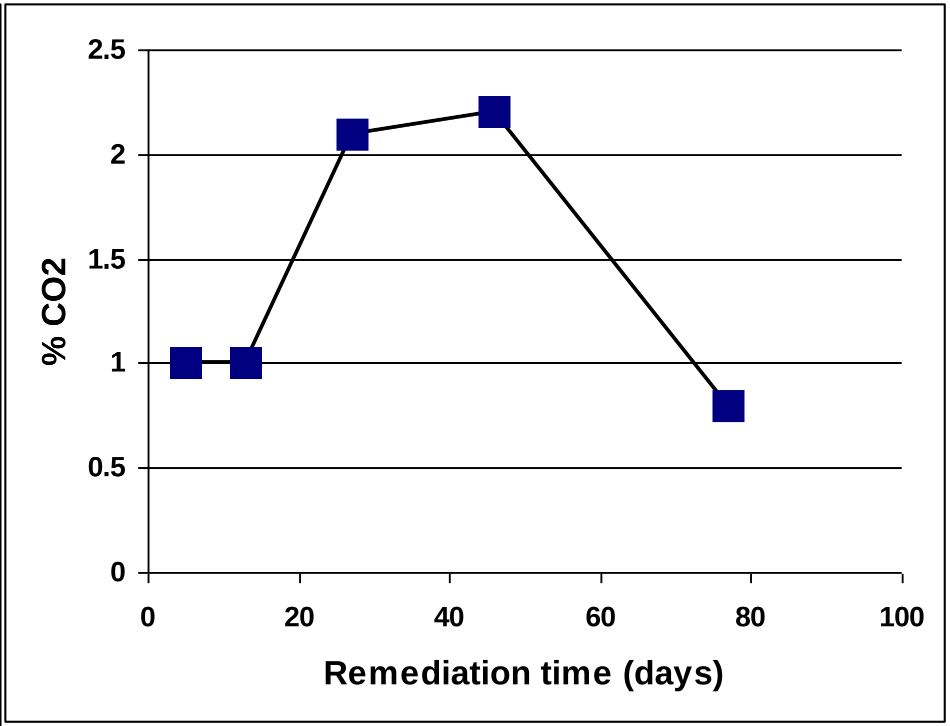
Figure 1: Insertion of Soil Gas Vapor Tip into the Subsurface with Electric Roto Hammer

Alternative means of soil gas analysis: Draeger tubes for CO_2, O_2, and petroleum hydrocarbons



Soil gas analysis from a landfarm





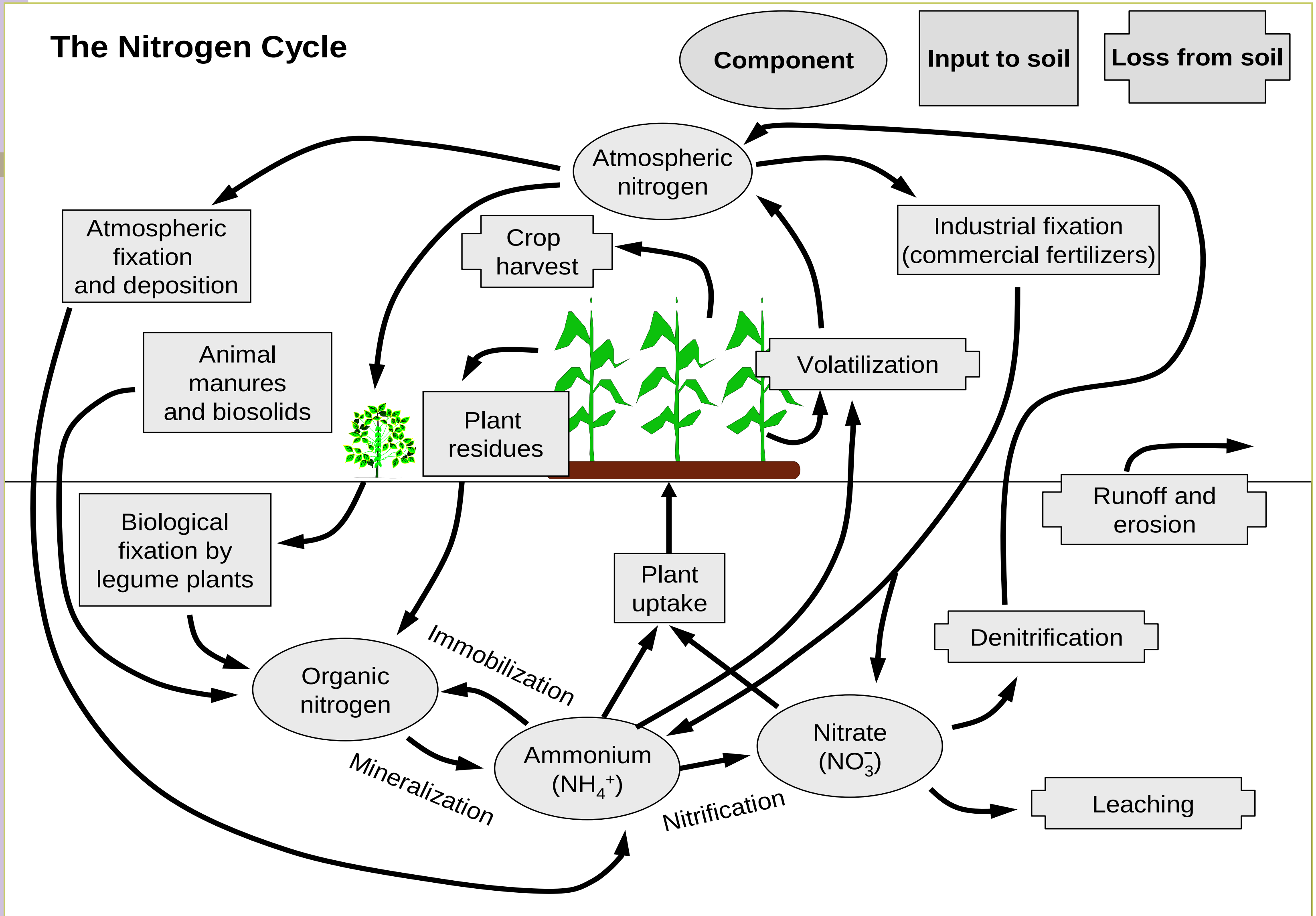
Depth = 6 inches

Occurrence, fate, and analysis of N, P nutrients in soil

Forms of soil nitrogen

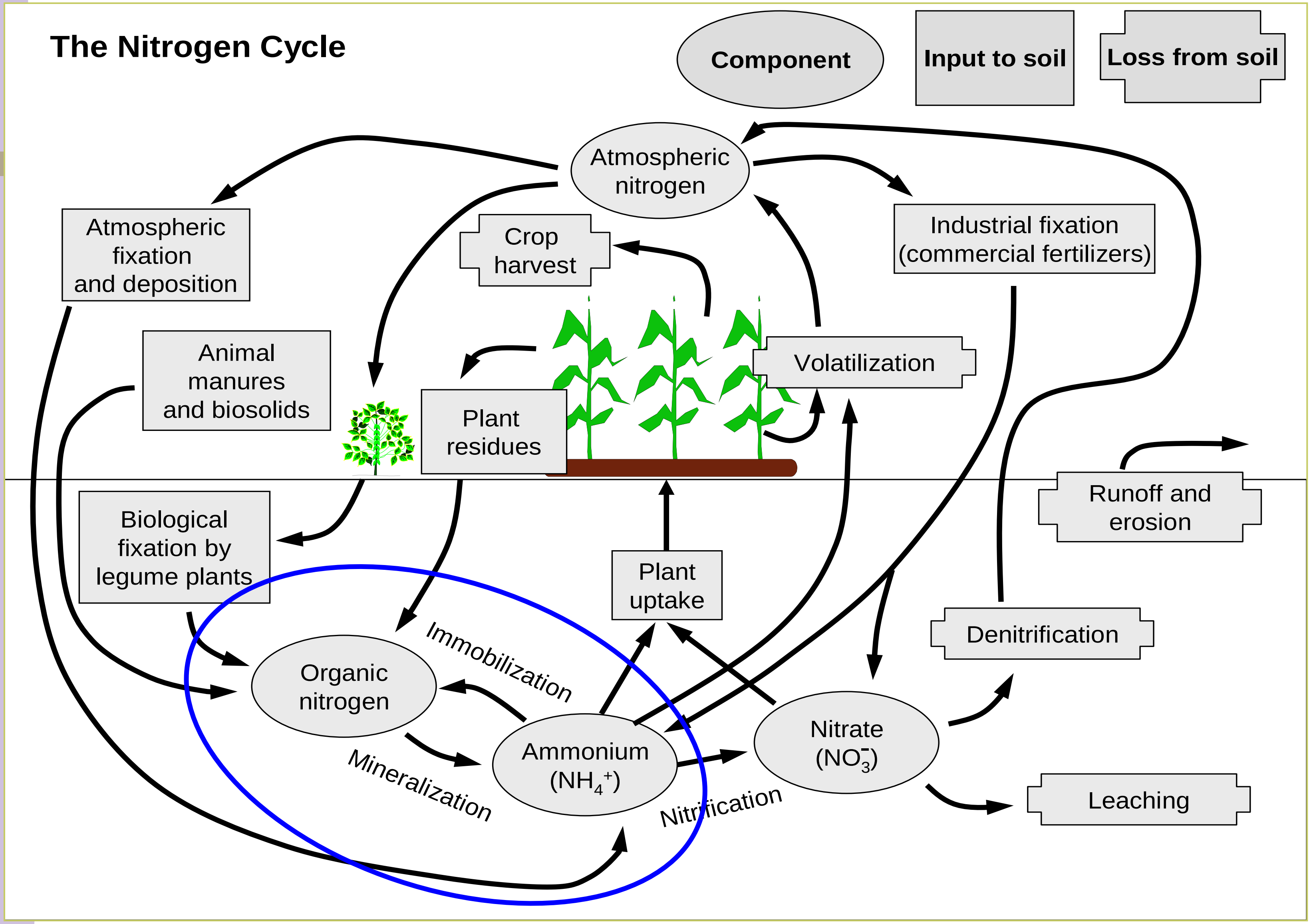
- Nitrogen concentrations in the top 1 ft of cultivated soil in the U.S. range from 0.03 to 0.4% by dry weight. 95% of that nitrogen is typically organic and 5% inorganic
  - Inorganic nitrogen is readily available to plants and microbes
    - ammonium (NH_4^+)
    - nitrate (NO_3^-)
    - nitrite (NO_2^-)
  - Organic forms of nitrogen must be broken down by the action of soil microorganisms to liberate inorganic nitrogen before the nitrogen is available to plants and microbes

The nitrogen cycle



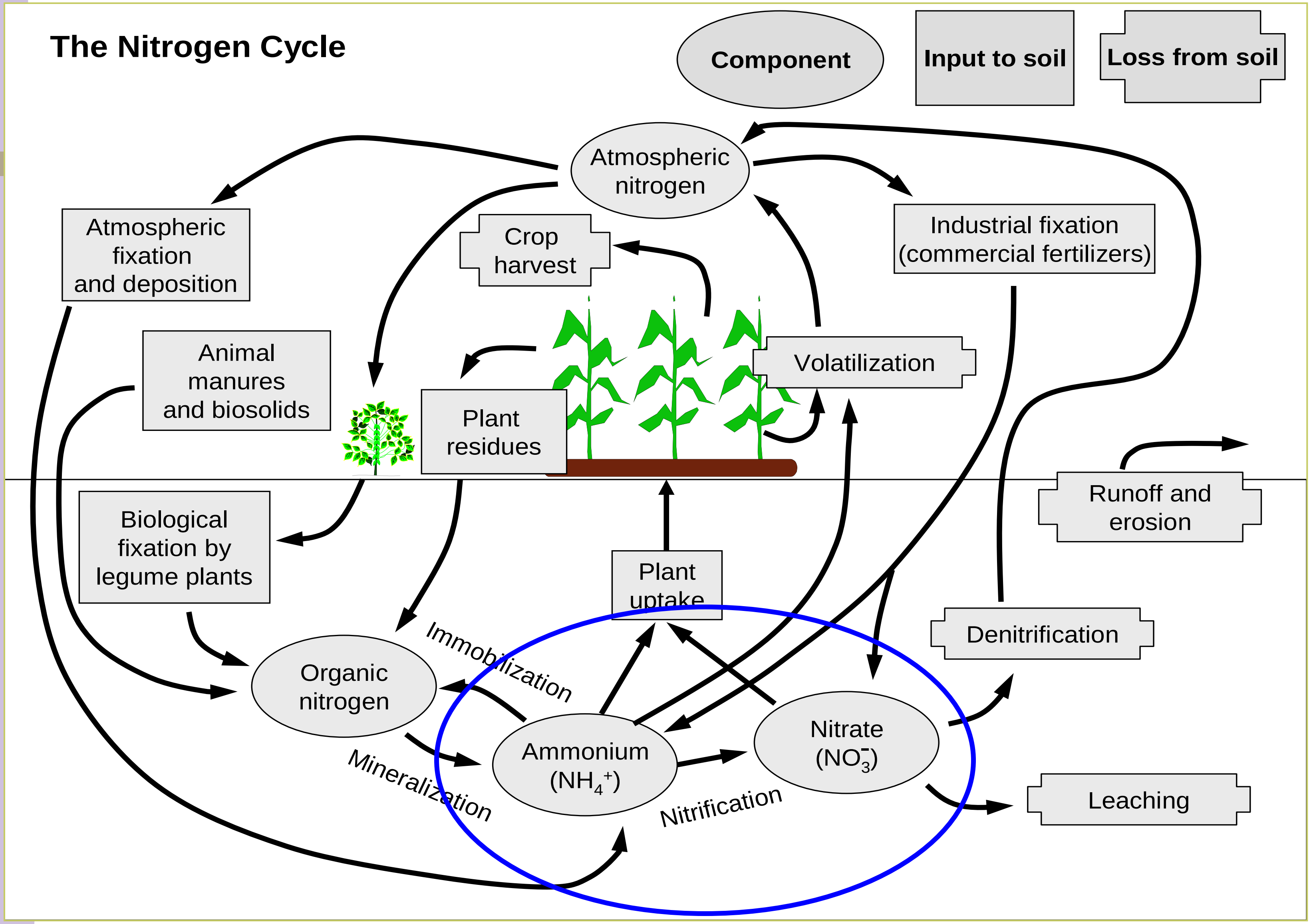
Modified from the Potash & Phosphate Institute web site at www.ppi-ppic.org

The nitrogen cycle



Modified from the Potash & Phosphate Institute web site at www.ppi-ppic.org

The nitrogen cycle



Modified from the Potash & Phosphate Institute web site at www.ppi-ppic.org

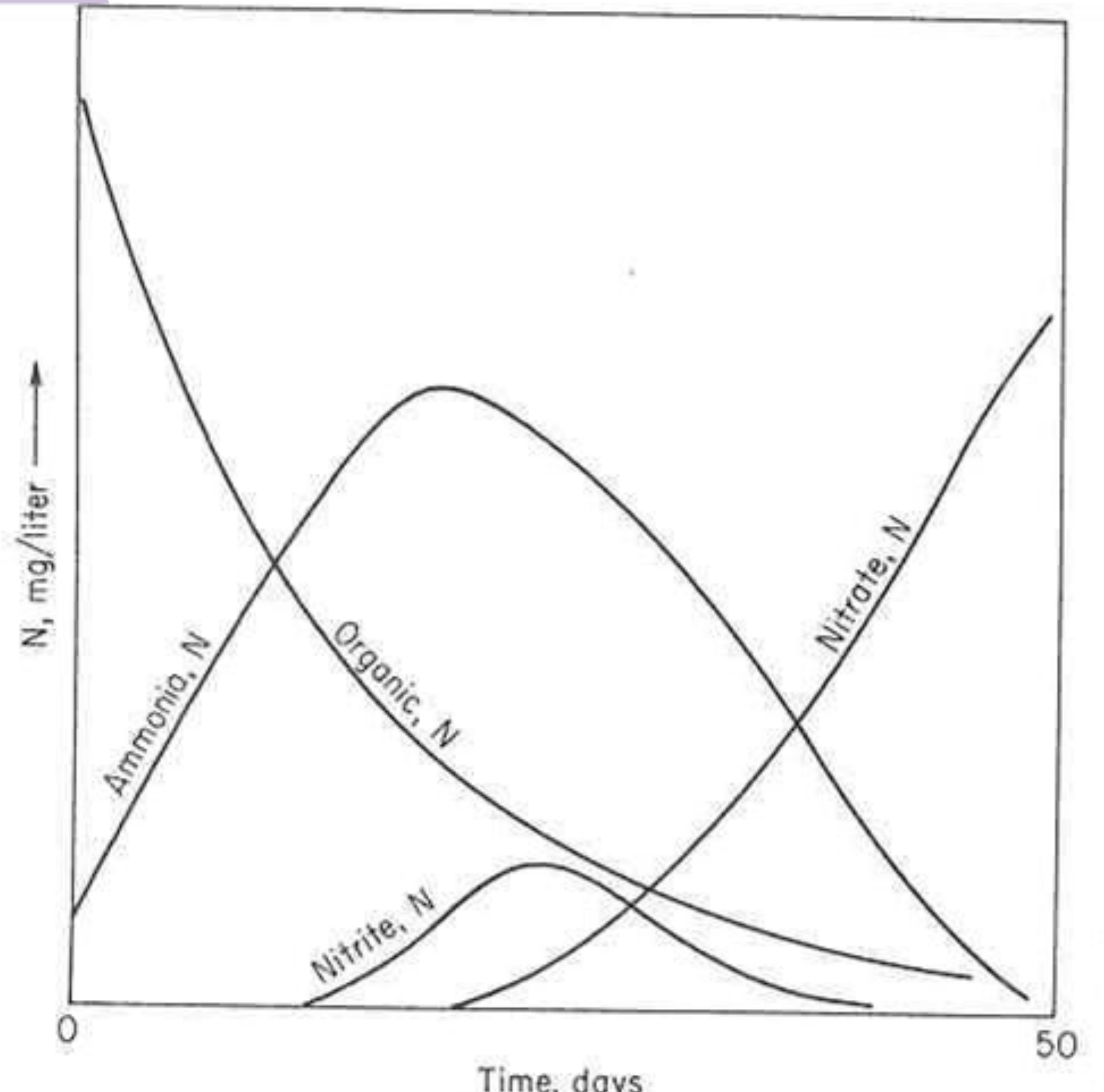
Nitrification

- Conversion of ammonium ion to nitrate is a two step process

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

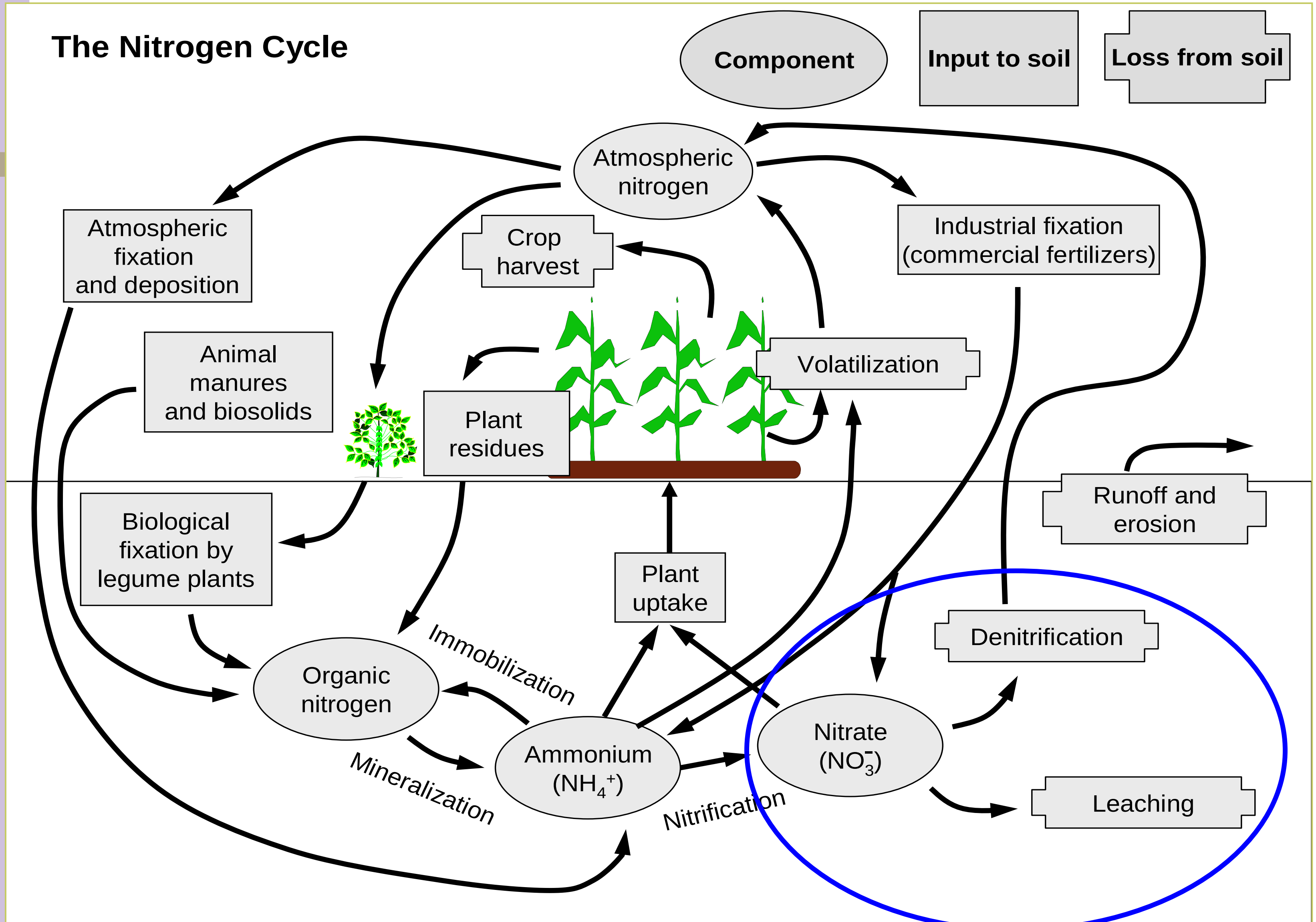
1 2

- Most of the time step 2 is faster that step 1 and nitrite (NO_2^-) never accumulates
- If the soil is slightly alkaline (pH > 7.5) the 2nd step slows down and nitrite accumulates in the soil
- Organic-N → NH_4^+ raises pH



Fate of organic nitrogen in soil

The nitrogen cycle



Modified from the Potash & Phosphate Institute web site at www.ppi-ppic.org

Denitrification

- Utilization of nitrate as a substitute for oxygen when not available by denitrifying bacteria

$$NO_3^- \rightarrow N_2$$

- Anaerobic conditions may be caused by:
  - **water logging soil**
  - insufficient aeration resulting in anaerobic microsites
  - Denitrification losses typically **5-35%**

Rates of denitrification losses

Soil temperature (^oF)	Days saturated	Loss of applied N
55-60	5	10%
	10	25%
75-80	3	60%
	5	75%
	7	85%
	9	95%

Measurement of mineral-N in soils

Extraction

- Bioavailable mineral-N = soluble N + exchangeable N
- Soil samples are kept on ice and extracted as soon as possible with 2M KCl (149 g of KCl dissolved in 1 L of water)
  - Example: 40 g wet soil is extracted with 200 mL of 2M KCl with shaking for 1 hr, samples is then filtered and analyzed for nitrate and ammonium (nitrite, if desired)

Measurement of mineral-N in soils

Analysis

- Usually colorimetric
  - Nitrate - Cd reduction method
  - Ammonium - Nessler's method
- Use spectrophotometer or color wheel (field kits) to measure intensity of color development
  - Intensity of color proportional to concentration of NO_3^- or NH_4^+ in extract

Typical ag lab price list for nutrient analysis

Soil Fertility	
Routine Soil Test	**$10.00**
pH, NO_3-N, P & K	
Nitrate-N or Ammonium-N	**$4.00**
NO_3-N or NH_4-N	
Nitrate-N and Ammonium-N	**$5.00**
NO_3-N and NH_4-N	
Secondary Nutrients	**$4.00**
Mg, Ca and SO_4	
Micronutrients	**$4.00**
Fe, Zn, Cu, B	

OSU Soils Lab

SOIL TEST REPORT

DR KERRY SUBLETTE
DEPT OF CHEMICAL ENGINEERING
UNIVERSITY OF TULSA
600 S COLLEGE AVE
TULSA, OK 74104

Name:

Location:

GS-I-NI-NUT-S7

Lab ID No.: 465221
Customer Code: 298
Sample No.: 101
Received: 7/12/2007
Report Date: 7/18/2007

- Routine Test -

pH:	5.6
Buffer Index:	6.8
NO_3-N(lbs/A)	
Surface:	6
Subsoil:	
Soil Test P Index:	6
Soil Test K Index:	119

- Secondary Nutrients -

SO_4-S(lbs/A)	
Surface:	
Subsoil:	
Ca (lbs/A):	
Mg (lbs/A):	

- Additional Tests -

OM (%):	1.52
TN (%):	0.09
NH_4-N (lbs/A):	9.49

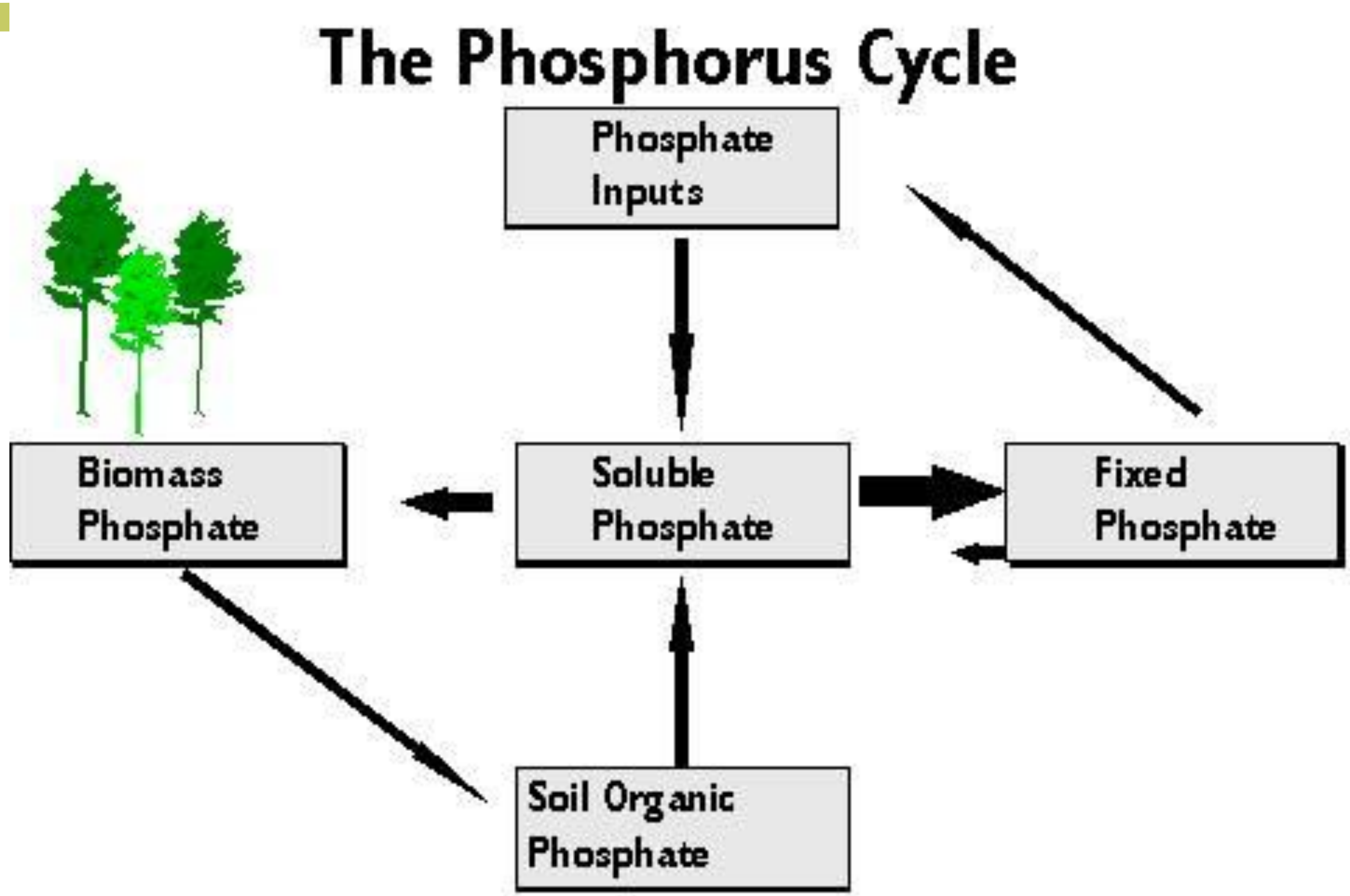
- Micronutrients -

Fe (ppm):	
Zn (ppm):	
B (ppm):	
Cu (ppm):	

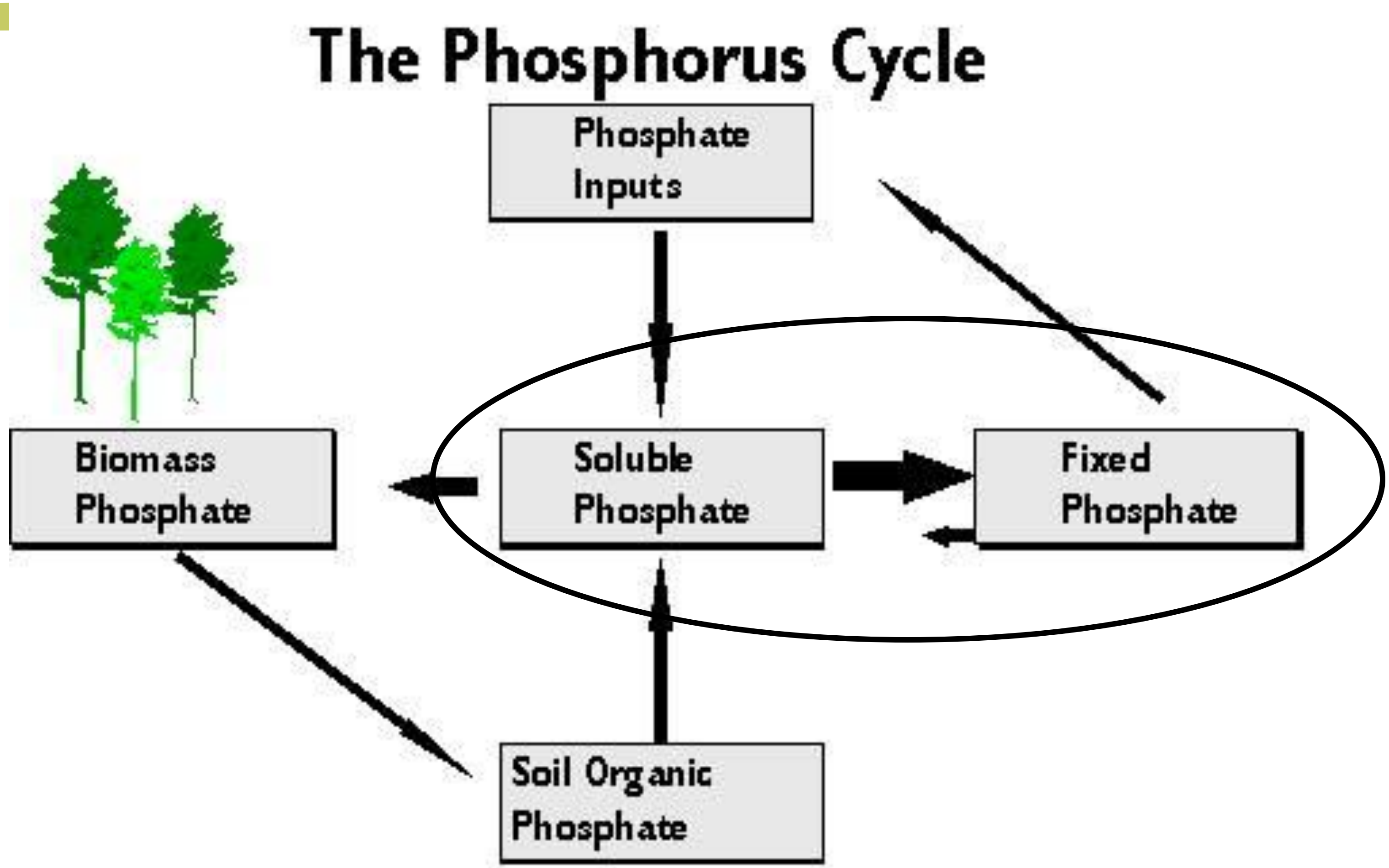
ppm = mg/kg = (lbs/acre)/2

(6.67-inch soil depth assumed)

Soil phosphorus

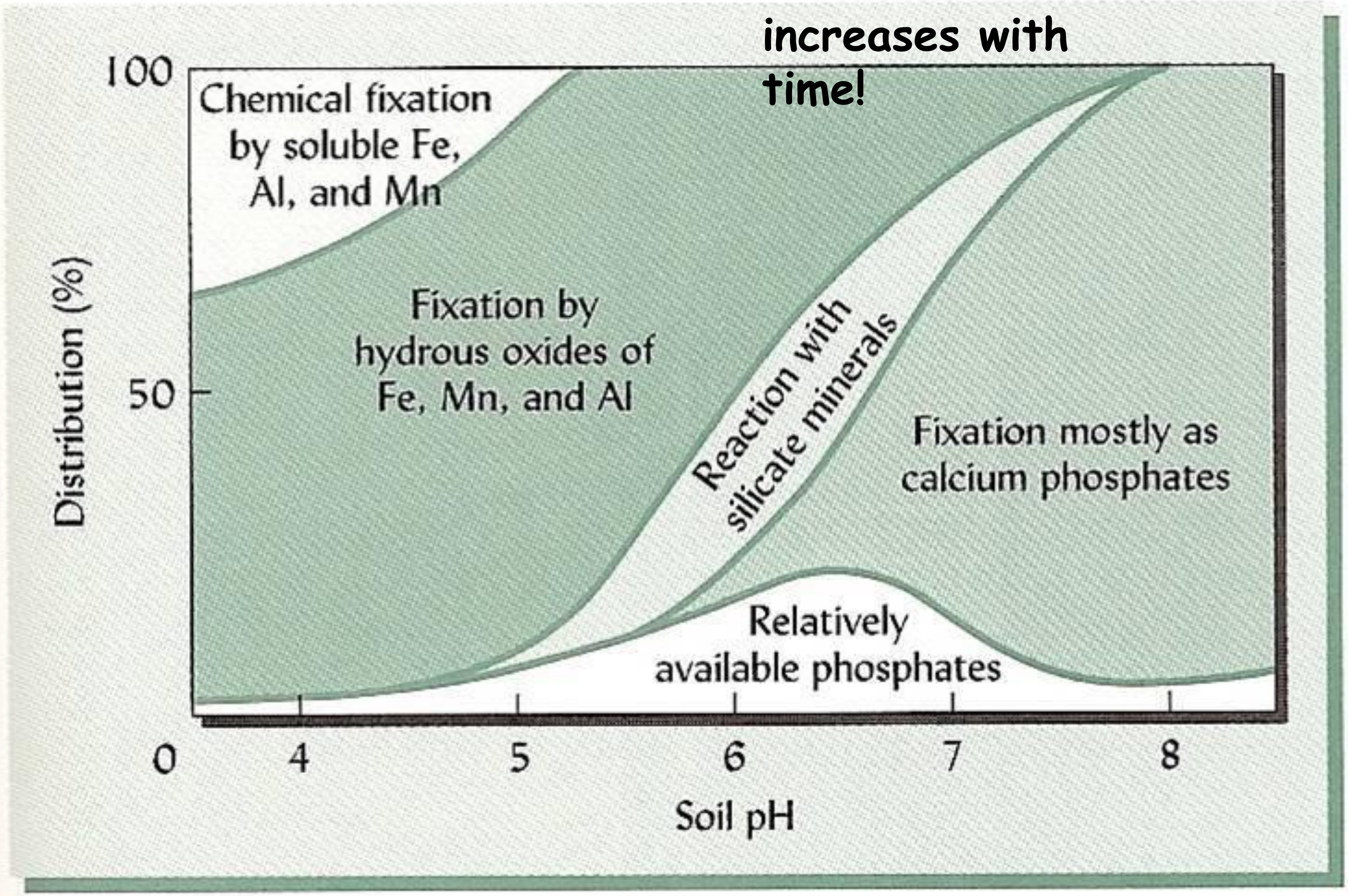


Soil phosphorus



Fixation of added soil phosphates

Note: Fixation increases with time!



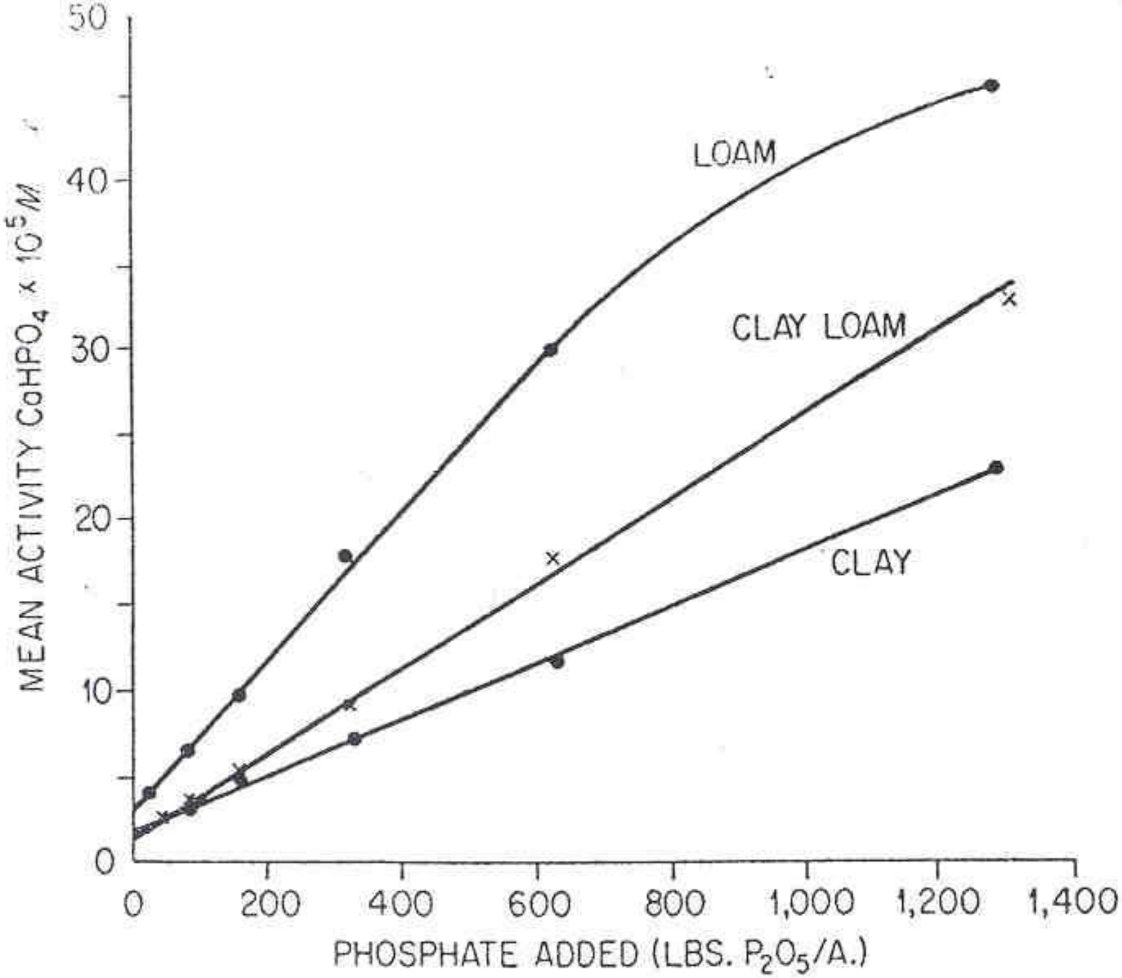


FIGURE 6-8. Phosphorus solubility (the mean activity of dicalcium phosphate in solution) as a function of the amounts of CSP added to three calcareous soils of different texture. [Cole et al., *SSSA Proc.*, **23**:119 (1959).]

Measurement of mineral-P in soils

Extraction

- Bioavailable mineral-P (phosphate) = soluble phosphate + exchangeable phosphate
- Soil samples are kept on ice and extracted as soon as possible with Mehlich III extractant
- Example: 5 g wet soil is extracted with 100 mL of Mehlich III extractant with shaking for 30 min; sample is then filtered and analyzed colorimetrically for bioavailable phosphate (phosphomolybdate method)

OSU Soils Lab

SOIL TEST REPORT

DR KERRY SUBLETTE
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- Secondary Nutrients -

SO_4-S(lbs/A)	
Surface:	
Subsoil:	
Ca (lbs/A):	
Mg (lbs/A):	

- Additional Tests -

OM (%):	1.52
TN (%):	0.09
NH_4-N (lbs/A):	9.49

- Micronutrients -

Fe (ppm):	
Zn (ppm):	
B (ppm):	
Cu (ppm):	

P and K soil test indices – OSU Soils Lab

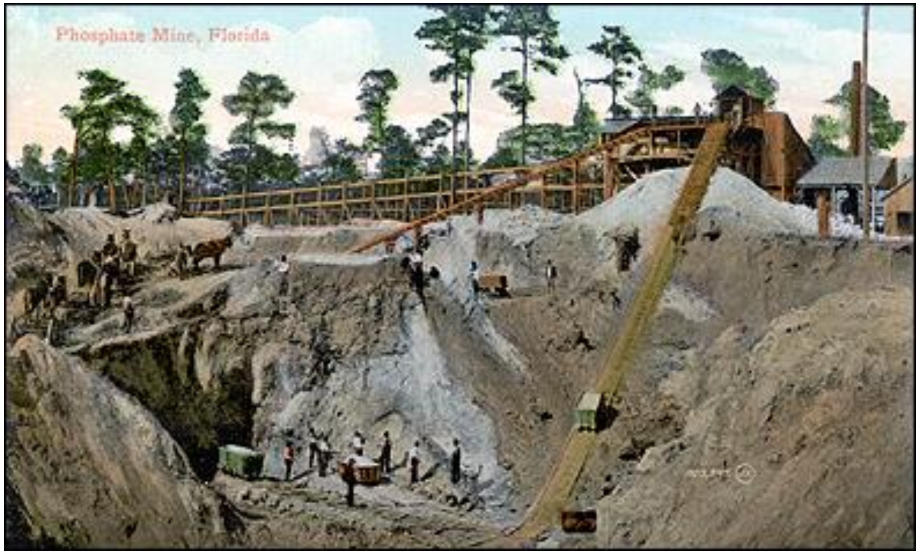
P Soil Test Index	Percent Sufficiency	P_2O_5 (lbs/acre)
0	30	80
10	50	60
20	70	40
40	95	30
65+	100	0

K Soil Test Index	Percent Sufficiency	K_2O (lbs/acre)
0	60	70
75	70	60
125	80	50
200	95	30
250+	100	0

These tables are for cool season grasses; tables differ for different plants/crops

Phosphorus fertilizers

- The original source of P fertilizer is rock phosphate mined in Florida and the Western U.S.
  - acid treated to increase phosphorus availability
  - reacted with ammonia to produce ammonium phosphates



Phosphorus fertilizers

- Some examples:
  - rock phosphate
  - super phosphate
  - triple super phosphate

Increasing wt % P

Potassium fertilizers

- Plants and microorganisms require relatively large amounts of potassium. Although there are large amounts of potassium (K) in soil, much of it is not bioavailable.
- It is customary to add K to soils when growing crops or when stimulating bioremediation of hydrocarbons.
- Potash (K_2O) is the most common commercial source of K fertilizer.

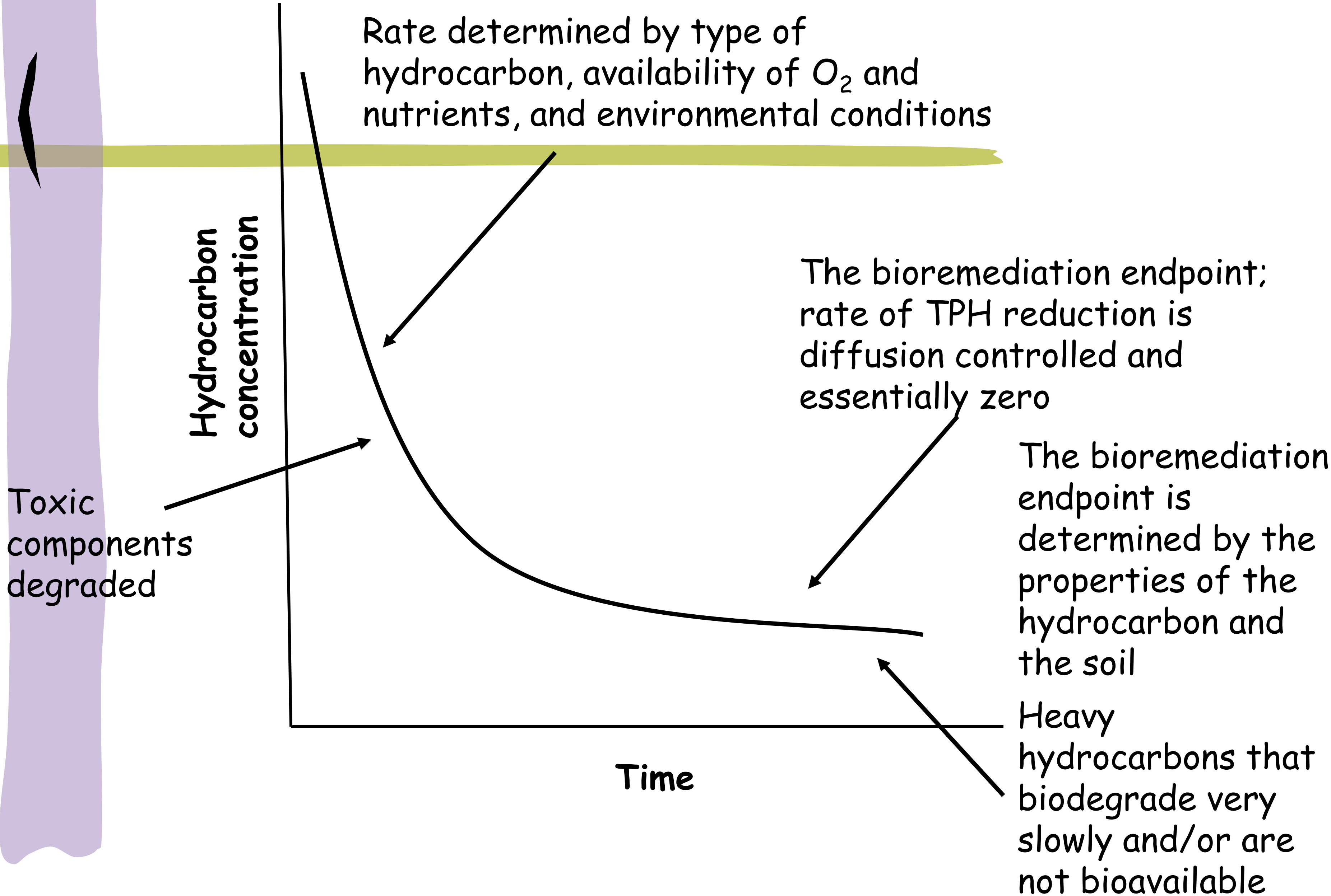
Fertilizer grading

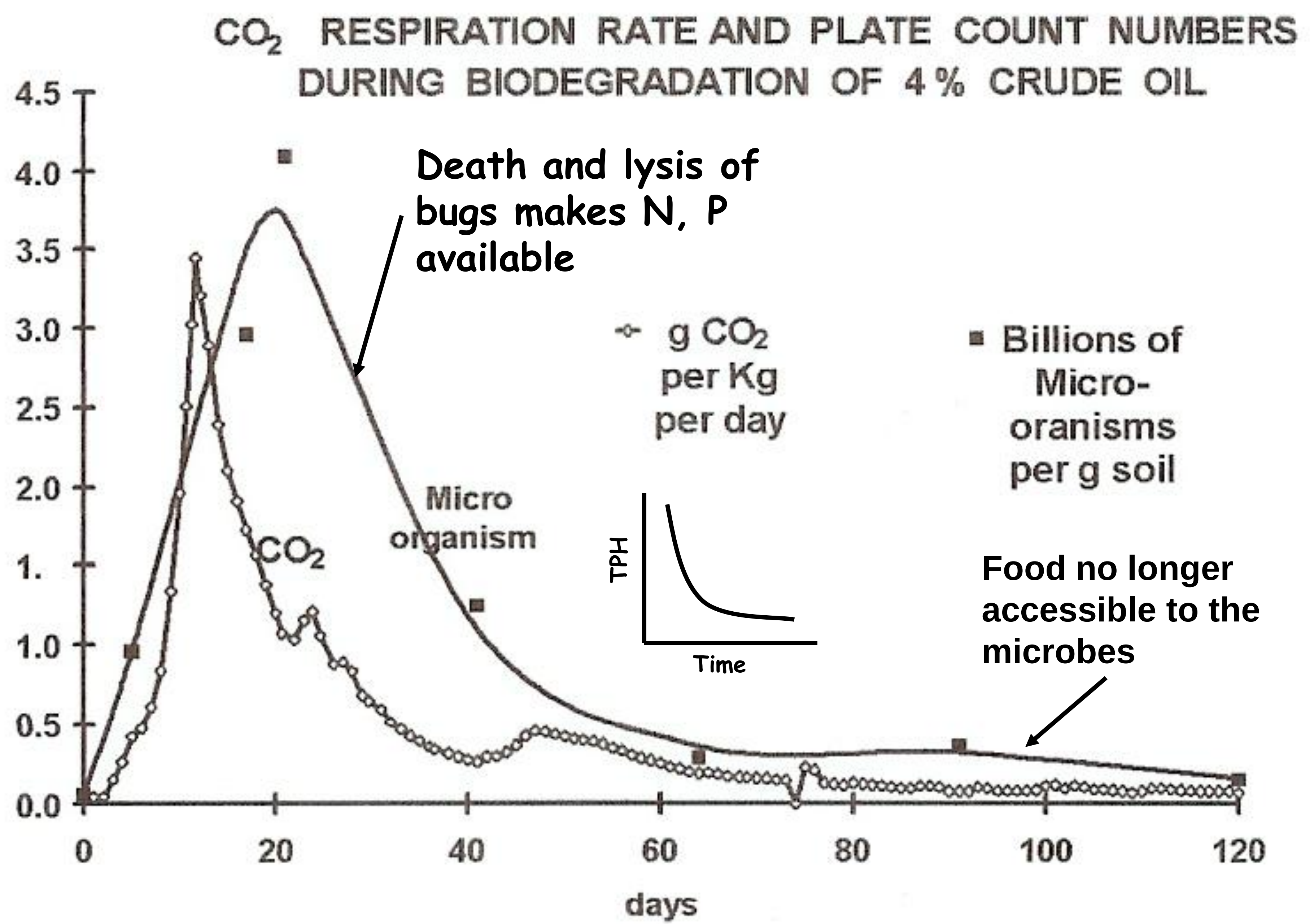
- Units are % by weight
  - N
  - P_2O_5
  - K_2O
- Be careful, sometimes the middle number refers simply to % weight available P.

$$\%P = 0.43 \times \%P_2O_5$$
$$\%K = 0.83 \times \%K_2O$$

A closer look at the process of hydrocarbon bioremediation in soil

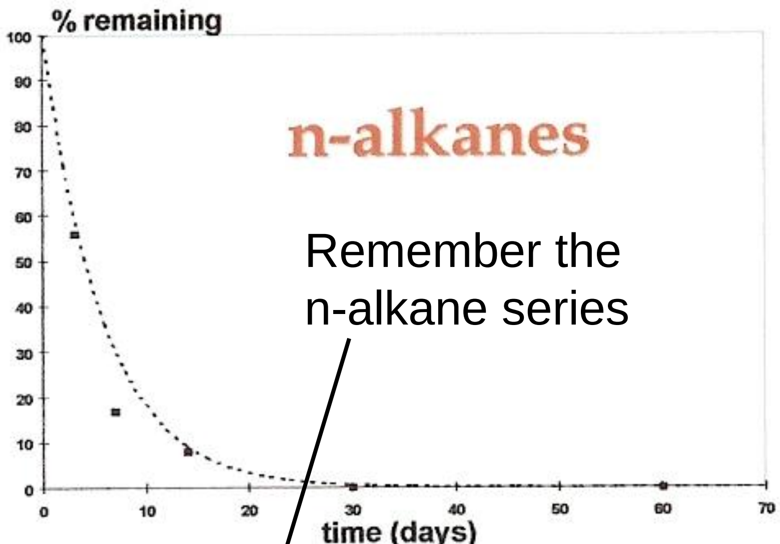
Bioremediation of hydrocarbons soil



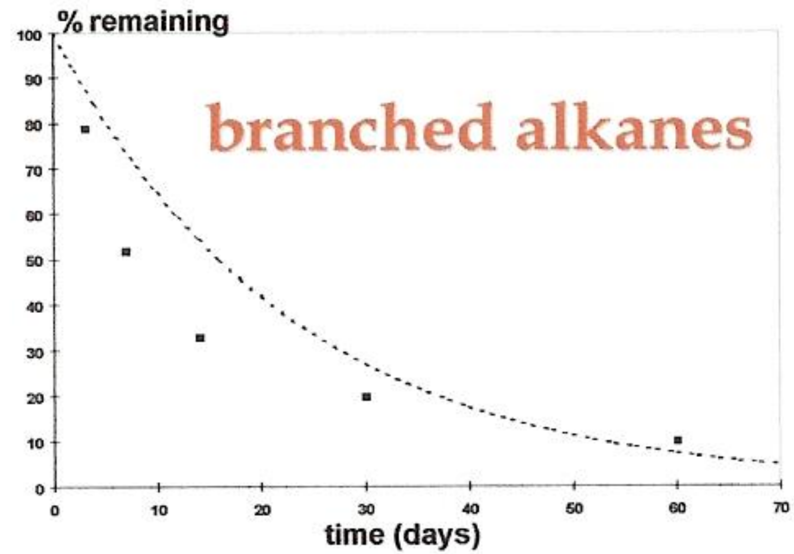


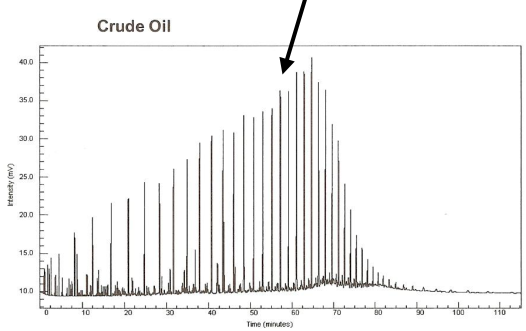
CO2 RESPIRATION RATE AND PLATE COUNT NUMBERS
DURING BIODEGRADATION OF 4 % CRUDE OIL
Death and lysis of bugs makes N, P available
g CO2 per Kg per day
Billions of Micro-oranisms per g soil
Micro organism
CO2
TPH
Time
Food no longer accessible to the microbes
4.5
4.0
3.5
3.0
2.5
2.0
1.
1.0
0.5
0.0
0
20
40
60
80
100
120
days

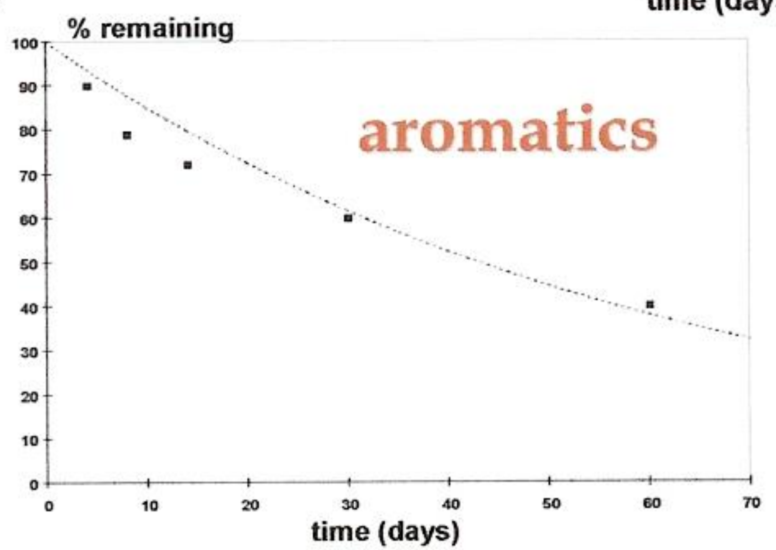
Different classes of hydrocarbons biodegrade at different rates

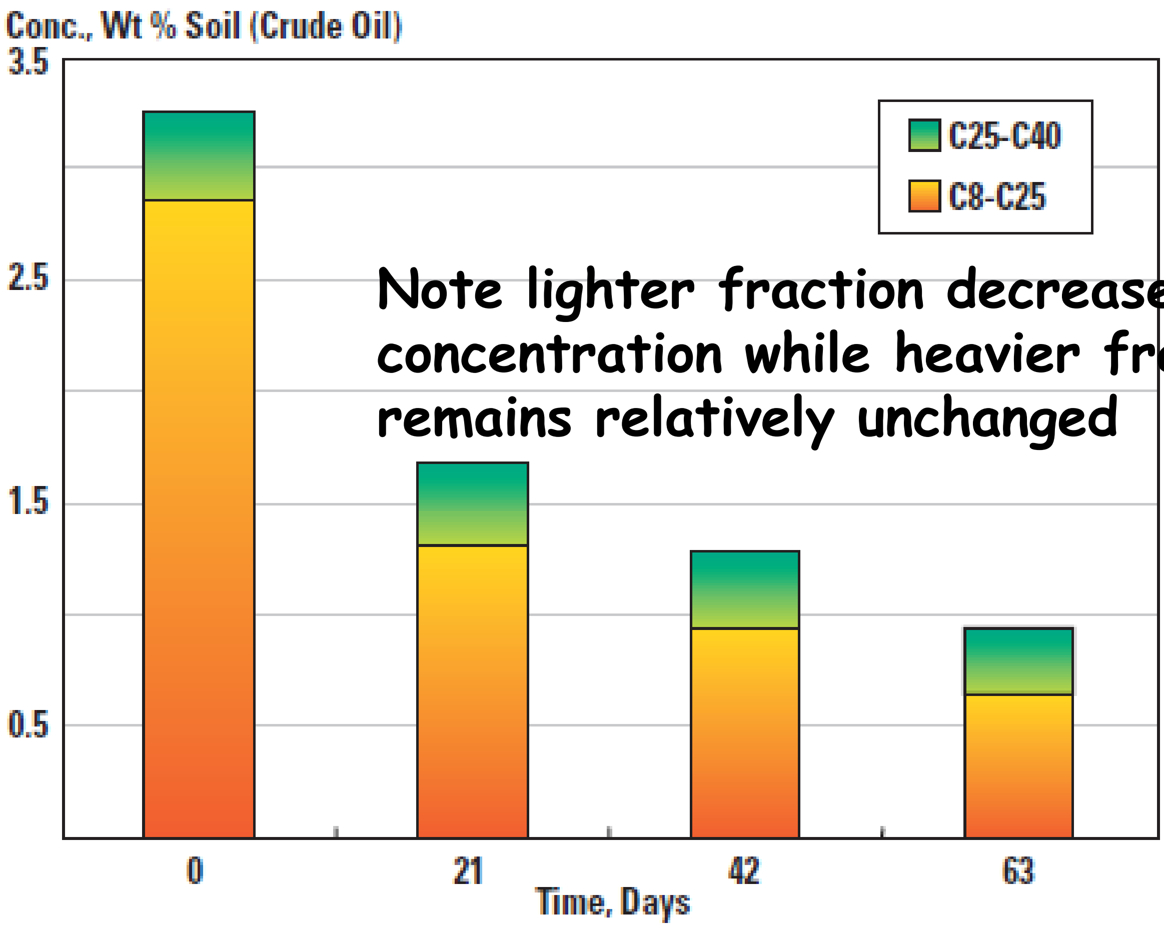


Remember the
n-alkane series







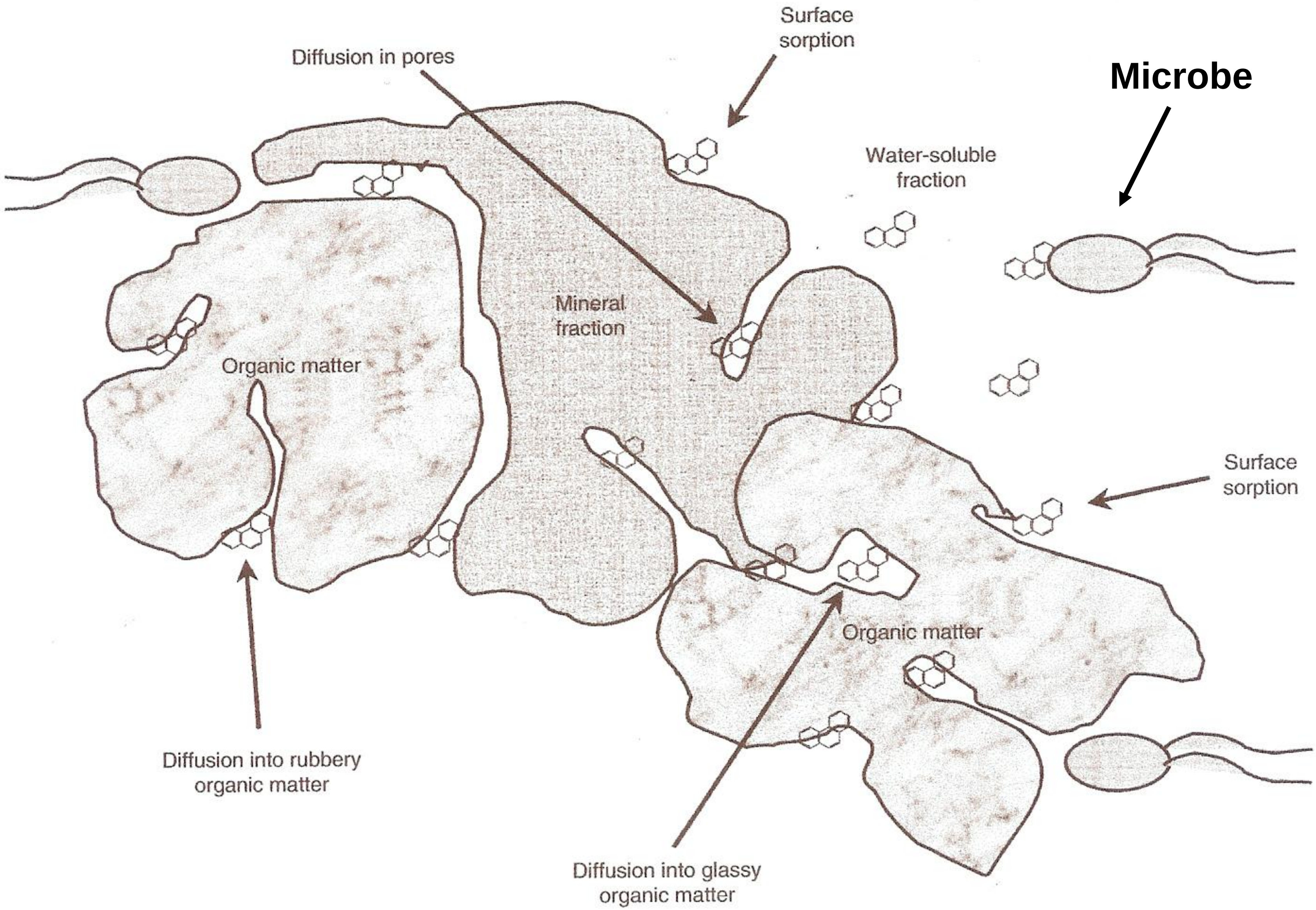


Note lighter fraction decreases in concentration while heavier fraction remains relatively unchanged

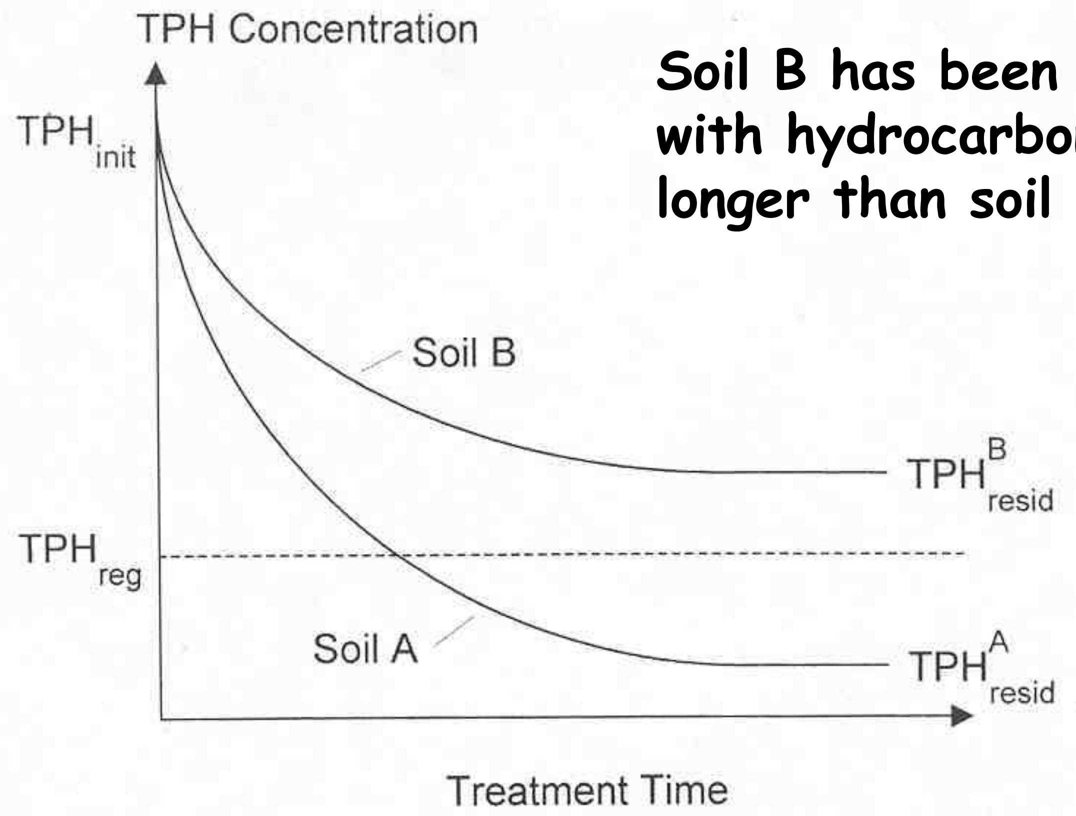
Figure 2. Changes in Carbon Range Due to Biodegradation

Source: Prince, Roger and Sara Mc Millen. Summary of PERF Bioremediation Projects. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

Hydrocarbons (especially heavy hydrocarbons) can become immobilized in soil and become difficult for the microbes to get to

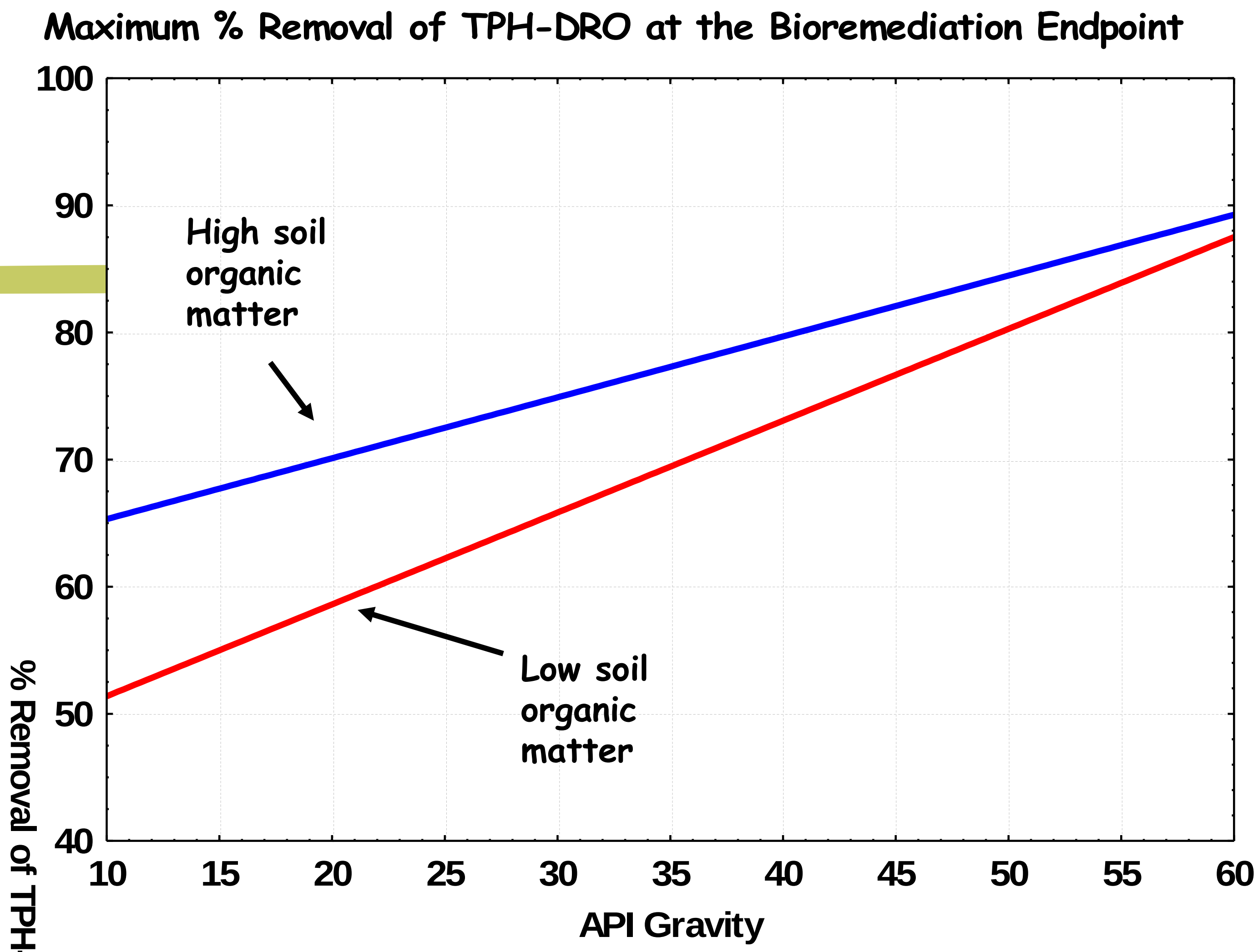


The effect of aging on bioremediation of hydrocarbons in soil



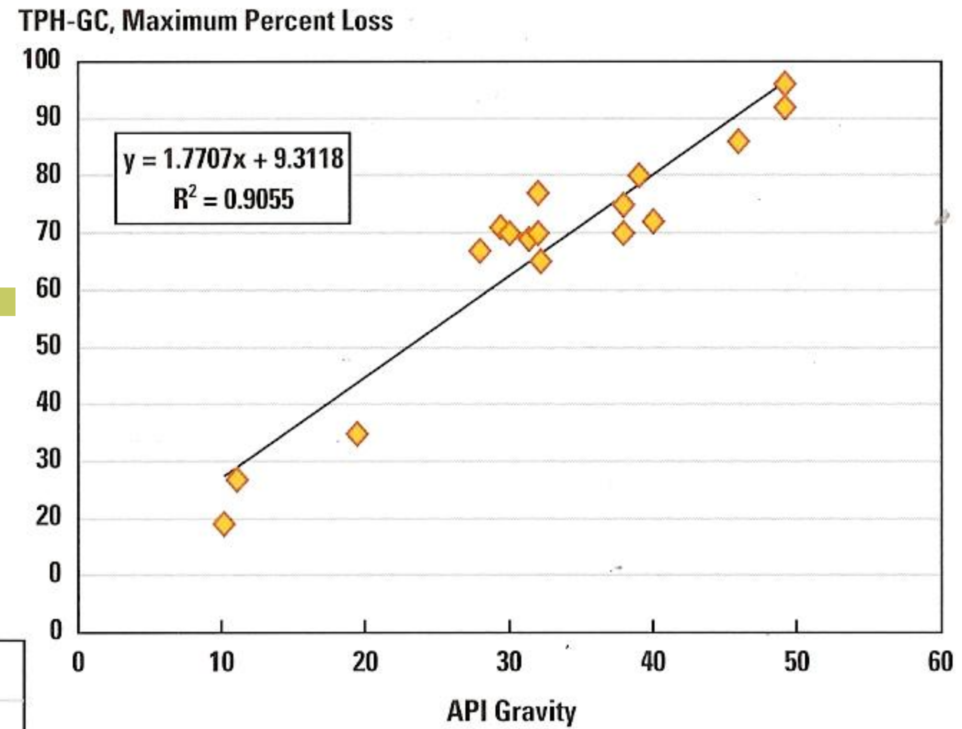
Soil B has been in contact with hydrocarbon for much longer than soil A

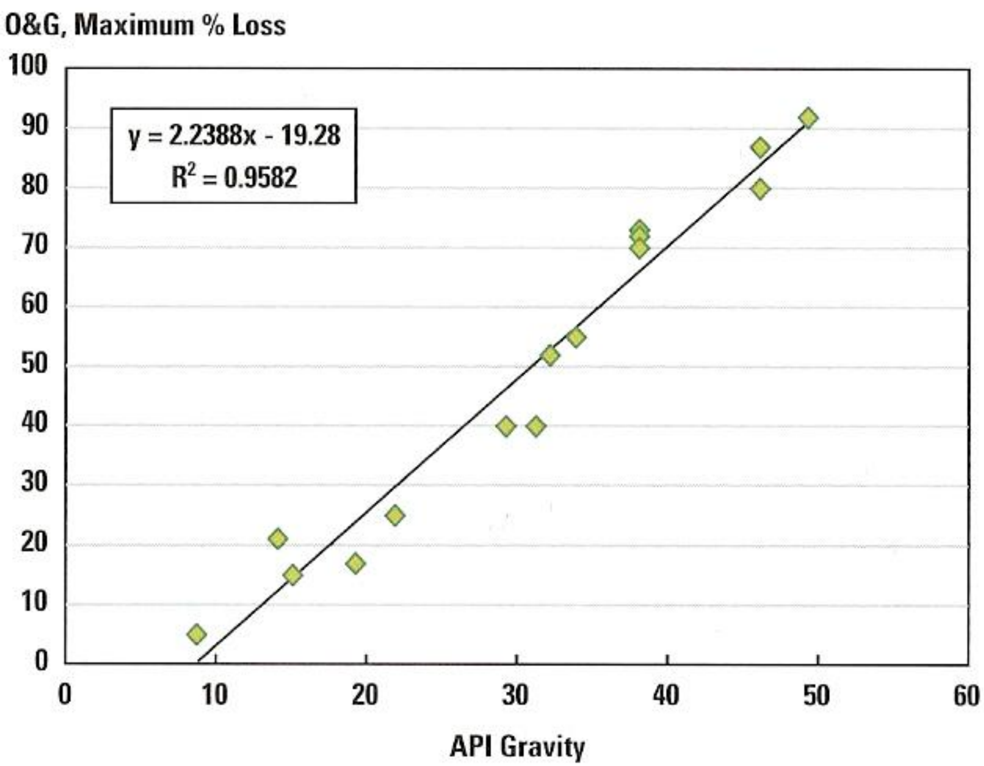
It is not possible to biodegrade all the hydrocarbons in soil; TPH is not zero at the bioremediation endpoint. At the biodegradation endpoint toxicity has been destroyed



Salinitro et al., Environmental Science and Technology, 31, 1769-1776 (1997)

Source: “A Summary of the DOE/PERF Bioremediation Workshop” (2003)





At the bioremediation endpoint

- The remaining hydrocarbon is
  - Not volatile
  - Adsorbed to soil particles
  - Low leaching
  - Low toxicity
  - Non-bioavailable

The methodology of biotreatment of petroleum contaminated soils - landfarming

What is landfarming?

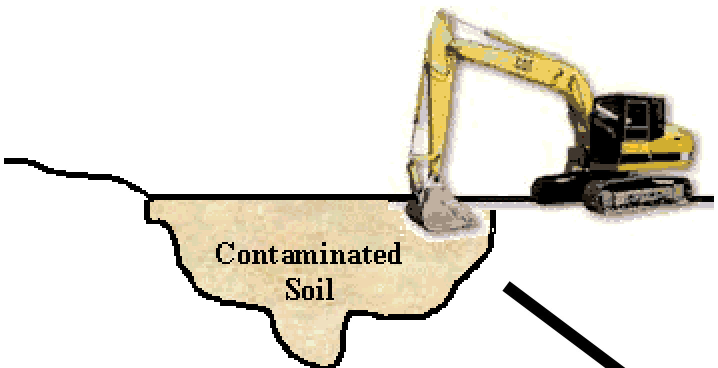
Facilitating the biodegradation of hydrocarbons in a **thin layer of soil** on the surface through

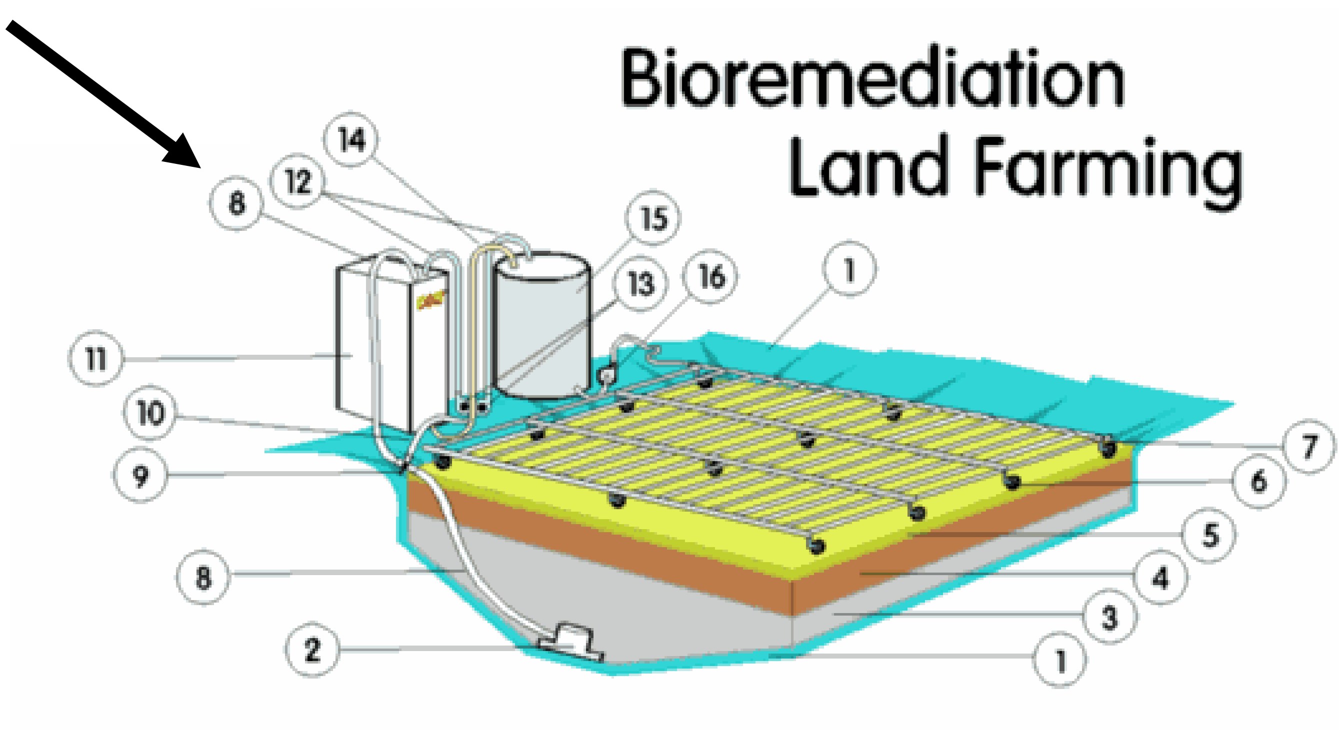
- the addition of soil amendments (N, P, organic matter)
- improving O_2 transfer into the soil
- maintaining proper moisture conditions

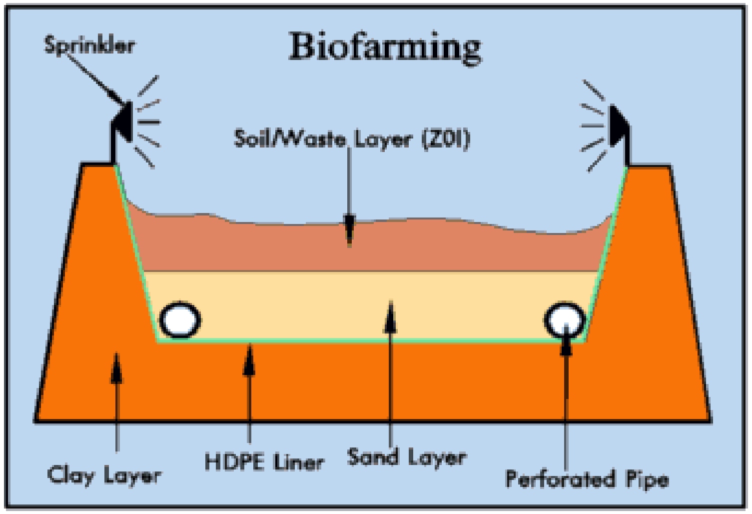
There are two types of landfarms:

- *in situ* – treatment occurs in place at the site of the spill
- *ex situ* – contaminated soil excavated and taken to a location remote from the site of the spill

Highly engineered *ex situ* landfarm
$$$



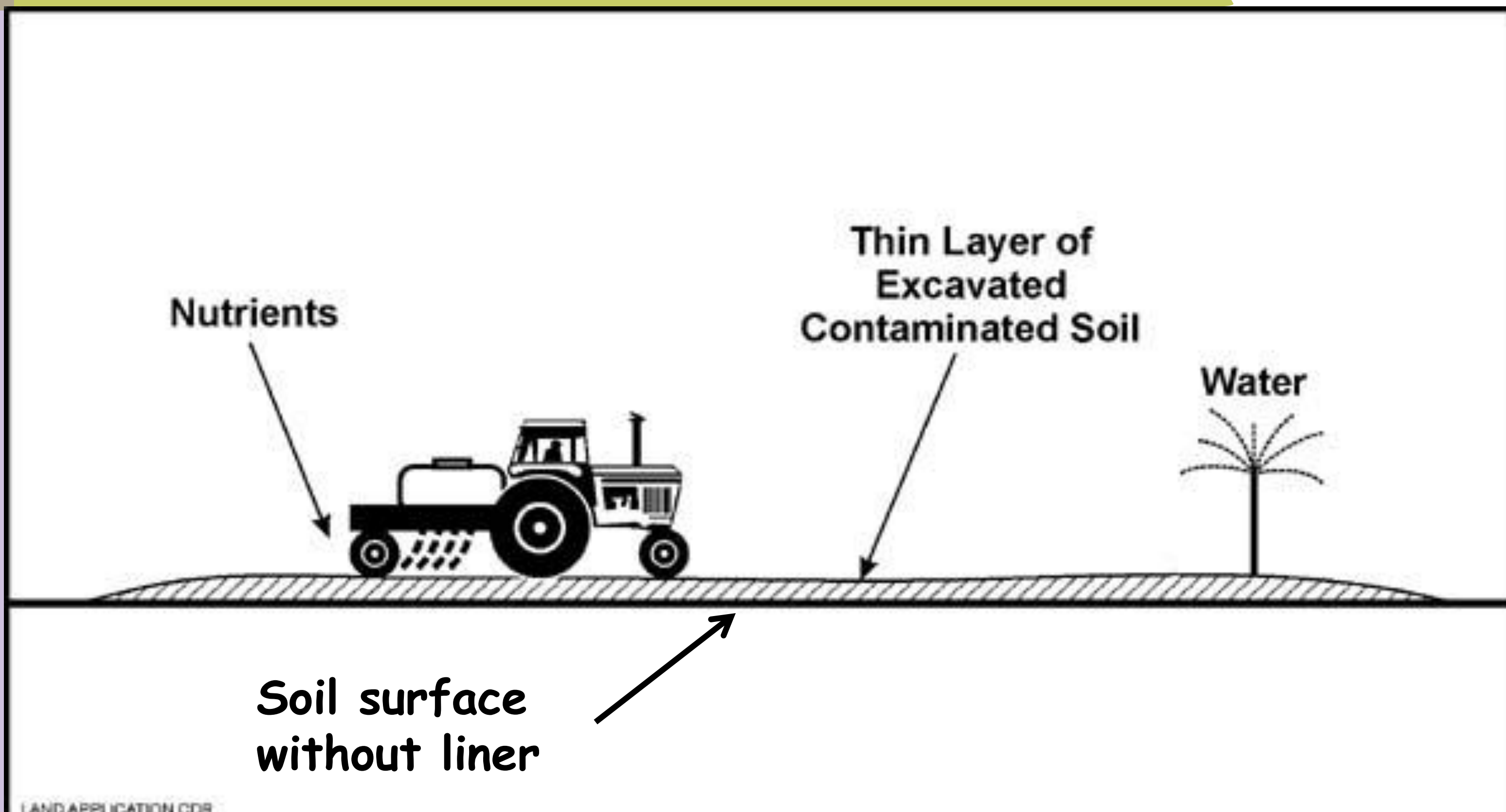




In land farming, waste or contaminated soil is aerobically treated above ground to biodegrade, transform, and immobilize contaminants. Source: Adapted from the U. S. EPA (8).

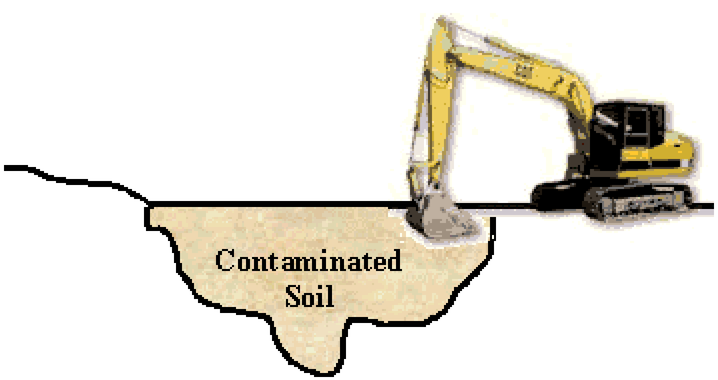
1. HDPE liner
2. Sump pump to collect leachate
3. Layer of pea gravel
4. Layer of polluted soil to be treated
5. Organic matter to retain moisture
6. Wheels on sprinkler piping system
7. Piping frame, aluminum or PVC pipes with frequent holes, sufficient to allow water, nutrients and bacteria to treat the land farm plot
8. Flexible leachate collection hose
9. Bypass valve that allows leachate to be circulated directly to water distribution tank, when sufficient nutrients are present in the leachate
10. Recirculation hose
11. Nutrient blending tank
12. Fresh water supply hoses
13. Pumps for fresh water
14. Treated water hose
15. Water distribution tank
16. Pump for distribution tank

Less intensive *ex situ* landfarming



In situ vs. *ex situ* landfarming

- ***Treating contamination in place***
  - Most of the cost associated with traditional cleanup technologies is associated with physically removing and disposing of contaminated soils. Because bioremediation can be carried out in place by delivering nutrients to contaminated soils, it does not incur removal-disposal costs.



In situ vs. *ex situ* landfarming

- ***Treating contamination in place, cont.***
  - *In situ* treatment reduces risk of exposure to toxic hydrocarbons
  - *In situ* treatment has a smaller carbon footprint
  - *In situ* treatment is considered more "sustainable" than *ex situ* treatment
    - *In situ* treatment reduces environmental stress. Because bioremediation methods minimize site disturbance compared with conventional cleanup technologies, post-cleanup costs can be substantially reduced.

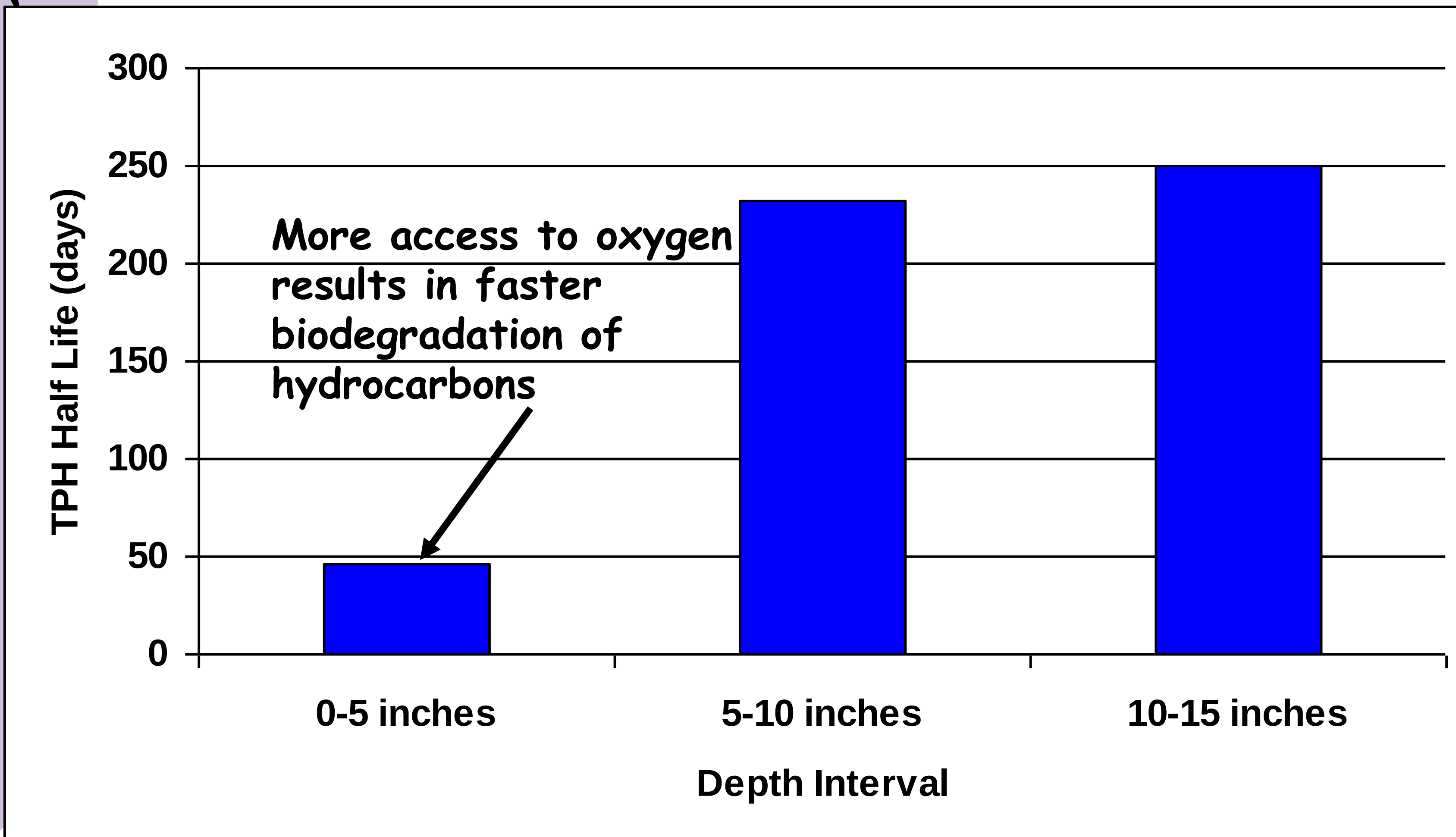
In situ vs. *ex situ* landfarming

In situ **treatment or** ***ex situ*** **treatment without a liner may be used when:**

- Contamination depth is less than 6-8 inches
- No shallow groundwater
- No significant slope
- Allowed by state regulatory agency

Biodegradation of diesel hydrocarbons in soil

Zytner, R.G., A Salb, T.R. Brook, M. Leunissen, and W.H. Stiver, Can. J. Civil. Eng., 28, 131-140 (2001)



Recommended practice for landfarming

Exempt waste amenable to landfarming

- Crude oil impacted soil
- Condensate impacted soil
- Drill cuttings
- Tank bottoms

Remember, the key to getting the most out of bioremediation is optimizing the *growth* of the microbes

- Getting the microbes together with the hydrocarbon
- Making sure the microbes have enough of the right nutrients
- Getting oxygen to the microbes
- Optimizing environmental conditions (to the extent we can)
- Moisture! Moisture! Moisture!

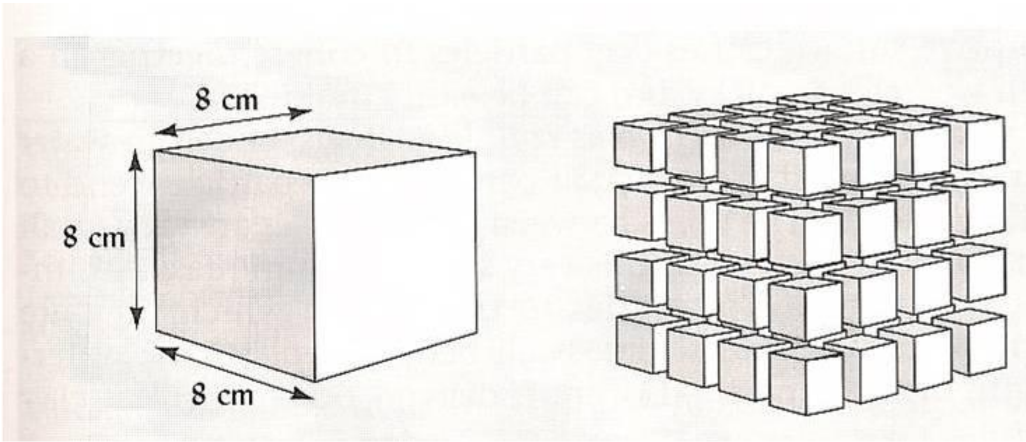
How do we put this into practice in a landfarm?

Recommended practice for landfarms

Getting the microbes together with the hydrocarbon

- Hydrocarbon loading (initial hydrocarbon concentration in the soil)
  - Biodegradation **rates** in a landfarm strongly correlate with the hydrocarbon interfacial area/mass ratio (i.e., the amount of hydrocarbon surface compared to the total hydrocarbon mass)

Low surface area to mass ratio



High surface area to mass ratio

Loading rates for *optimum* rates of biodegradation rates (API > 20°)

Avg. soil temperature (°C)	Loading rate (%TPH)
> 22	5
15 -22	4
8-15	3
< 8	1-2

Loading rates for *optimum* rates of biodegradation rates (API < 20°)

Avg. soil temperature (°C)	Loading rate (%TPH)
> 22	4
15 -22	3
8-15	2
< 8	1

Reference

Conversion of TPH units

What does 4% TPH mean?

TPH concentrations are almost always given on a dry weight basis, i.e., concentration of TPH per unit weight of dry contaminated soil. So a TPH concentration of 4% means that 4% of the dry weight of a contaminated soil is hydrocarbon detected by whatever method the lab uses.

Remember that ppm TPH in soil is how many parts hydrocarbon in 1 million parts of hydrocarbon + dry contaminated soil. So what is 4% of a million? It's 40,000. So 4% TPH is the same as 40,000 ppm.

Remember a ppm in soil is the same as mg/kg. So a TPH concentration of 4% is 40,000 mg hydrocarbon per kg of dry contaminated soil or 40,000 mg/kg.

A simple formula: % X 10,000 = mg/kg or ppm

Recommended practice for landfarms

Getting the microbes together with the hydrocarbon

- If the initial TPH concentration is too high for optimum treatment, you can dilute the contaminated soil with uncontaminated soil to increase rates of bioremediation:
  - Utilize full 6-8 inch depth
  - Utilize surrounding soil as a diluent
  - **Rule of thumb: dilute until the soil no longer glistens**
  - Dilution is not treatment! Destruction of toxicity is treatment.

Recommended practice for landfarms

Making sure the microbes have enough of the right nutrients

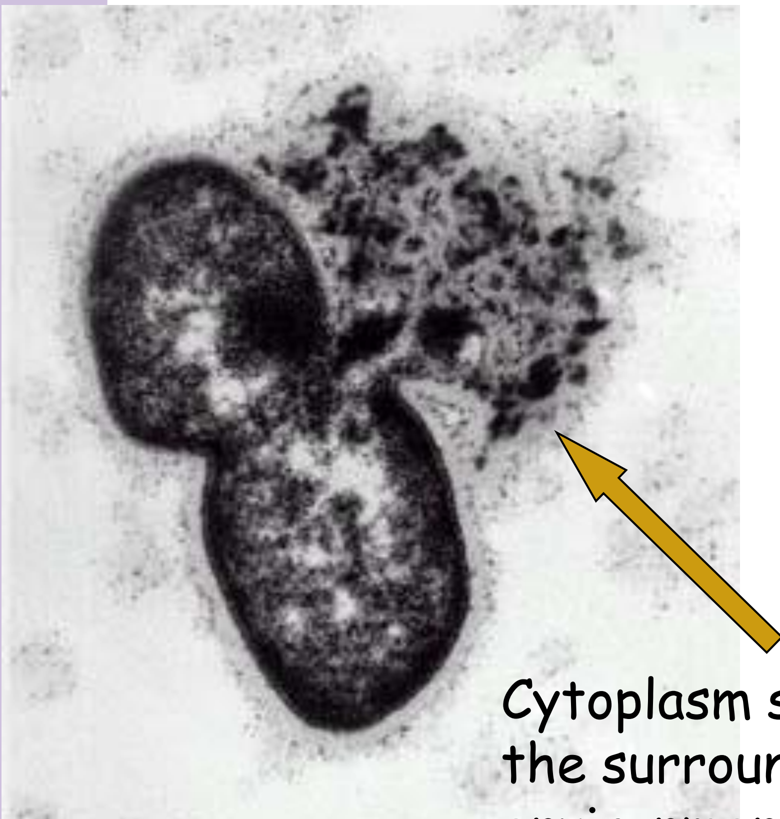
- How much fertilizer is required to support biodegradation of hydrocarbons?
  - An often quoted EPA recommendation is C:N:P:K of 100:10:1:1 (weight ratio).
    - The concentration of C in a contaminated soil is frequently assumed to be 80% of the TPH concentration
    - This ratio comes from a purely theoretical calculation

EPA recommendation is problematic!

- Does not account for
  - Recycling of nutrients
  - P sequestration
  - NO_3^- losses from leaching
  - NO_3^- losses from denitrification
- Often misinterpreted as a guideline for one-time fertilizer addition. Results in:
  - Excess salinity
  - Greater potential for nitrate run-off

You can have too much of a good thing!

One reason the EPA guidelines don't work



Cytoplasm spilling into the surrounding environment acting as nutrients for other microbes

When conditions are good for bioremediation, there is a bloom of hydrocarbon degrading bacteria. When there is no longer enough food to support such a large population they begin to die off. Lysis of dead cells releases nutrients for recycle.

How do you avoid excess salinity and minimize fertilizer losses?

- Split additions of N recommended, initial C:N of 150:1 but check for salinity potential from fertilizer
  - Prevents nitrite accumulation when NH_4^+ is used
  - Limits NO_3^- losses (and potential impact on surface waters)
  - Can prevent excess salinity
- N:P of 5:1
- N:K of 5:1

Safe fertilizer additions – based on seed/fertilizer recommendations (cereal grains)

Soil texture	lbs N/1000 ft^2
Sandy loam	2.8
Loam to clay loam	3.4
Clay to heavy clay	4.1

Based on:

1) ammonium nitrate
2) adequate soil moisture for seed germination

Safe fertilizer additions – based on limited data from literature*

Water content (%)	lbs N/1000 ft^2
5	1.8
10	3.4
15	5.0
20	6.3

Based on:
1) ammonium nitrate
2) 750 mg N/kg soil water

*Walworth, J.L., C.R. Woolard, J.F. Braddock, and C.M. Reynolds, J. Soil Contamination, 6: 465-480 (1997)

Safe fertilizer additions – based on 4 mS/cm maximum salinity of soil solution

Water content (%)	lbs N/1000 ft^2
5	2.0
10	3.8
15	5.6
20	7.1

Based on:

1) ammonium nitrate
2) 845 mg N/kg soil water

Factors affecting salinity resulting from fertilizer additions

- Soil moisture – lower soil moisture results in higher salinity of soil solution
- Salt index of the fertilizer
- Soil texture – clays and soil organic matter buffer the effects of fertilizer salts; sandy soils particularly susceptible to over fertilization

Fertilizer salt index

- The fertilizer salt index is a relative measure of the salinity produced by a fertilizer in soil water
- Sodium nitrate is the standard against which all other fertilizer are compared
  - Salt index (SI) for $NaNO_3$ = 100
- A fertilizer with a SI < 100 generates fewer ions in soil solution than the same weight of $NaNO_3$ and a fertilizer with SI > 100 produces more ions in soil solution than the same weight of $NaNO_3$
- The SI cannot predict amount of fertilizer that will do injury to plants (or microbes); it is for comparison of fertilizers only

Salt indices of common fertilizers

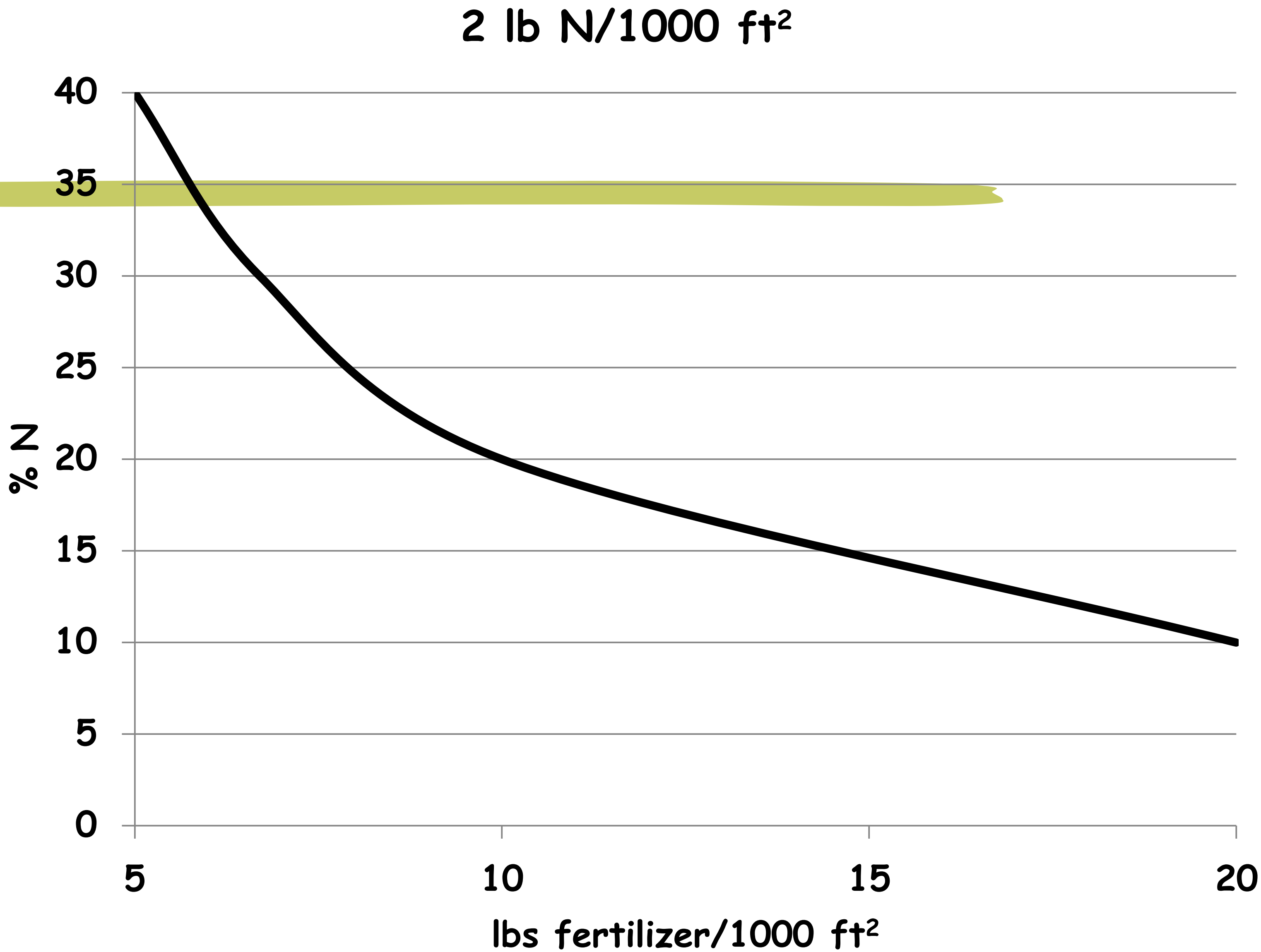
Fertilizer	SI	Fertilizer	SI
Ammonium nitrate (33% N)	104	Calcium nitrate (15.5% N)	65
Urea (46%)	71	Monoammonium phosphate (MAP) (11% N)	26
Ammonium sulfate (21% N)	88	Triple super phosphate (45% P_2O_5)	10
Diammonium phosphate (DAP) (18% N)	29		
Muriate of potash	32		

See the Appendix for a detailed fertilizer calculation for landfarming based on C:N:P:K ratios

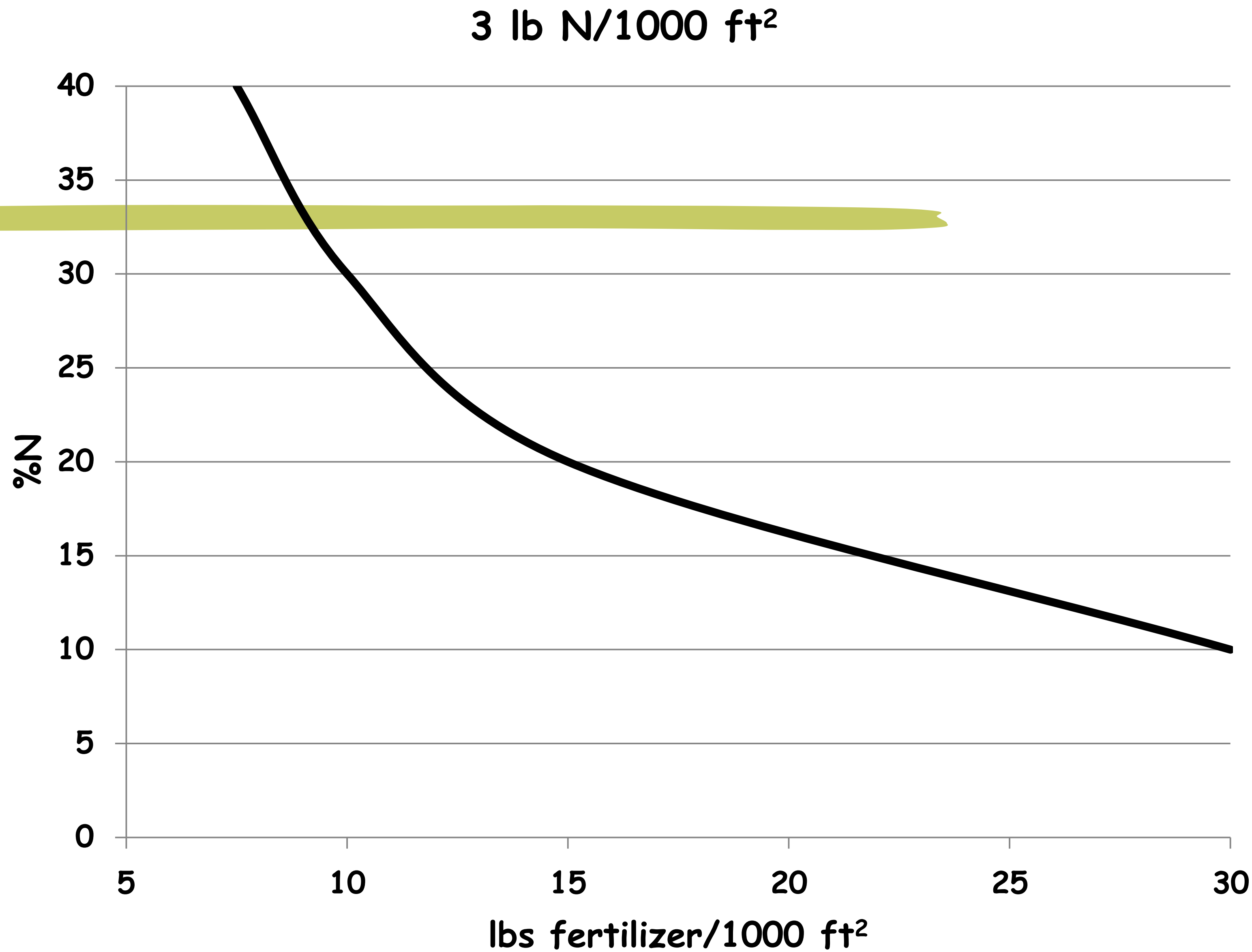
Simple fertilizer guidance

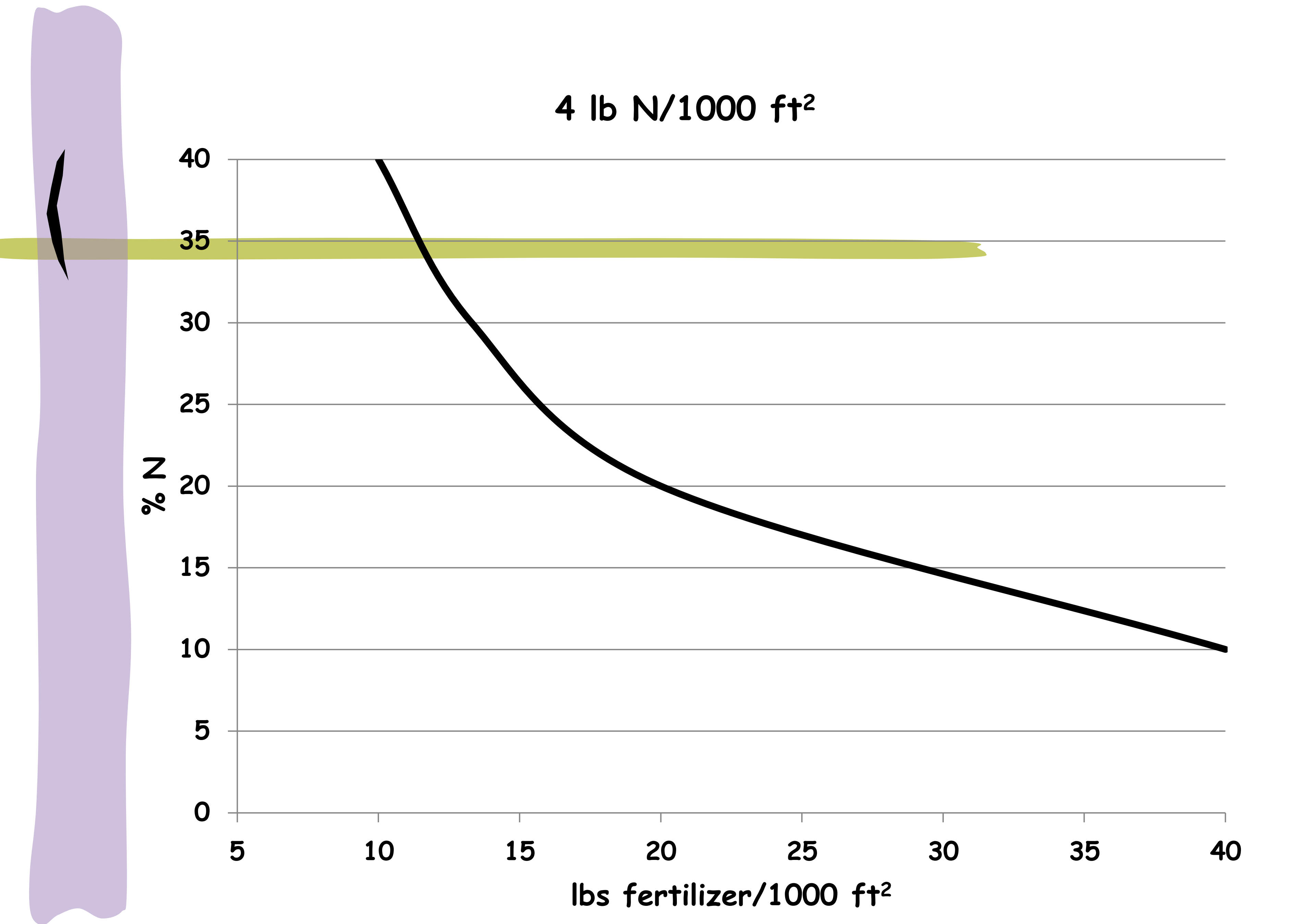
Fertilizer applications

- 13:13:13 or comparable composite fertilizer
- Use the spill area as a surrogate for the TPH inventory
- Use 3-4 lb N/1000 ft^2 each of three applications (30 days apart) during **warm weather**
  - Less with sandy soil
  - Less fertilizer and less frequent application when relatively dry
  - Use organic matter to increase moisture holding capacity of the soil



2 lb N/1000 ft²
40
35
30
25
20
15
10
5
0
% N
5
10
15
20
lbs fertilizer/1000 ft²


3 lb N/1000 ft²
%N
40
35
30
25
20
15
10
5
0
5
10
15
20
25
30
lbs fertilizer/1000 ft²

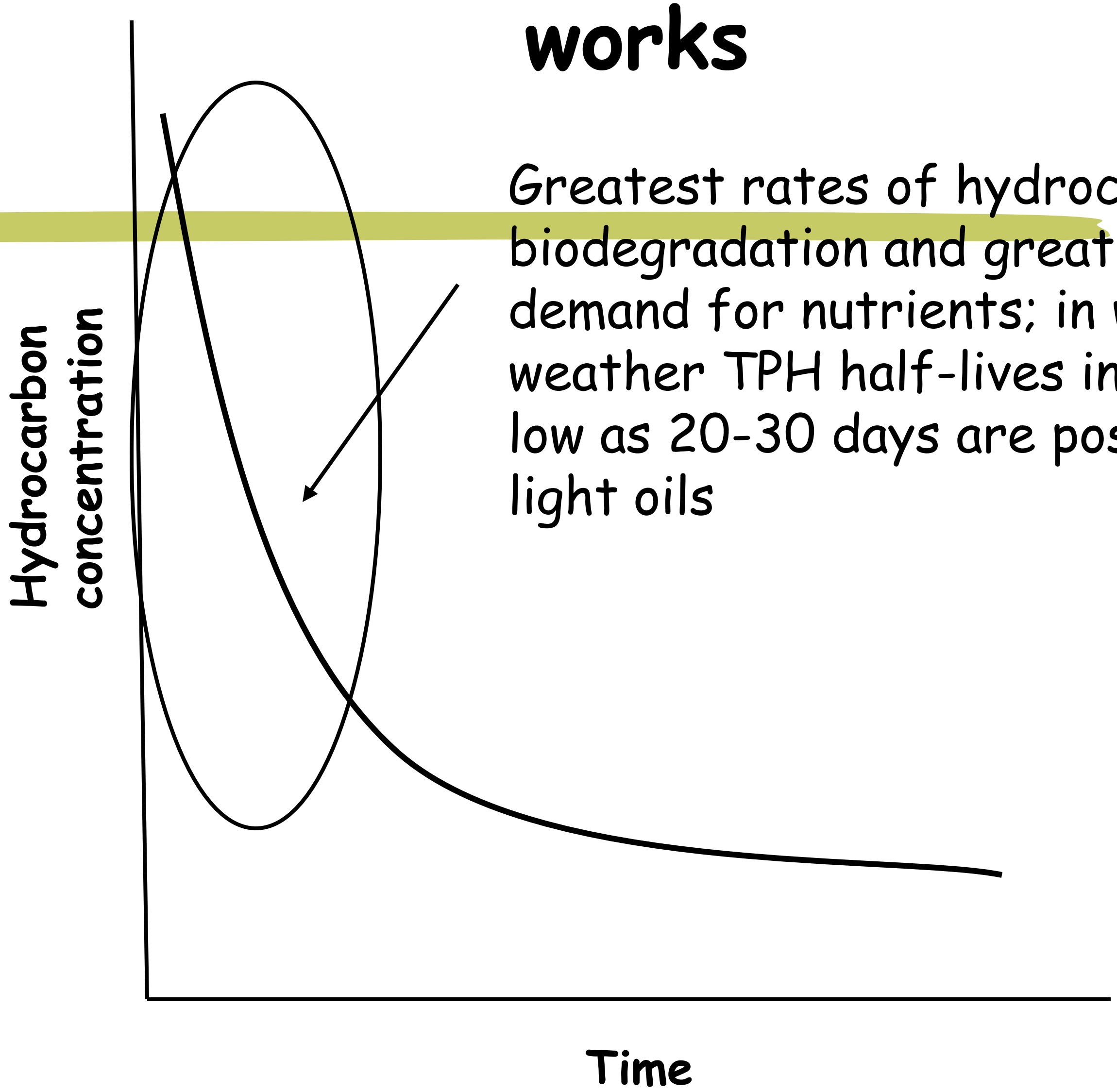


4 lb N/1000 ft²
40
35
30
25
20
15
10
5
0
% N
5
10
15
20
25
30
35
40
lbs fertilizer/1000 ft²

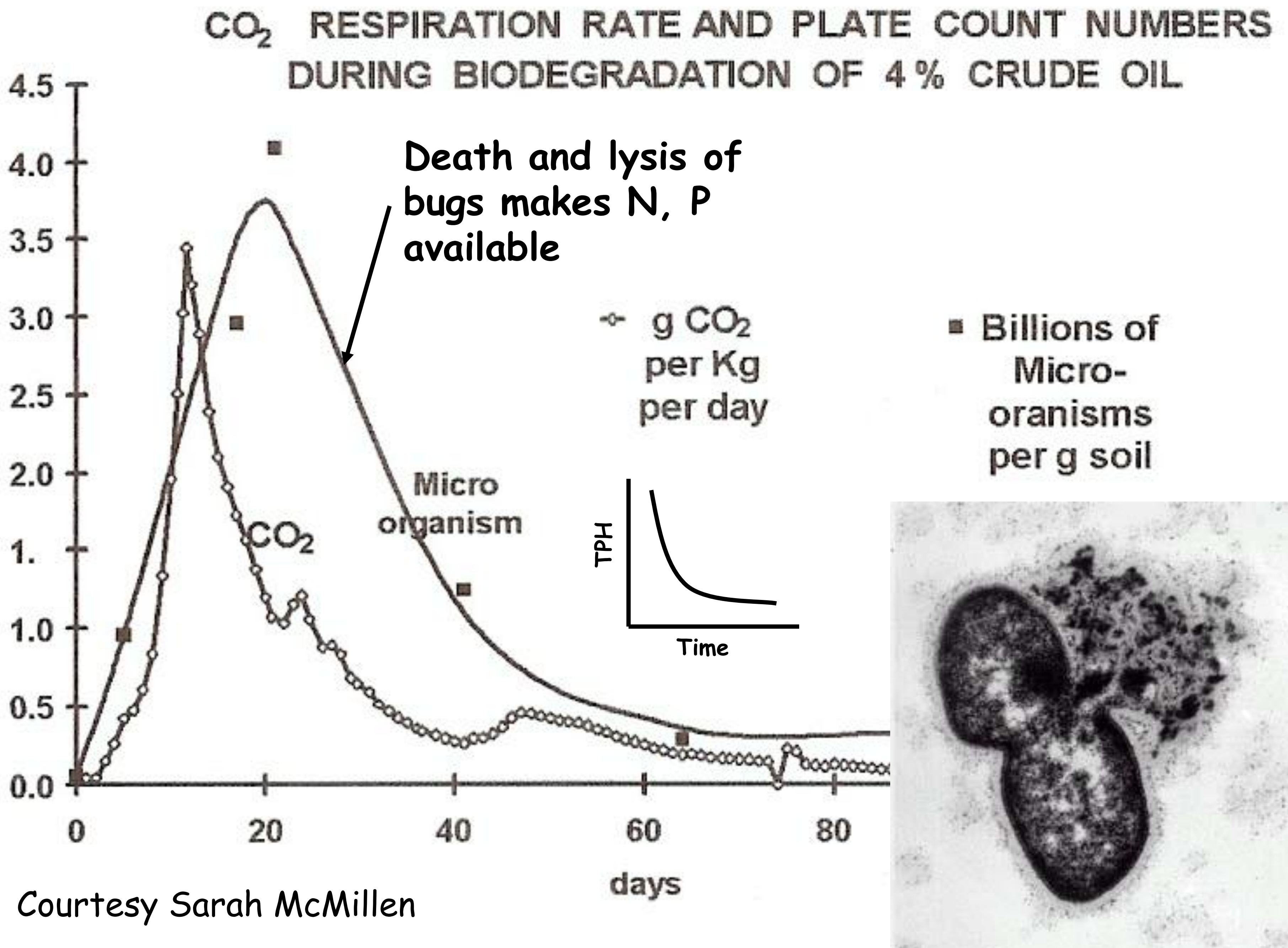
Compound fertilizers

- Base fertilizer additions on the first number (%N) in the fertilizer rating to prevent burning
  - The second and third numbers in the fertilizer rating should be at least 20% of the first number (to provide sufficient phosphorus and potassium)

Simple fertilizer guidance: why it works

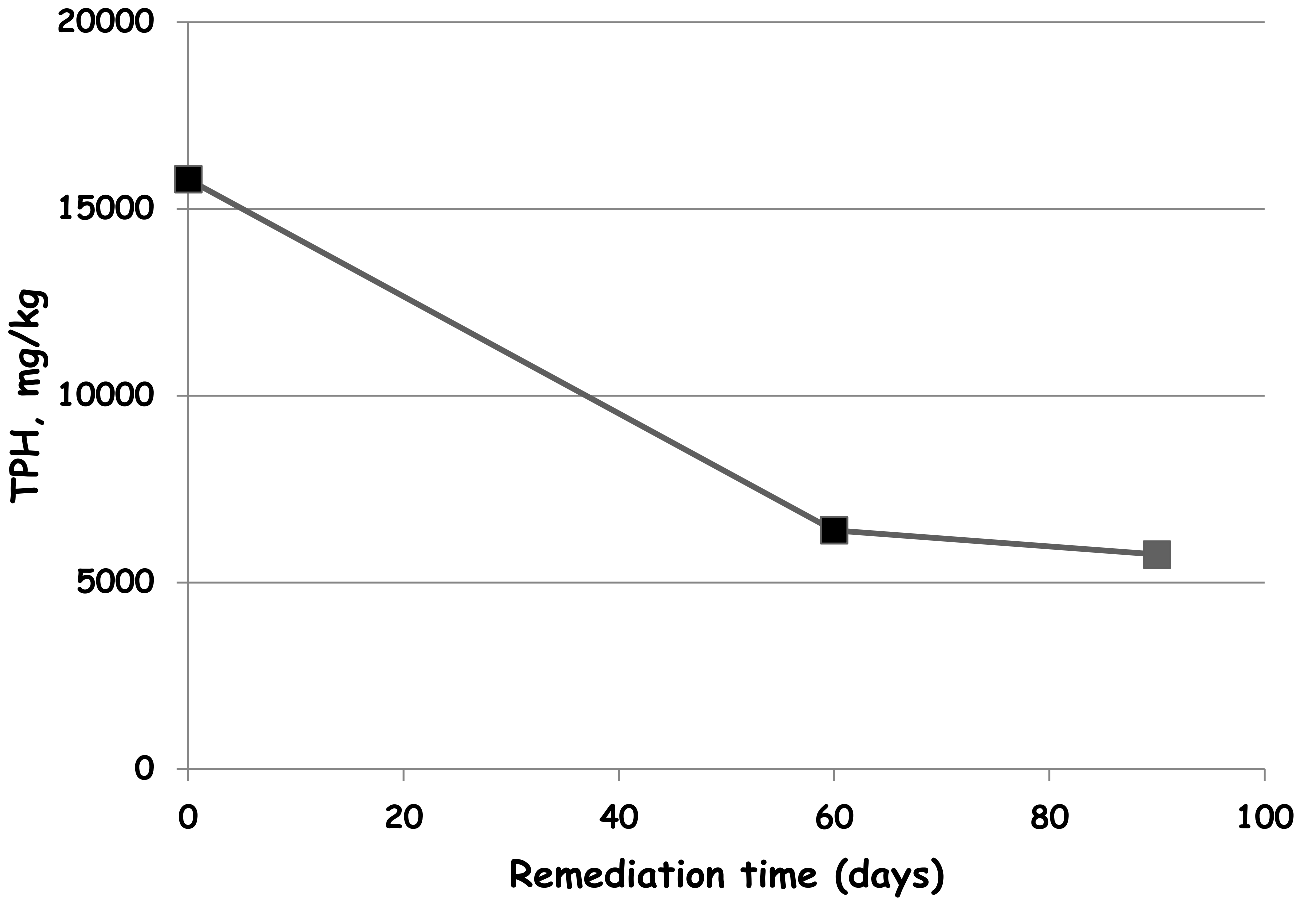


Greatest rates of hydrocarbon biodegradation and greatest demand for nutrients; in warm weather TPH half-lives in soil as low as 20-30 days are possible for light oils

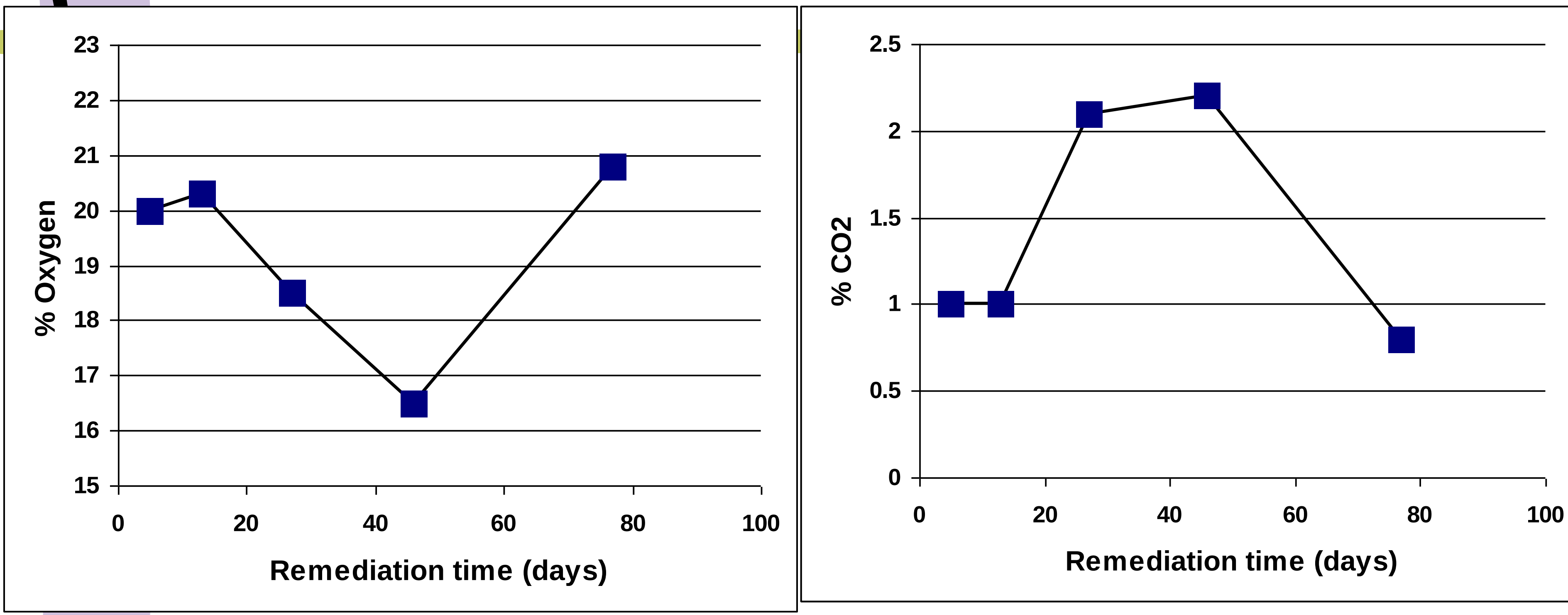


Courtesy Sarah McMillen

Landfarming of 40° API crude oil

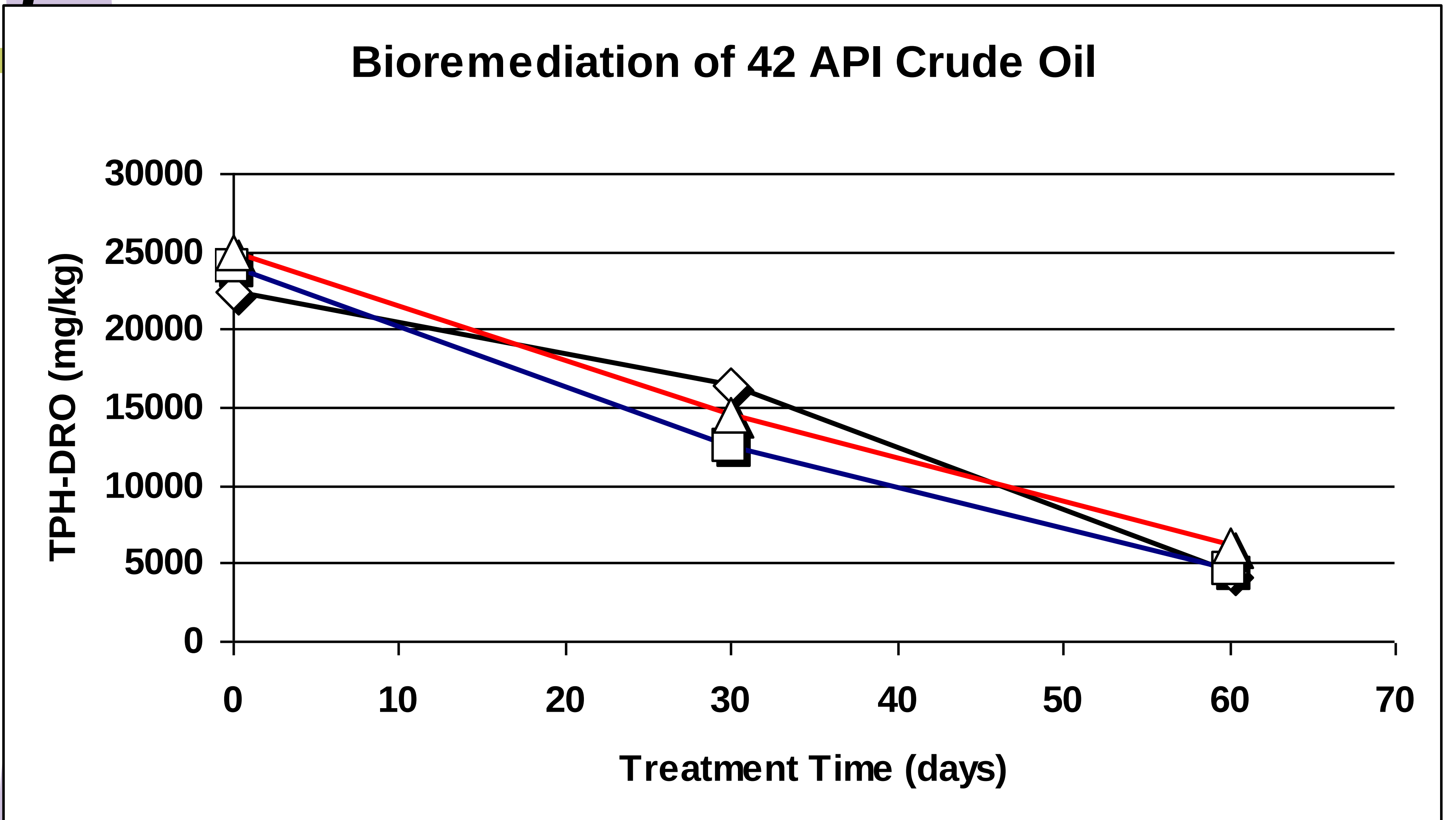


Soil gas analysis from a landfarm during biodegradation of 40° API crude oil



Repeating fertilizer additions after 30 and 60 days should be sufficient considering the recycling of nutrients as long as tilling and watering is continued and the soil is warm. When you can no longer smell hydrocarbons after warming the soil in your hands you are likely near the endpoint.

Fertilizer addition at 0, 30, and 60 days during warm weather

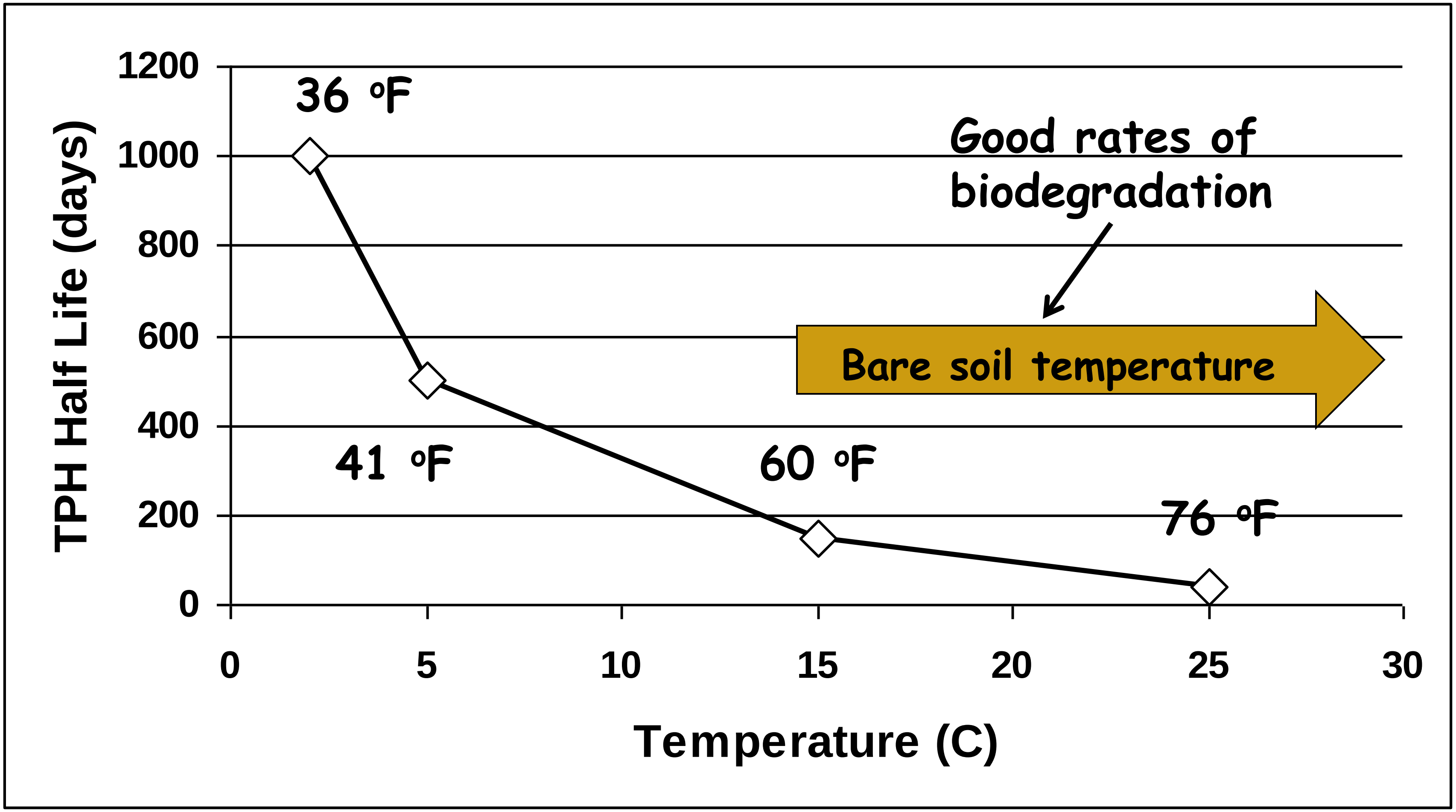


Simple fertilizer guidance, cont.

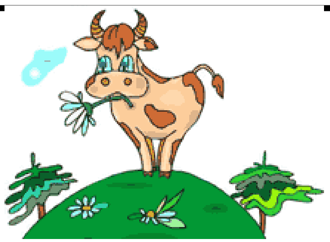
- Don't add fertilizer during the cold months (December-February)
- If you can't make three fertilizer additions before it gets cold start fertilizer additions again when it starts to warm up in March
- Decrease fertilizer rate and frequency in dry weather

Biodegradation rates of diesel hydrocarbons increase with temperature

Zytner, R.G., A Salb, T.R. Brook, M. Leunissen, and W.H. Stiver, Can. J. Civil. Eng., 28, 131-140 (2001)



Fresh Cow Manure	Composted Cow Manure[4]
Wet with strong odor	Earthy smell[1] Moist-dry
High nutrient concentrations	Low nutrient concentrations 1-3% N, 0.5-1% P_2O_5, 1-2% K_2O (Releases about 10% of its nutrients per year)
High tendency to "burn"[2]	No burning, safe fertilizer
High salinity	Usually much lower salinity[3]
Weed seeds and pathogens	Weed seeds and pathogens killed by composting
Biodegradation in soil tends to deplete oxygen	No negative effect on soil gas oxygen



[1]If it smells of ammonia its not done yet.

[2]Must let fresh manure age 60-90 days to prevent burning.

[3]Check salinity before use. Make a paste with distilled water and measure EC. If EC < 10 mmhos/cm it's OK to use if you till it in 6-8 inches.

[4]Density 15-25 lbs/ft^3 or 400-675 lbs/yd^3 dry; X 2 moist

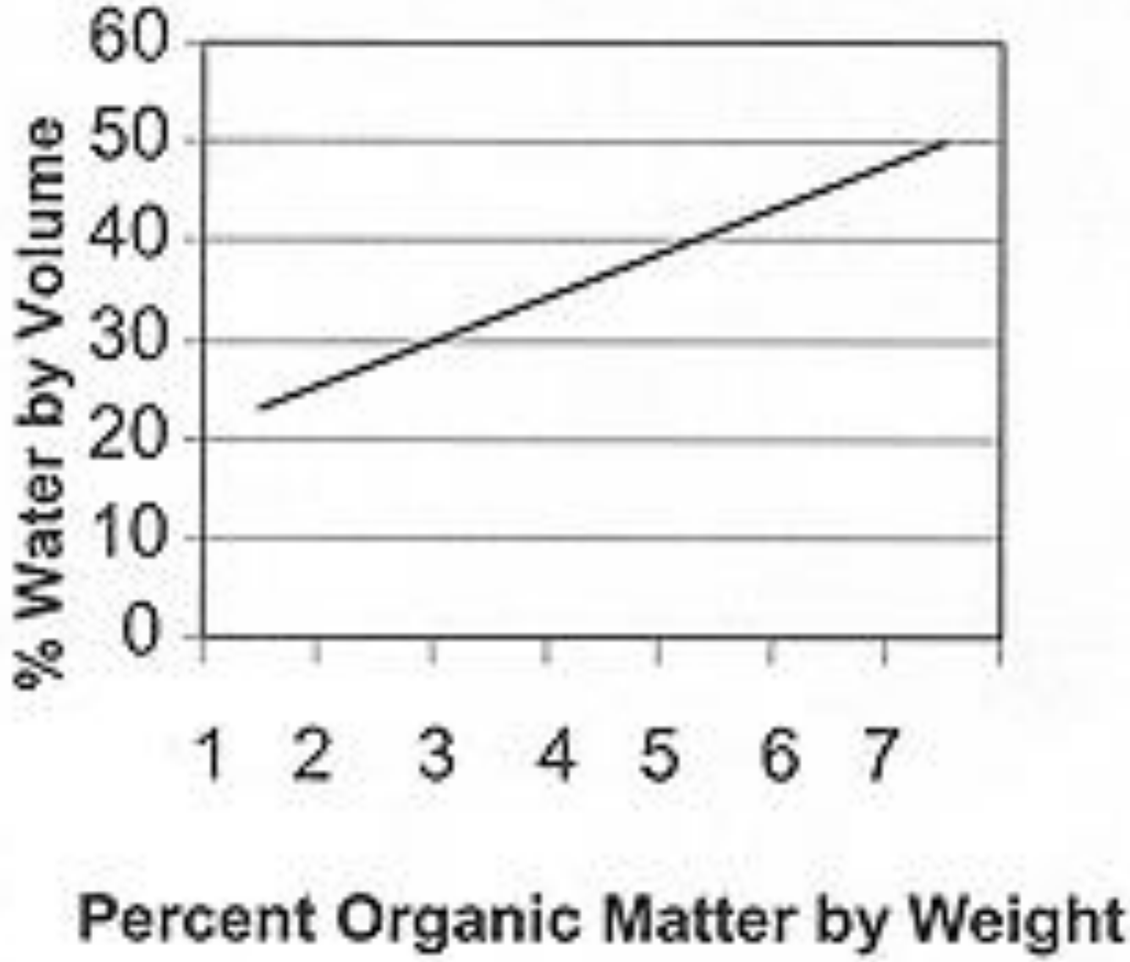
Monitoring nutrients

- If following simple fertilizer guidance check nutrients around 30 days after the last of the three fertilizer additions
  - Total the ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N):
  - mg/kg (or ppm) N X 0.05 = lb N/1000 ft^2
  - Make up N as required if N is deficient
  - Use a compound fertilizer

Recommended practice for landfarms

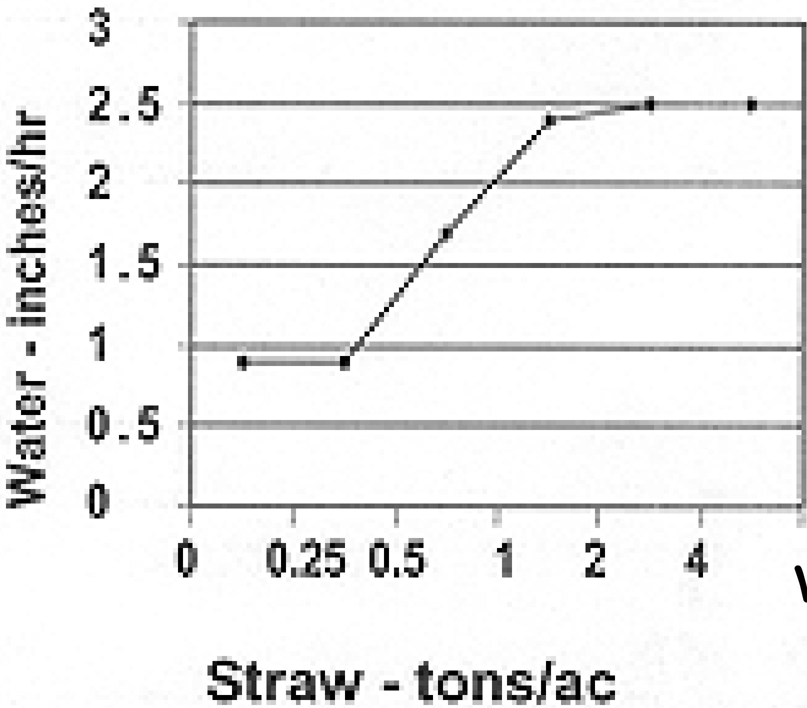
Increasing oxygen transfer into soil

- Organic matter
  - Blending organic matter into landfarm soil has several benefits including:
    - Aeration of the soil environment
    - Improved moisture retention
    - Improved soil structure
    - Establishing a fertility base to improve re-vegetation upon closure



For every 1% of soil organic matter, the soil can hold 16,000 gallons of plant-available water per acre of soil down to one foot deep

Effect of organic matter on infiltration rate



Water entry into the soil after 1 hour

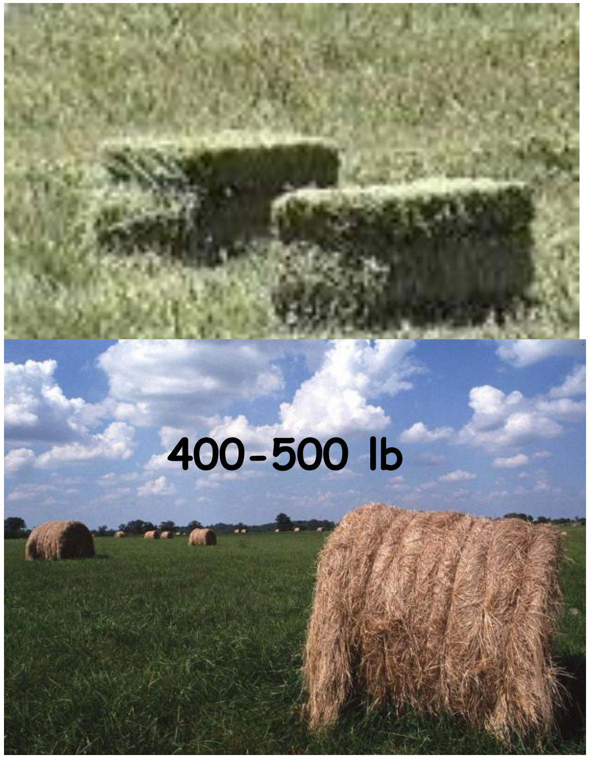
Manure Rate (Tons/acre)	Inches of water
0	1.2
8	1.9
16	2.7

Recommended practice for landfarms

- What organic matter?
  - Two types needed **ideally**
    - Stable organic matter – fertility base
      - Composted manure
      - Composted agricultural waste (cotton burr, for example)
    - Easily biodegraded – build strong soil aggregates
      - Hay (best if already rotting)
  - Contain no pesticides or herbicides

Recommended practice for landfarms

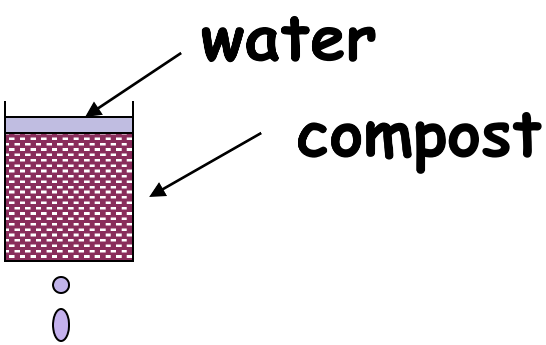
- How much organic matter?
  - Compost*
    - Cover site with about 1 inch and work into soil
    - For 1000 ft^2 this requires about 3 yd^3 of compost
  - Hay
    - 5 small square bales per 1000 ft^2 (about 300 lb)



*Check salinity before use. Make a paste with distilled water and measure EC. If EC < 10 mS/cm it's OK to use if you cultivate it in 6-8 inches.

Organic matter – How much?

- Coffee can test
  - Poke a hole in the bottom of a 2-lb coffee can and fill the can with landfarm soil after tilling in organic matter
  - Try to pour water through the soil. If water does not flow through almost immediately you don't have enough organic matter



Recommended practice for landfarms

Increasing oxygen transfer into soil

- Loosening the soil: cultivation
  - The benefits of cultivation of the landfarm include:
    - Maintaining a soil structure in the landfarm conducive to good oxygen transfer from the atmosphere
    - Vertical mixing of the soil ensures that the entire soil depth spends some time in the upper most active zone of biodegradation
    - More uniform distribution of nutrients in the soil profile
    - Overcome surface crusting

Cultivation on a small scale

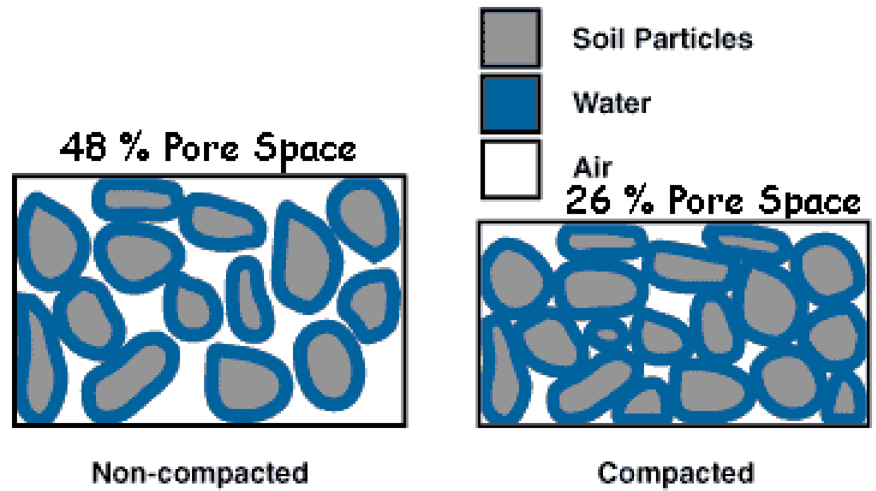
Cultivation on a large scale

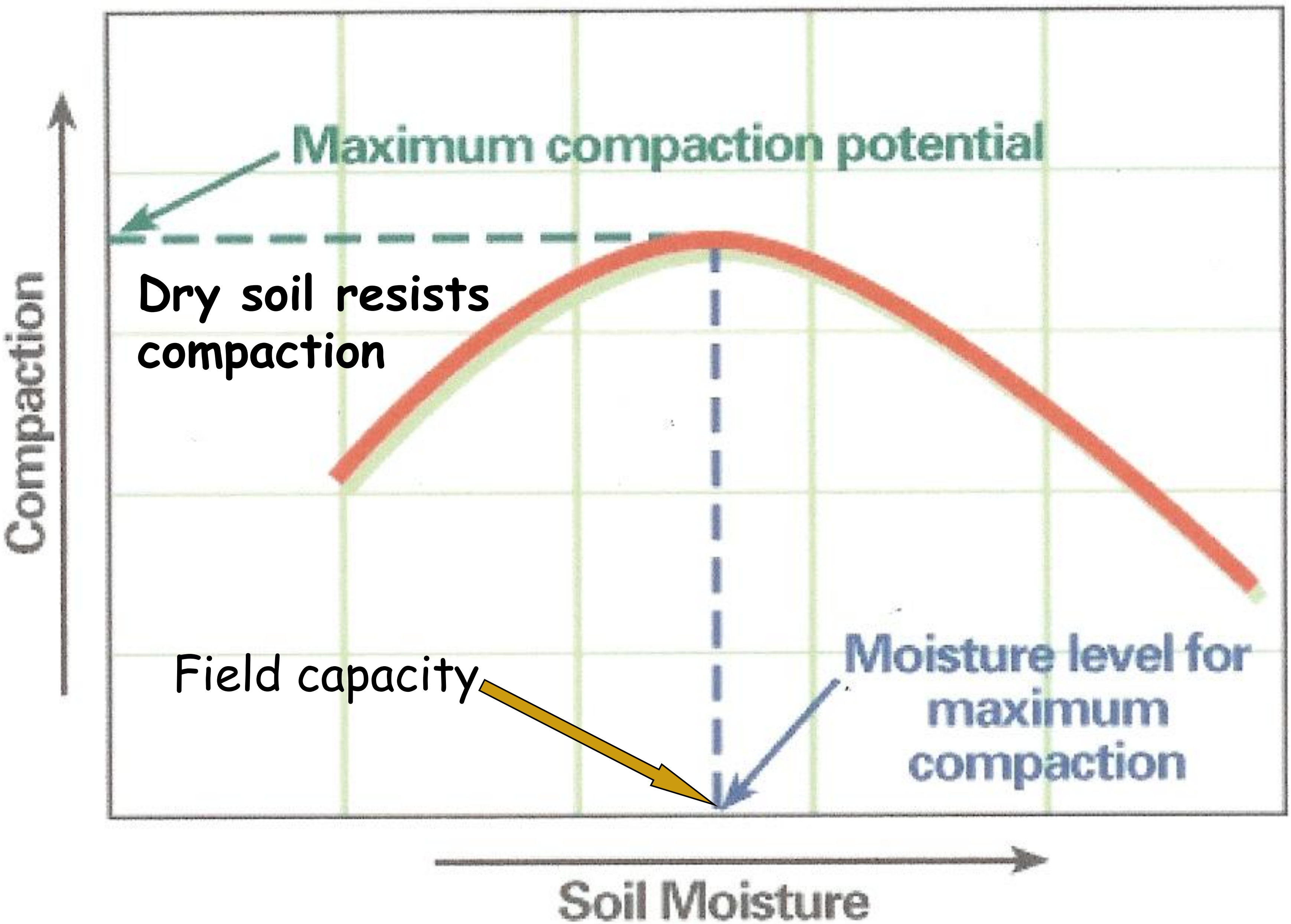
Recommended practice for landfarms

- Cultivation
  - The landfarm should be cultivated at least twice monthly for the first 2-3 warm months then once monthly thereafter with a cultivation depth equal to the depth of the contaminated soil.
  - Soil should be at or near the lower end of the recommended soil moisture range before cultivation - **cultivating wet soil can cause soil compaction**

Soil compaction

- Soil compaction causes
  - Poor aeration and oxygen transfer
  - Waterlogging
  - Excessive runoff and erosion
  - Excessive soil strength limiting root growth during revegetation




Maximum compaction potential
Dry soil resists compaction
Compaction
Field capacity
Moisture level for maximum compaction
Soil Moisture

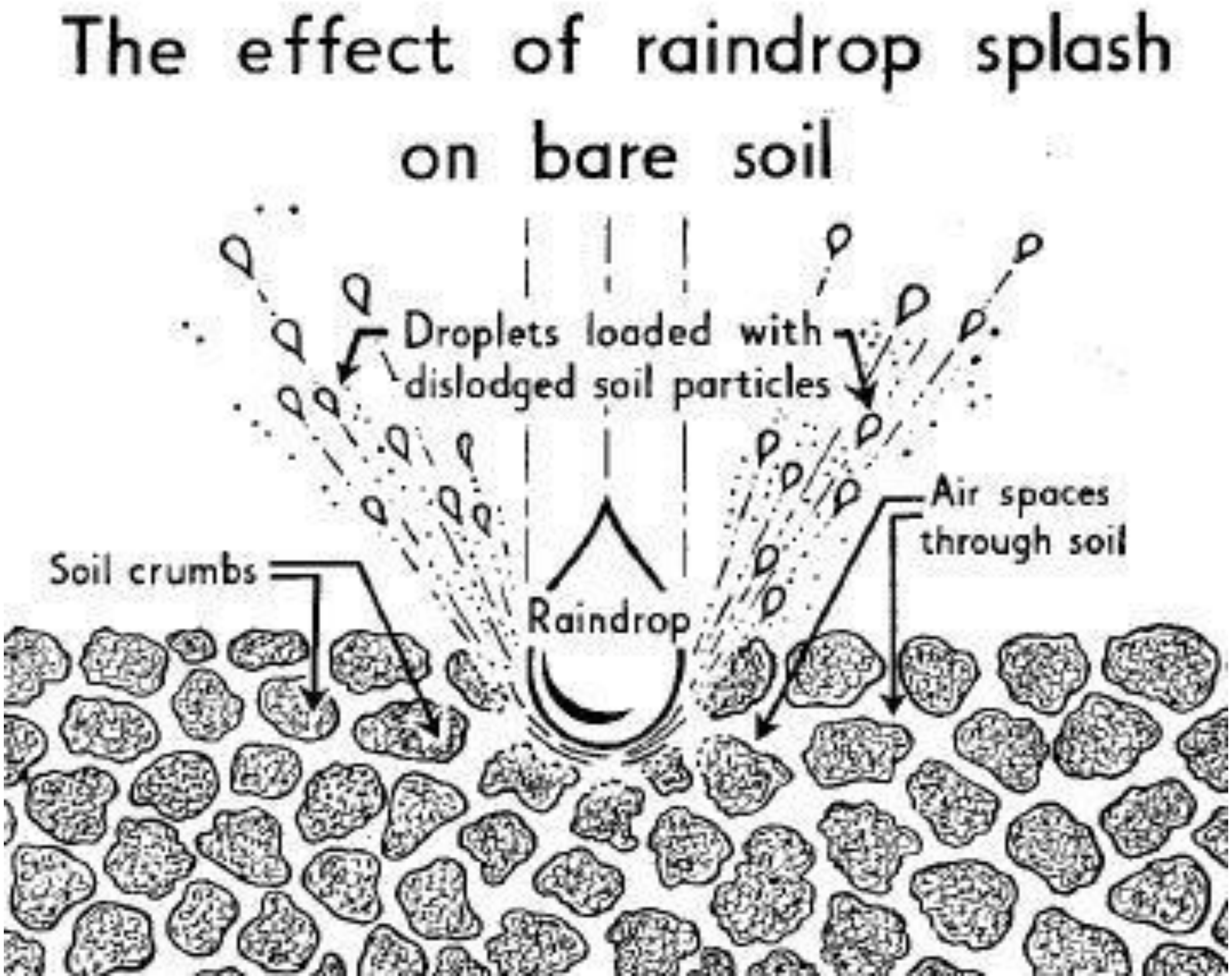
Compacted soil Normal soil

Additional tips for preventing soil compaction

- Limit vehicular traffic
- **Keep out livestock**

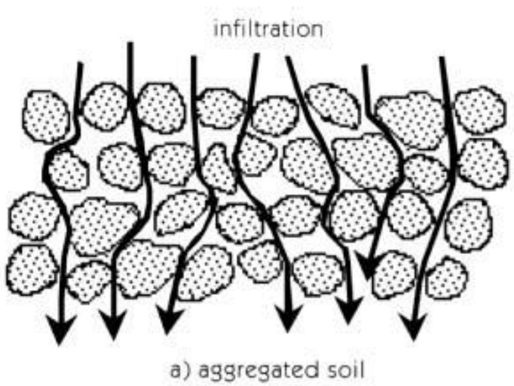
Livestock damage

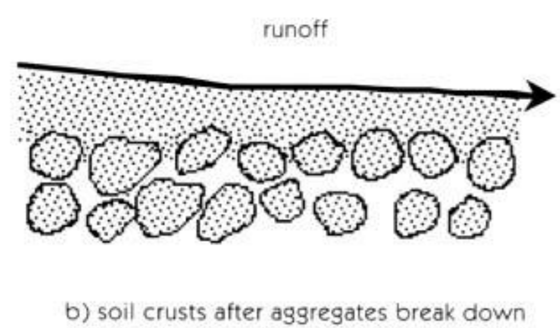
Another cultivation-moisture issue: the effect of rain drops on soil infiltration and oxygen transfer



Effect of rain on soil infiltration and oxygen transfer

- Rain drops strike the soil surface at about 20 mph
- Soil aggregates are separated due to beating of rain drops
- Surface soil macropores fill with soil particles forming a **surface crust** and reducing infiltration
- Anaerobic zone produced under the surface crust
- Reduced soil infiltration also causes surface flow and erosion
- The more intense the rain the greater the damage

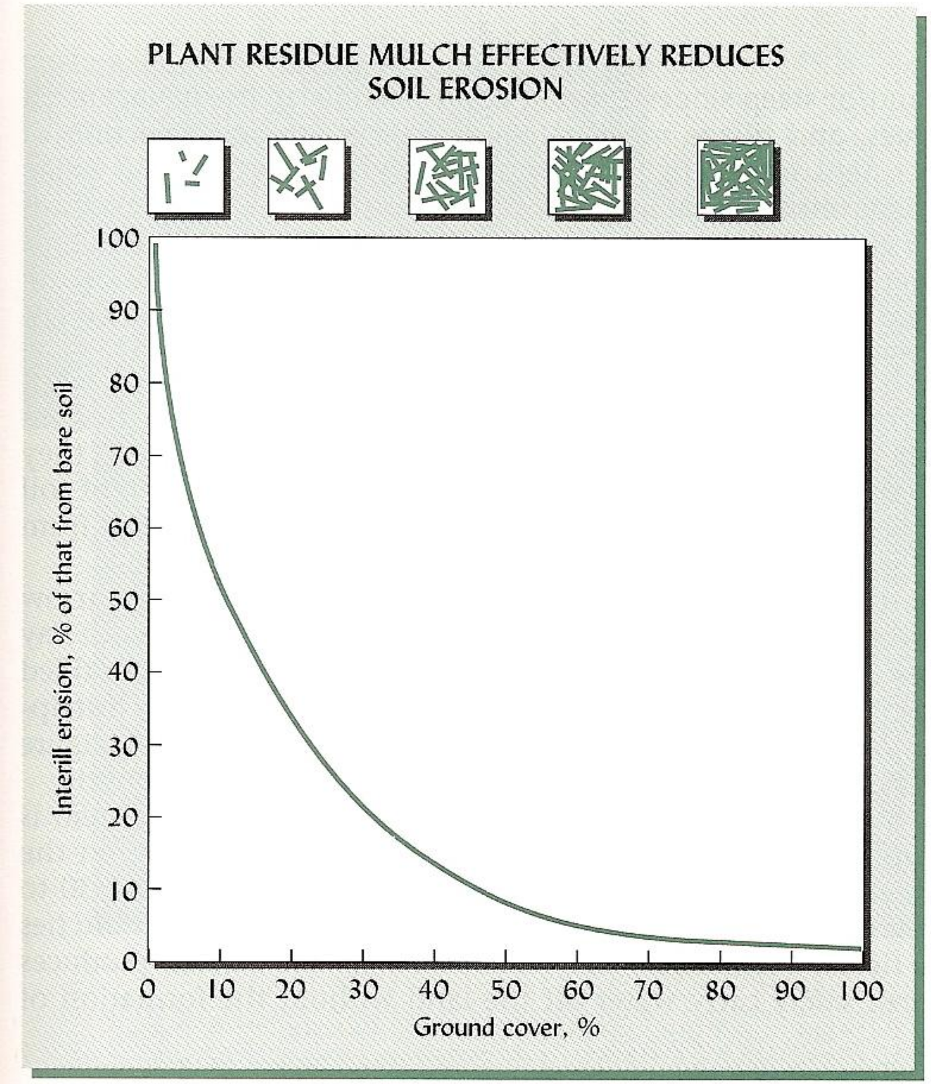




What types of soils tend to form surface crusts and how is crusting prevented?

- Tendency to form crusts
  - High silt or clay content
  - Low organic matter concentrations and weak aggregates
- Preventing soil crusting
  - High concentrations of decaying organic matter in the soil
  - **Light** surface dressing or mulch of organic matter
    - Too much and you limit oxygen transfer
  - Coordinate cultivation with rainfall events
    - After a heavy rain let the soil dry out then till

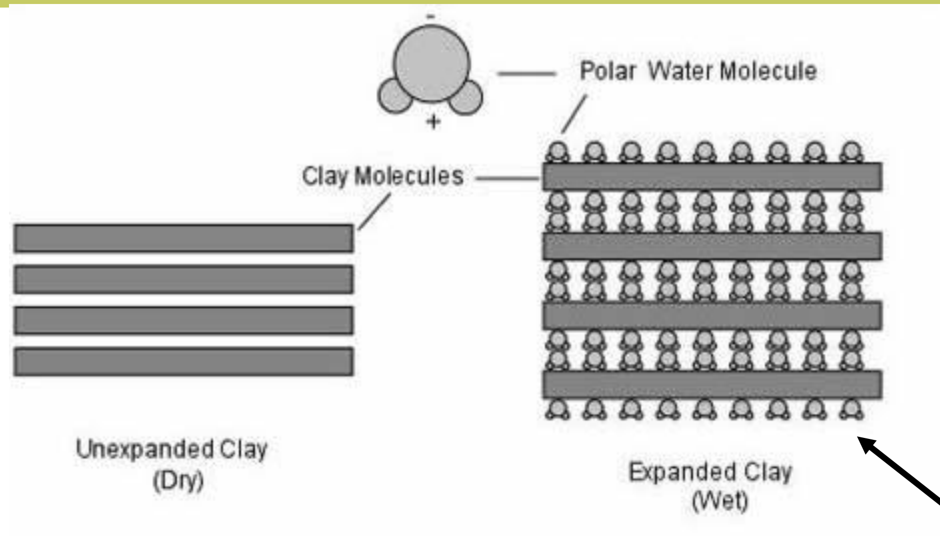
Surface dressing of organic matter reduces crusting and erosion



A little goes a long way; too much and you limit oxygen transfer into the soil

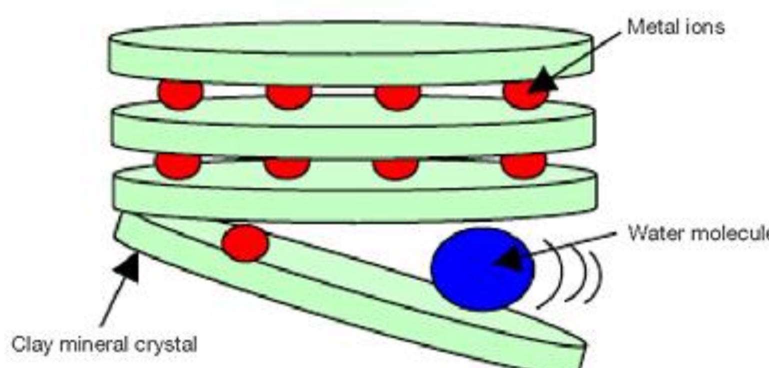
FIGURE 17.11 Reduction in interrill erosion achieved by increasing ground cover percentage. The diagrams above the graph illustrate 5, 20, 40, 60, and 80% ground cover. Note that even a light covering of mulch has a major effect on soil erosion. The graph applies to interrill erosion. On steep slopes, some rill erosion may occur even if the soil is well covered. [Generalized relationship based on results from many studies]

Another moisture issue: Certain clays swell when wet with fresh water with low electrolyte concentrations (as in rainfall and many irrigation waters)



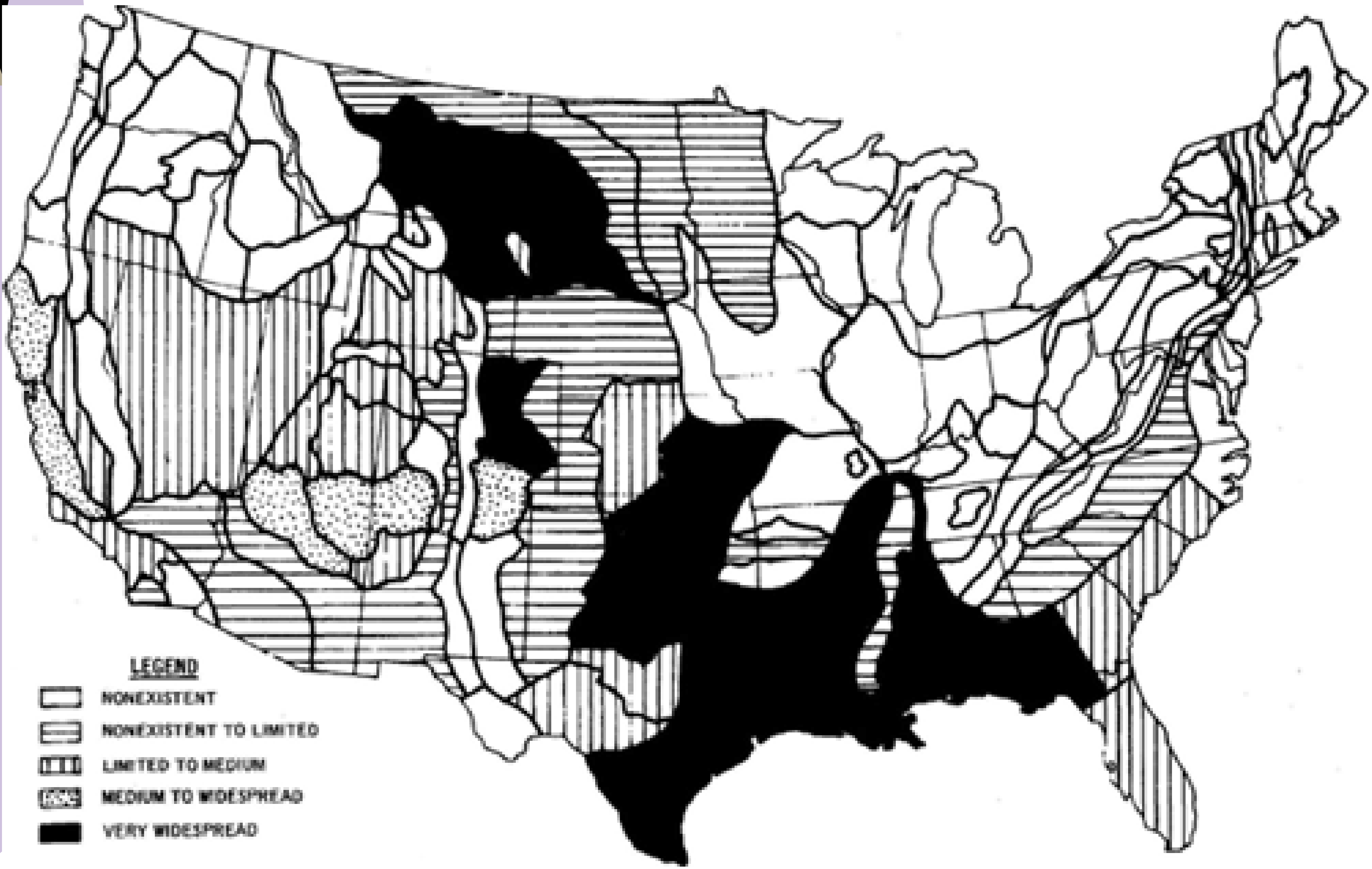
Seals off the surface and reduces infiltration


SMECTITE (2:1)
KAOLINITE (1:1)

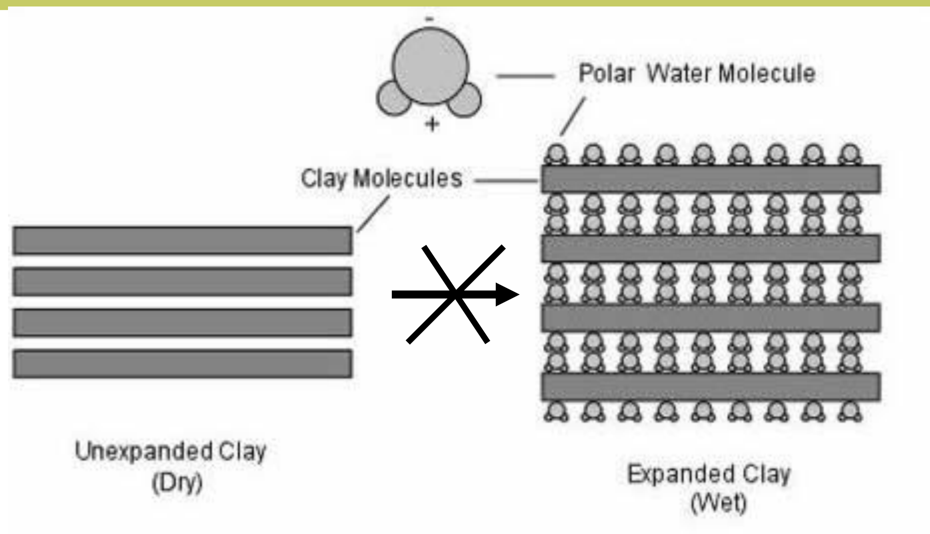

Metal ions
Water molecule
Clay mineral crystal

Typical sign of clays with high swelling potential

Distribution of swelling soils in the US

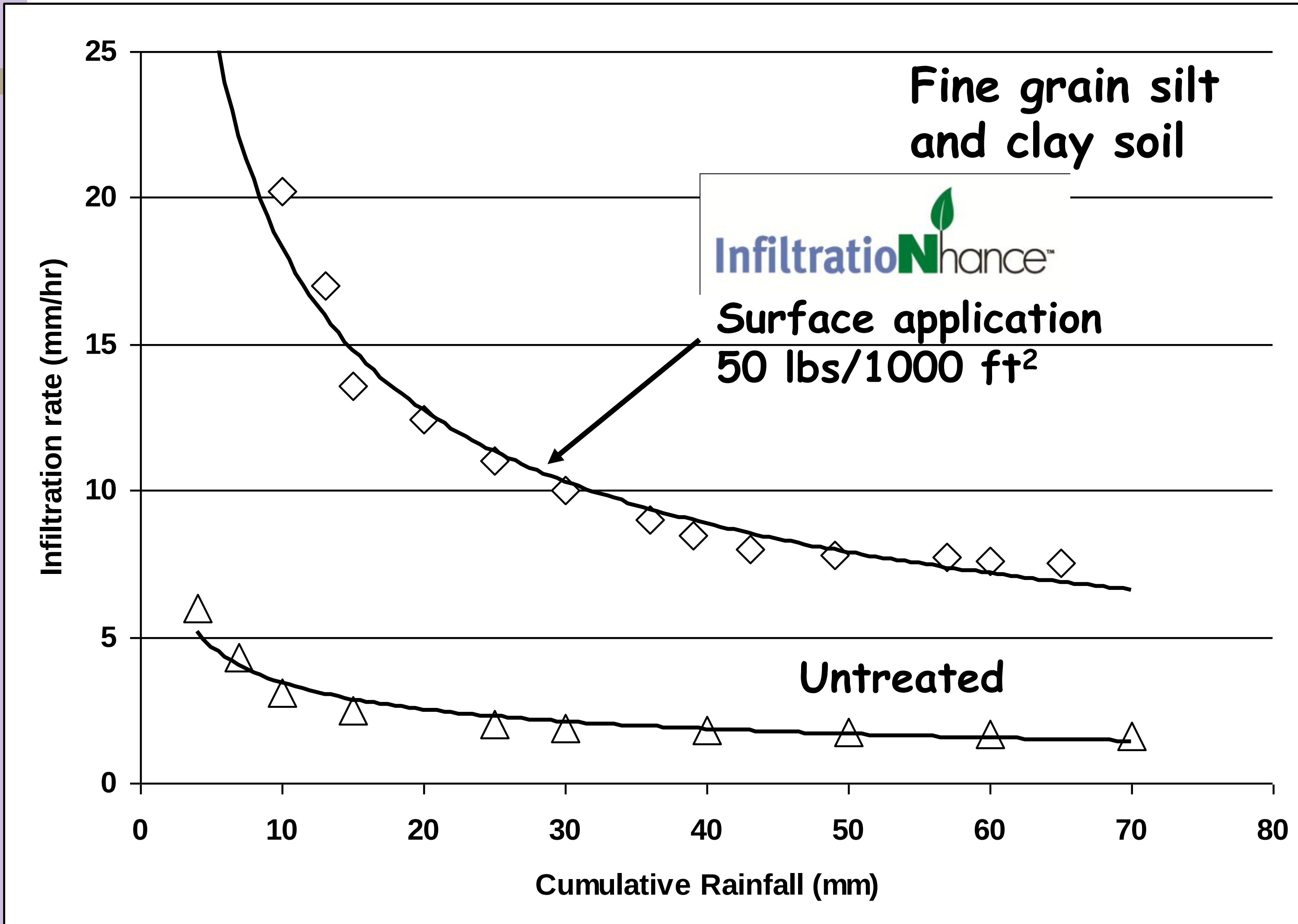


Preventing swelling and the resulting reduction in infiltration rates



Prevented by low concentrations of electrolytes – surface applied minerals with low solubility

Surface application of electrolyte source inhibits swelling and maintains good rates of water infiltration into the soil



Recommended practice for landfarms

Optimizing environmental conditions (to the extent we can)

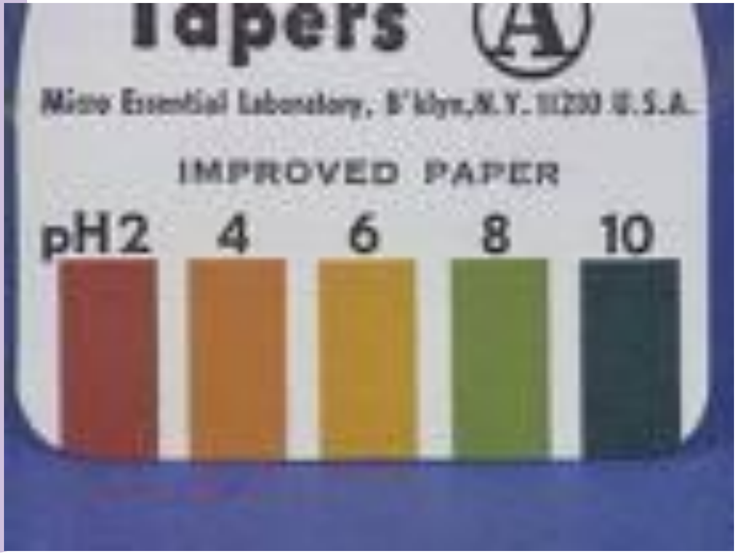
- pH
  - Optimum pH for biodegradation is typically 6-8 (unless the soil was originally outside this range normally)
  - Monitored monthly for the first 2-3 warm months and adjust if necessary

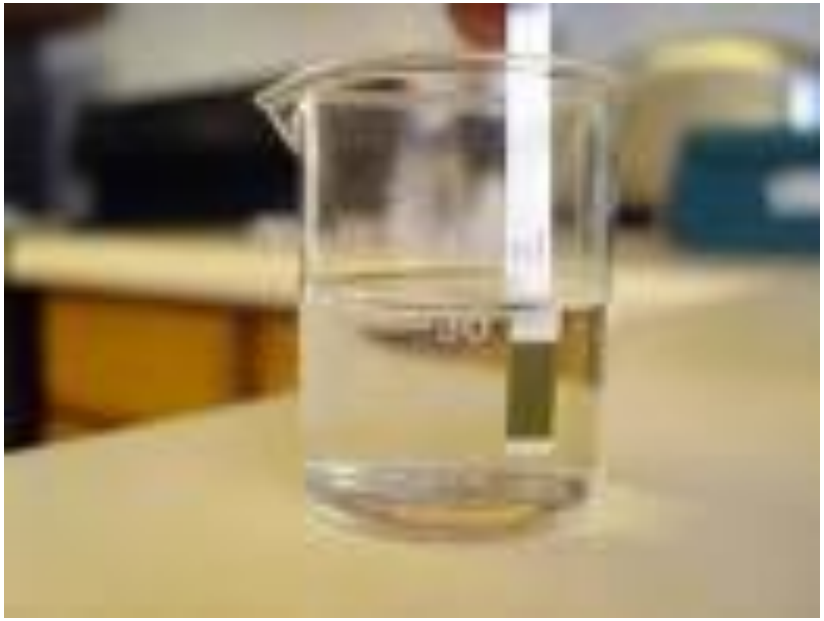
Measuring soil pH

- In a clean cup mix approximately equal volumes of soil and water
  - Distilled or deionized water from the grocery store or, better yet, use rain water
- Stir for about 30 sec, wait a few minutes and then repeat; finally allow the soil to settle
- Measure the pH of the water layer (it's OK if it's cloudy) using a pH meter, pH paper, or field kit

pH paper

pH paper contains dyes that are different colors at different pH values



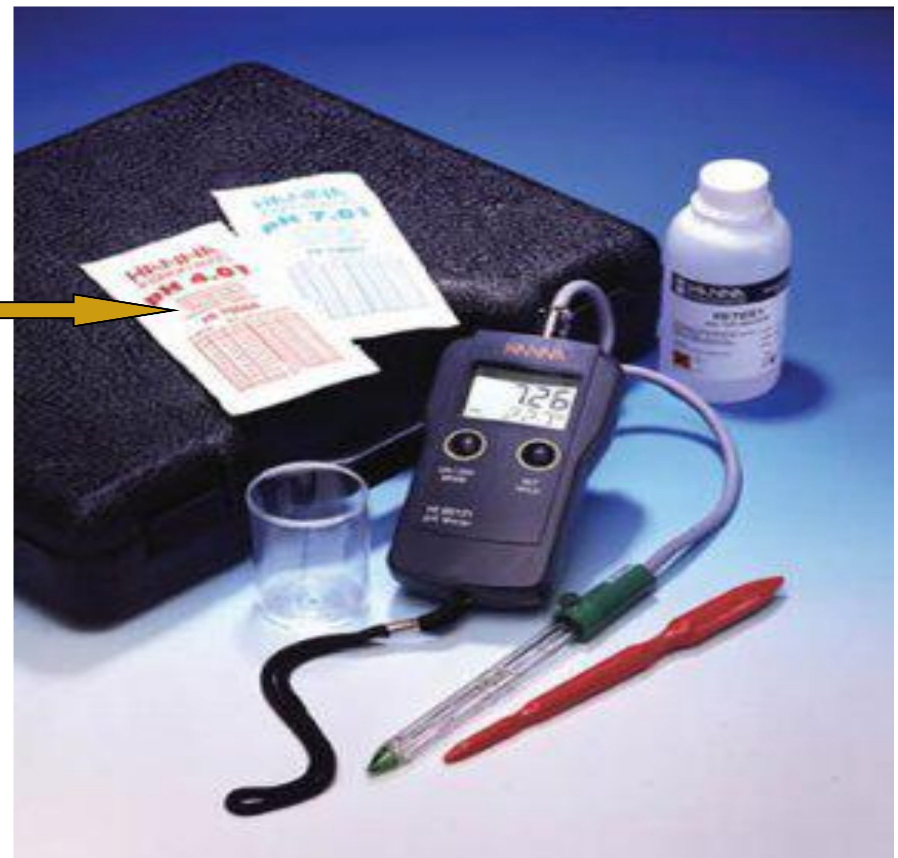


Soil pH field test kit

Sold in lawn and garden supply stores; same basic method as pH paper

pH meter

Buffers to standardize meter



Recommended practice for landfarms

- Adjusting pH
  - Neutralizing acidity (to raise pH):
    - Ag lime- dolomite or calcitic limestone crushed and ground to a specified fineness
      - Calcitic limestone – crystalline calcium carbonate ($CaCO_3$)
      - Dolomite – mixture of calcium carbonate and magnesium carbonate

Recommended practice for landfarms

- Adjusting pH, cont.
  - Increasing acidity (to lower pH):
    - Elemental sulfur (see tables)
    - Aluminum sulfate [$Al_2(SO_4)_3$]
    - Ferrous sulfate ($FeSO_4$)*

If pH is outside of the 6-8 range and the surrounding unimpacted soil is also outside of the 6-8 range in the same direction, do not try to adjust soil pH. If you do try to adjust pH get help from your local ag lab

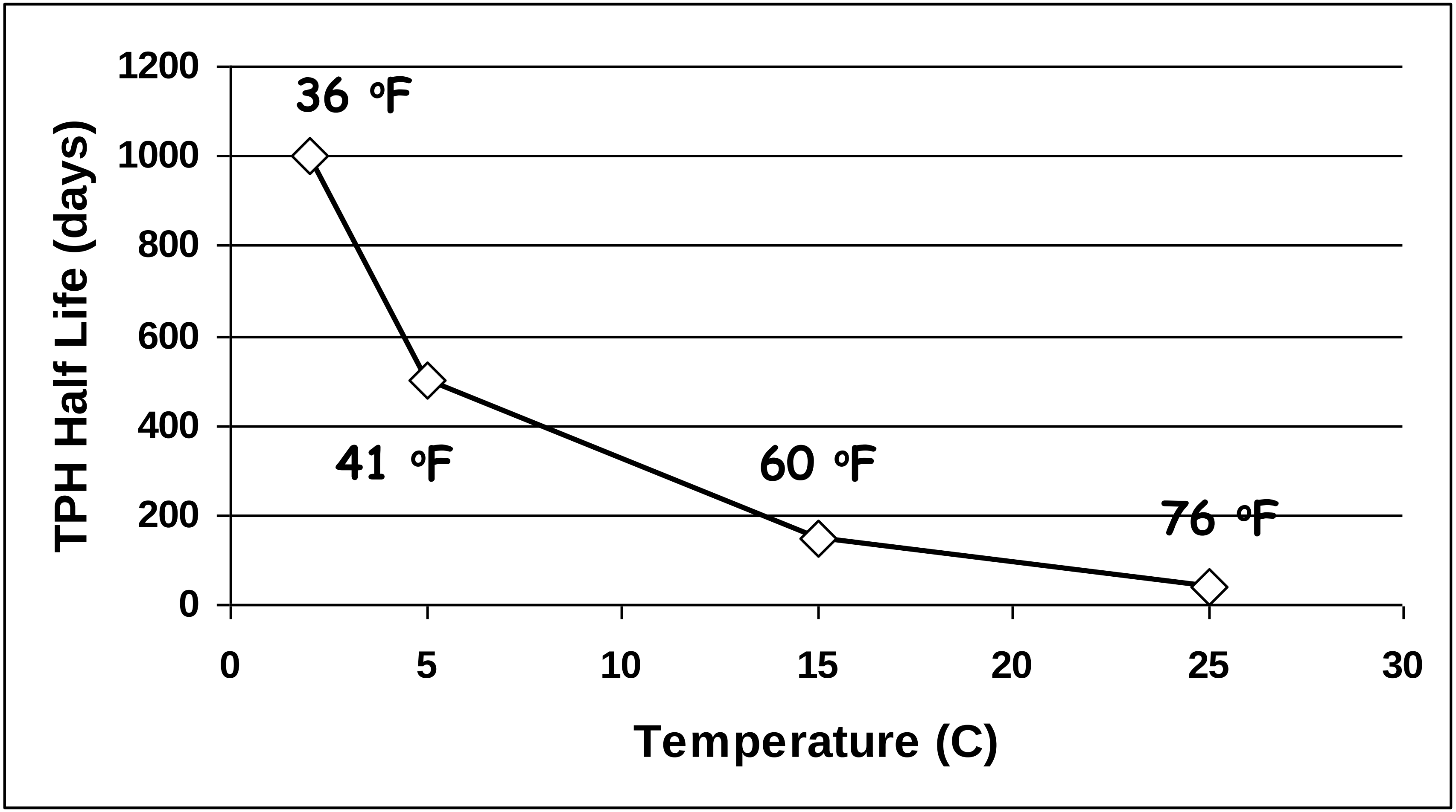
Recommended practice for landfarms

Optimizing environmental conditions (to the extent we can)

- Temperature
  - The landfarm operator has little control of temperature in the landfarm
  - Biodegradation rates decrease with decreasing soil temperature
  - In cold periods the operator should expect slower rates of hydrocarbon removal and slower rates of nutrient utilization
    - Less need for monitoring
    - Less need for cultivation

Biodegradation rates of diesel hydrocarbons increase with temperature

Zytner, R.G., A Salb, T.R. Brook, M. Leunissen, and W.H. Stiver, Can. J. Civil. Eng., 28, 131-140 (2001)



Recommended practice for landfarms

Moisture! Moisture! Moisture!

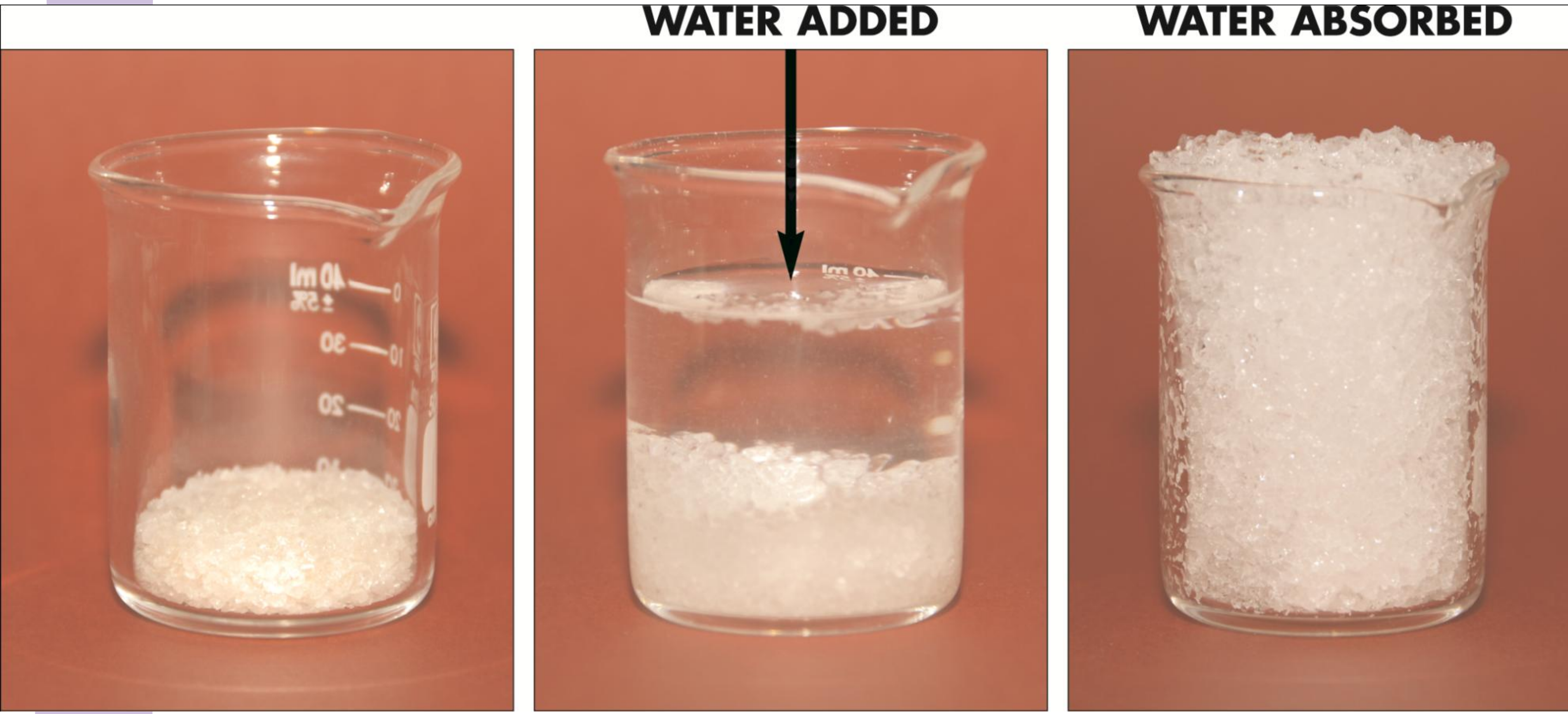
- Moisture
  - Optimum soil moistures for biodegradation are typically 60-80% of field capacity
    - This gives plenty of moisture for microbes but allows large macropores to exist in the soil for oxygen transfer
    - Saturation results in anaerobic conditions
    - Monitor as often as possible

Here's a large project in a dry climate using irrigation equipment to supply moisture

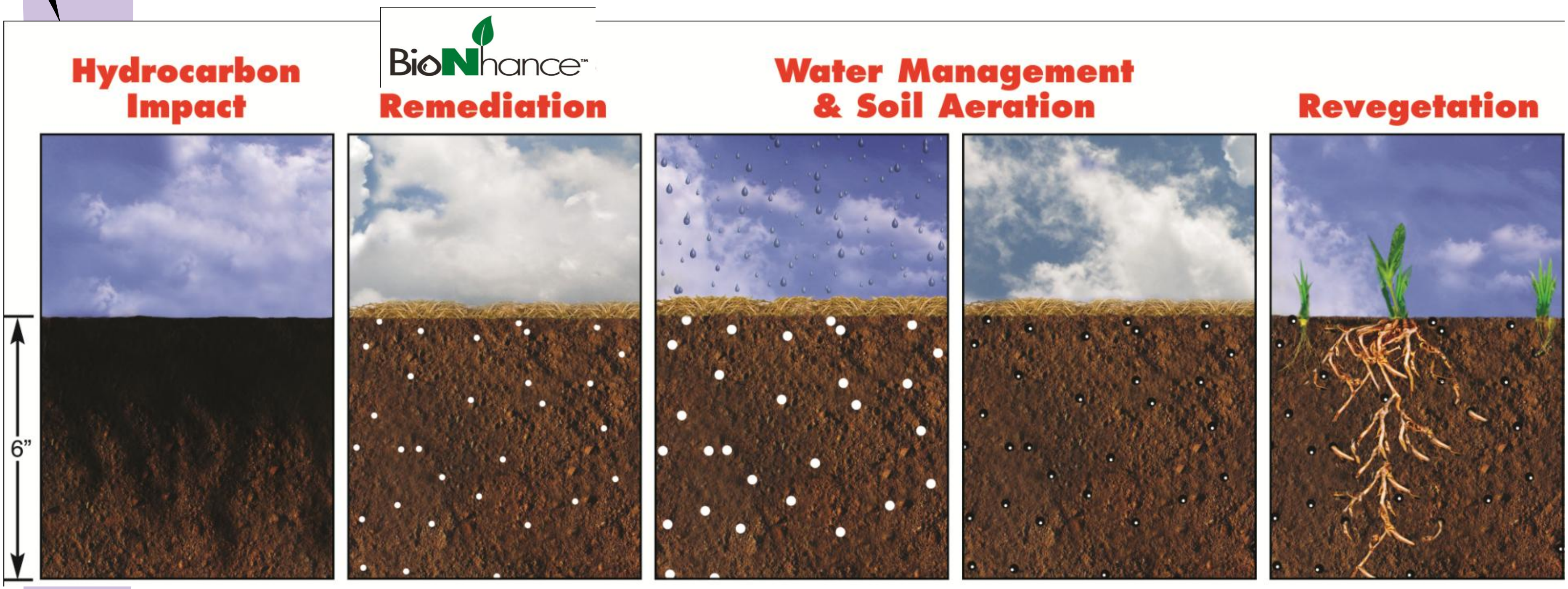
Recommended practice for landfarms

- Moisture
  - A convenient way of measuring soil moisture

When water is in short supply: The power of hydrogels



BioNhance captures water and aerates the soil



Recommended practice for landfarms
TPH monitoring

- Hydrocarbon analysis for operational monitoring
  - Following the course of hydrocarbon degradation
  - Determining the bioremediation endpoint
  - Several commercial field kits available that are satisfactory for operational monitoring
    - Don't change methods in mid-process
  - Rule of thumb – when you can no longer smell hydrocarbon in the soil you are at or near the endpoint.

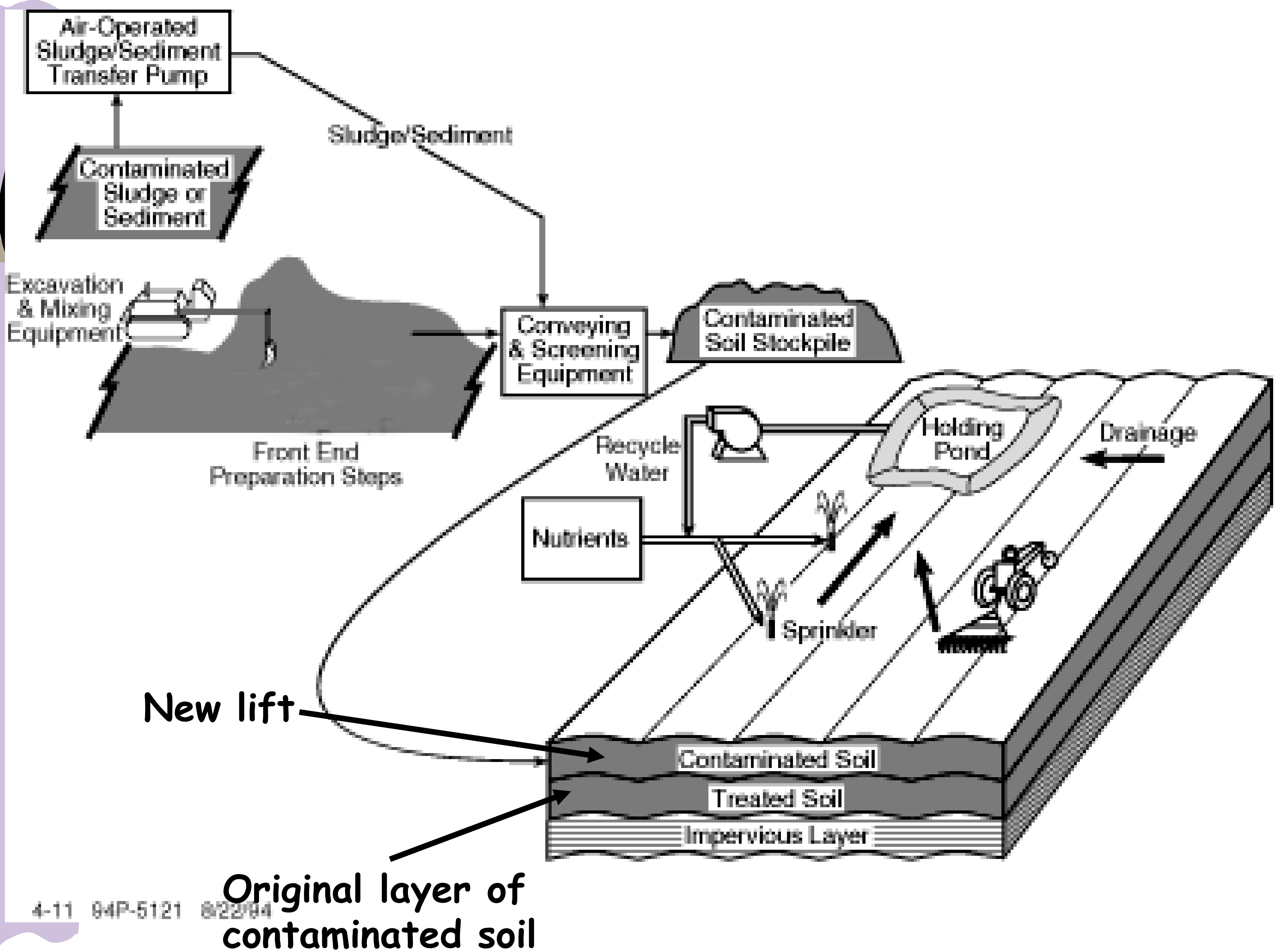
Recommended practice for landfarms

- Hydrocarbon analysis closure
  - Requires at a minimum a measurement of TPH by a method specified by the regulatory authority
  - May also require measurements of individual components (Contaminants of Concern) where risk of human exposure is significant.
  - Regulators may also specify the number of grab or composite samples required

Recommended practice for landfarms

Multi-lift *ex situ* landfarm

- *Ex situ* landfarm will be required when
  - Contamination is deeper than the soil can be effectively cultivated (typically 6-8 inches)
  - Shallow groundwater
  - Too much slope
  - Required by regulator
- Adding the another lift in an *ex situ* landfarm
  - Each new lift should be cultivated to incorporate the top two inches of the lift below
  - This will provide an inoculum of microbes already adapted to the hydrocarbon and environmental conditions.



Air-Operated Sludge/Sediment Transfer Pump
Contaminated Sludge or Sediment
Sludge/Sediment
Excavation & Mixing Equipment
Conveying & Screening Equipment
Contaminated Soil Stockpile
Front End Preparation Steps
Recycle Water
Holding Pond
Drainage
Nutrients
Sprinkler
New lift
Contaminated Soil
Treated Soil
Impervious Layer
Original layer of contaminated soil
4-11 94P-5121 8/22/94

When will I be finished?

- Hydrocarbon degradation rates in landfarms are dependent on:
  - Type of hydrocarbon and hydrocarbon concentration
  - Oxygen transfer into the soil
    - Soil type
    - Organic matter
    - Cultivation frequency
  - Nutrients
  - Soil moisture
  - Temperature

Recommended practice for landfarms

Bioaugmentation

- Soils usually have indigenous microorganisms that are effective degraders of hydrocarbons if all other growth requirements are met (highest populations are found in soils with long-term exposure to hydrocarbons)
- Non-indigenous microorganisms **rarely** compete well with indigenous populations
- The only type of "bioaugmentation" that makes sense is using good quality topsoil or landfarm soil to augment a contaminated poor quality soil

Commercial bugs are adapted to this environment

Not this environment

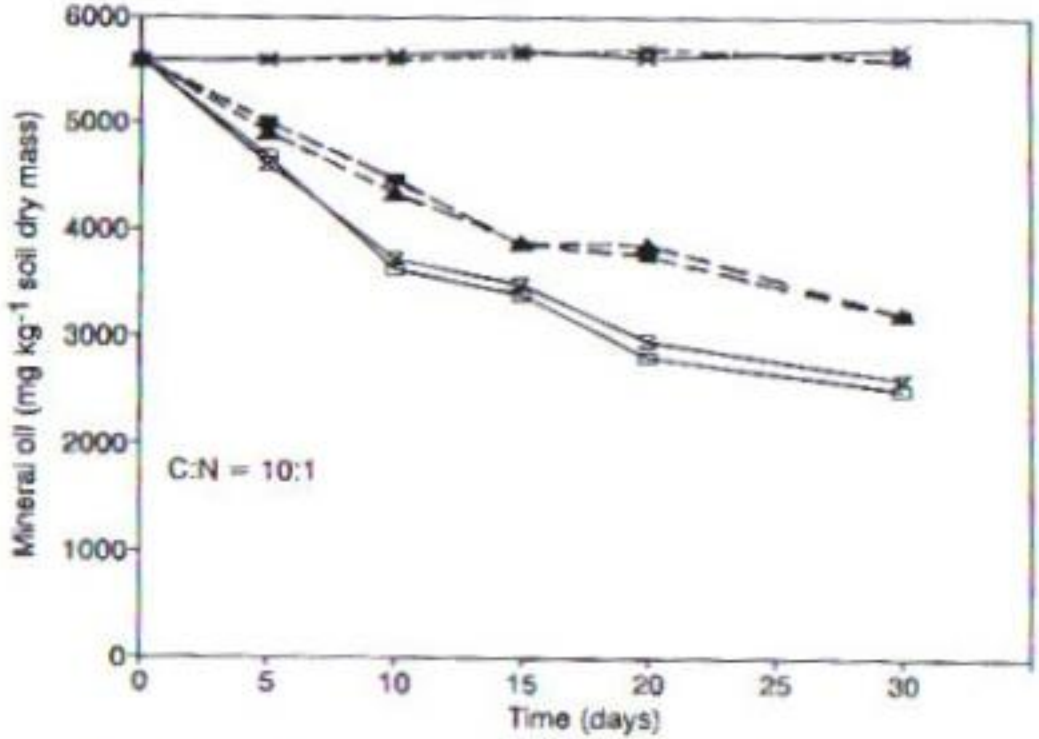


Fig. 3. Bioremediation of a diesel-oil contaminated soil at 10°C and 25°C in the presence of fertilizer at a C : N ratio of 10 : 1. Poisoned control (abiotic elimination): 10°C (---✱---), 25°C (—×—); non-inoculated soil (activity of indigenous soil microorganisms); 10°C (---■---), 25°C (—□—); inoculated soil: 10°C (---▲---), 25°C (—⌧—).

Margesin, R. and F. Schinner, Laboratory bioremediation experiments with soil from a diesel-oil contaminated site – Significant role of cold-adapted microorganisms and fertilizers, *J. Chem. Tech. Biotechnol.,***70**, 92-98 *1997)

Quote from EPA fact sheet

- "Since oil-degrading bacteria usually grow at the expense of one or more components of the crude oil, and these organisms are ubiquitous, there is usually no reason to add hydrocarbon-degrading bacteria unless the indigenous bacteria are incapable of degrading one or more of the contaminants. The size of the hydrocarbon-degrading bacterial population usually increases rapidly in response to oil contamination and it is difficult, if not impossible, to increase the microbial population over that which can be achieved by biostimulation alone."

Low-tech *in situ* bioremediation protocol
Site characterization

- Has the source been removed?
- Type of hydrocarbon? API gravity? How much? Any brine?
  - Can frame expectations of rate and extent of bioremediation
- Depth of contamination?
  - *In situ* vs. *ex situ*
- Hydrocarbon concentration?
  - Spill area as surrogate for initial TPH concentration
  - What type of sampling and TPH analysis will be required for closure?
  - Will dilution be necessary?
    - Does the soil still glisten after blending in amendments?
    - Source of clean soil?

Low-tech *in situ* bioremediation protocol
Site characterization

- Soil characteristics
  - Salinity (EC)
    - Drainage?
  - Texture
    - Rope test – special attention to bulking agents with high clay
  - What amendments will be used?
    - Fertilizers
      - 13:13:13 or comparable composite fertilizer
      - How much for first application?
        - Based on area and maximum nitrogen application rates (3-4 lb per 1000 ft^2)
      - How will nutrients be monitored?
        - None (or simple field kit)

Low-tech *in situ* bioremediation protocol
Site characterization and moisture

- Soil characteristics, cont.
    - Bulking agents
        - Hay or straw only?; Compost?
        - Source?
        - How much will be needed?
    - Equipment for blending and cultivating?
        - Tilling only
- Source of moisture?
    - Rainfall only if sufficient
    - Maximizing infiltration and minimizing losses
        - Light top dressing
        - Hydrogels in arid climates
    - Swelling clays?
        - Surface applied electrolyte
- Down gradient receptors?
- When to start?

Low-tech *in situ* bioremediation protocol
Site preparation

- Establish remediation goals with regulator and landowner
  - Required TPH?
  - Timeframe?
  - Can the landowner provide equipment, hay, etc.?
  - Revegetation
- Site preparation
  - Till in amendments
    - Fertilizer
    - Organic matter (hay, compost)
  - Dilute with clean soil as necessary until the soil no longer glistens
  - Surface treatment for swelling clays if necessary

Low-tech *in situ* bioremediation protocol

Site preparation and maintenance

- Site preparation, cont.
  - Top dress with **thin** layer of organic matter after tilling in nutrients and organic matter
- Site maintenance
  - Reapply and till in fertilizer based on maximum nitrogen application after 30 and 60 days (unless too dry)
  - Till at least twice a month for the first 2-3 months and monthly thereafter
  - Reapply light top dressing of organic matter after any tilling
- Closure
  - Final TPH analysis as directed by regulator after a smell test indicates bioremediation endpoint has been achieved
- Revegetation
  - Natural

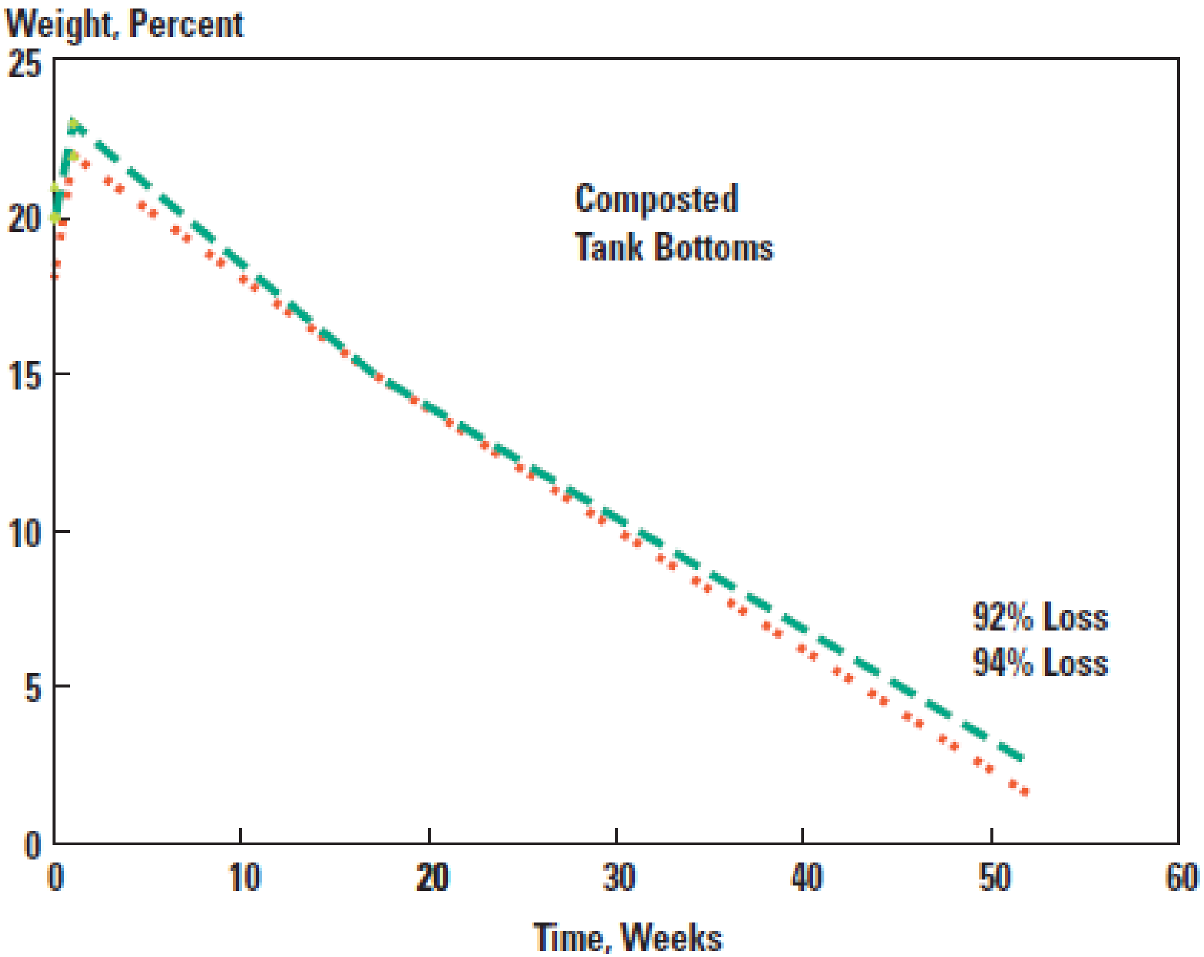
Composting vs. landfarming

- Composting
  - Windrows
  - Microbial heating
  - Smaller footprint
  - Greater requirements for organic matter for aeration
- Landfarming
  - Thin layers
  - No microbial heating
  - Larger footprint
  - Less organic matter required for aeration

Figure 12. Steam Rising From Compost During Tilling

Source: Sara Mc Millen, Ross Smart, Rene Bernier and Rob Hoffmann. "Biotreating E&P Wastes: Lessons Learned From 1992-2002." Presentation at the 2002 Integrated Petroleum Conference, Albuquerque, NM.

Figure 6. Biodegradation Results From Composting Tank Bottoms



Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

See the appendix for more information about composting of hydrocarbons

What do you do about
1) deep contamination
2) weathered hydrocarbon?

Deep or highly weathered contamination

- Highly weathered hydrocarbon
  - High fractions of heavy hydrocarbons and high TPH at bioremediation endpoint
- Deep contamination
  - Excavation required for *ex situ* landfarming
    - Cost of excavation increases with depth and volume of contaminated soil
    - Risks of exposure increase

Deep or highly weathered contamination

- The solution: *In situ* treatment by **chemical oxidation** (ISCO) + biodegradation
  - Chemical oxidation can completely oxidize some hydrocarbons (to carbon dioxide) and partially oxidize others
  - The partially oxidized hydrocarbons are now highly biodegradable
- The ISCO must be effective in oxidizing hydrocarbons but not kill the bugs
  - No excessive heat
  - No pH extremes
- And no sodium!
  - (SAR)

The Cool-Ox® process

(Producing hydrogen peroxide in situ)

$$CaO_2 + H_2O \rightarrow Ca(OH)_2 + H_2O_2$$

(Chelates activate intrinsic catalysts)

$$H_2O_2 + Fe^{+2} \rightarrow (OH)^- + [OH]^\bullet + Fe^{+3}$$

$$H_2O_2 + Fe^{+3} \rightarrow (OH)^- + [OOH]^\bullet + Fe^{+2}$$

(Radicals react with contaminants)

$$[OH]^\bullet \;\&\; [OOH]^\bullet + C_x \rightarrow C_x(OH)_y$$

The Cool-Ox® process

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$$H_2O_2 + Fe^{+3} \rightarrow (OH)^- + [OOH]^\bullet + Fe^{+2}$$

Highly biodegradable

(Radicals react with contaminants)

$$[OH]^\bullet \,\&\, [OOH]^\bullet + C_x \rightarrow C_x(OH)_y$$

Advantages of Cool-Ox over other chemical oxidation processes

- Sustained oxidation
  - CaO_2 dissolves slowly
- pH 8
  - No corrosion
  - Compatible with biodegradation
- No heat
- No sodium (does not raise the SAR, in fact it **reduces SAR**)
- **Transient** generation of surfactants
- Less up front site characterization needed

FIGURE 2
EB Concentrations With Time In The Slurry Reactors.

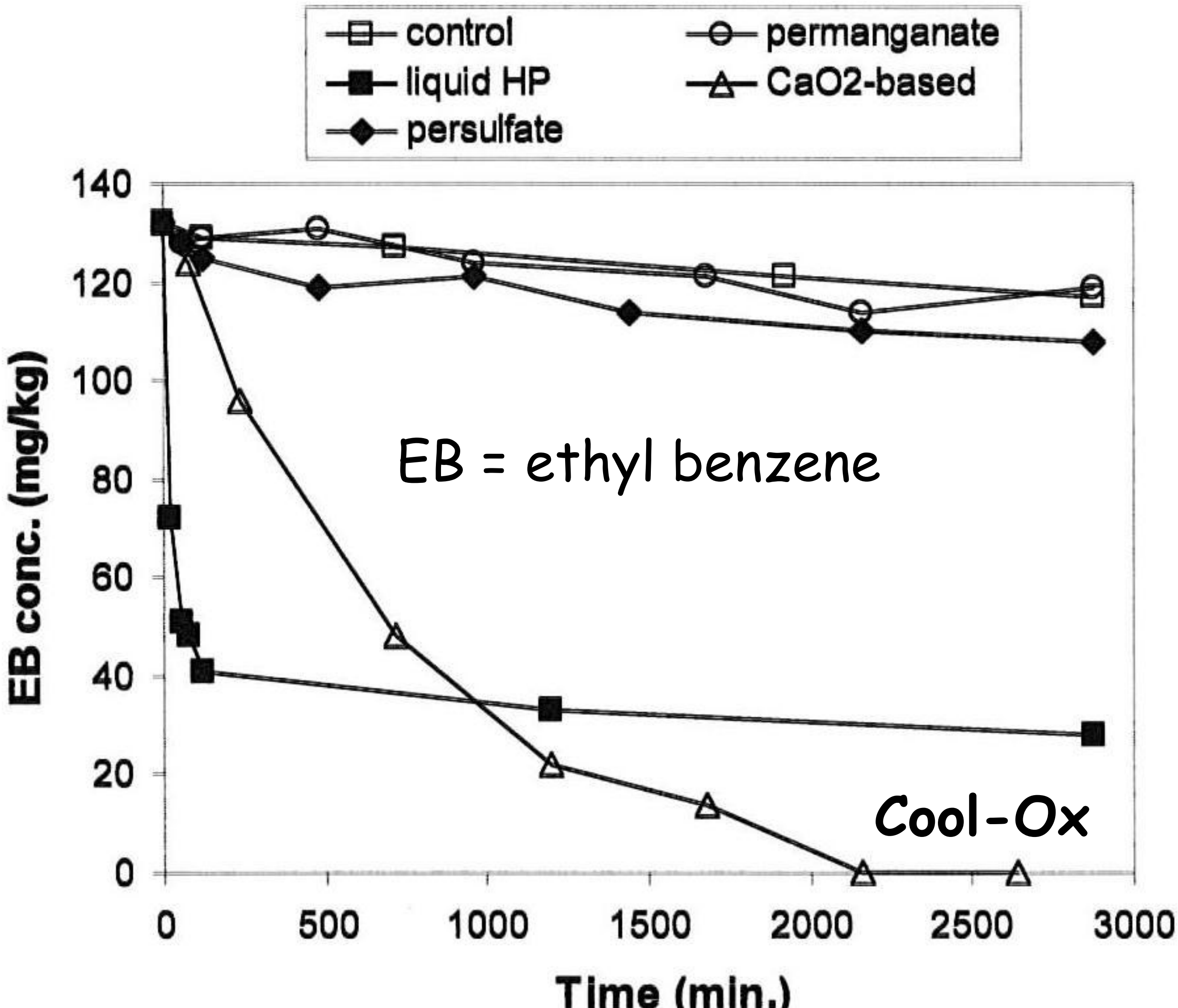
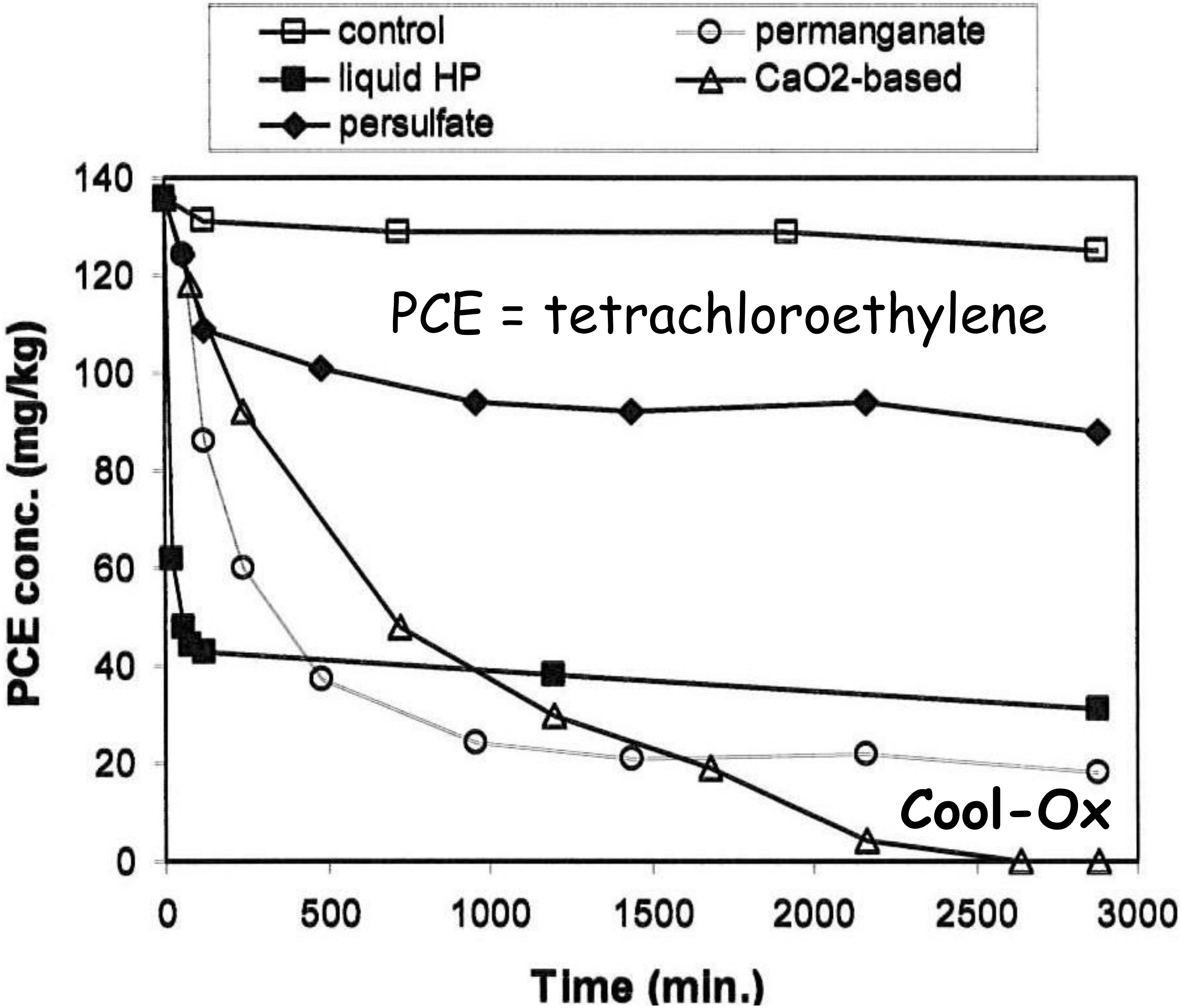
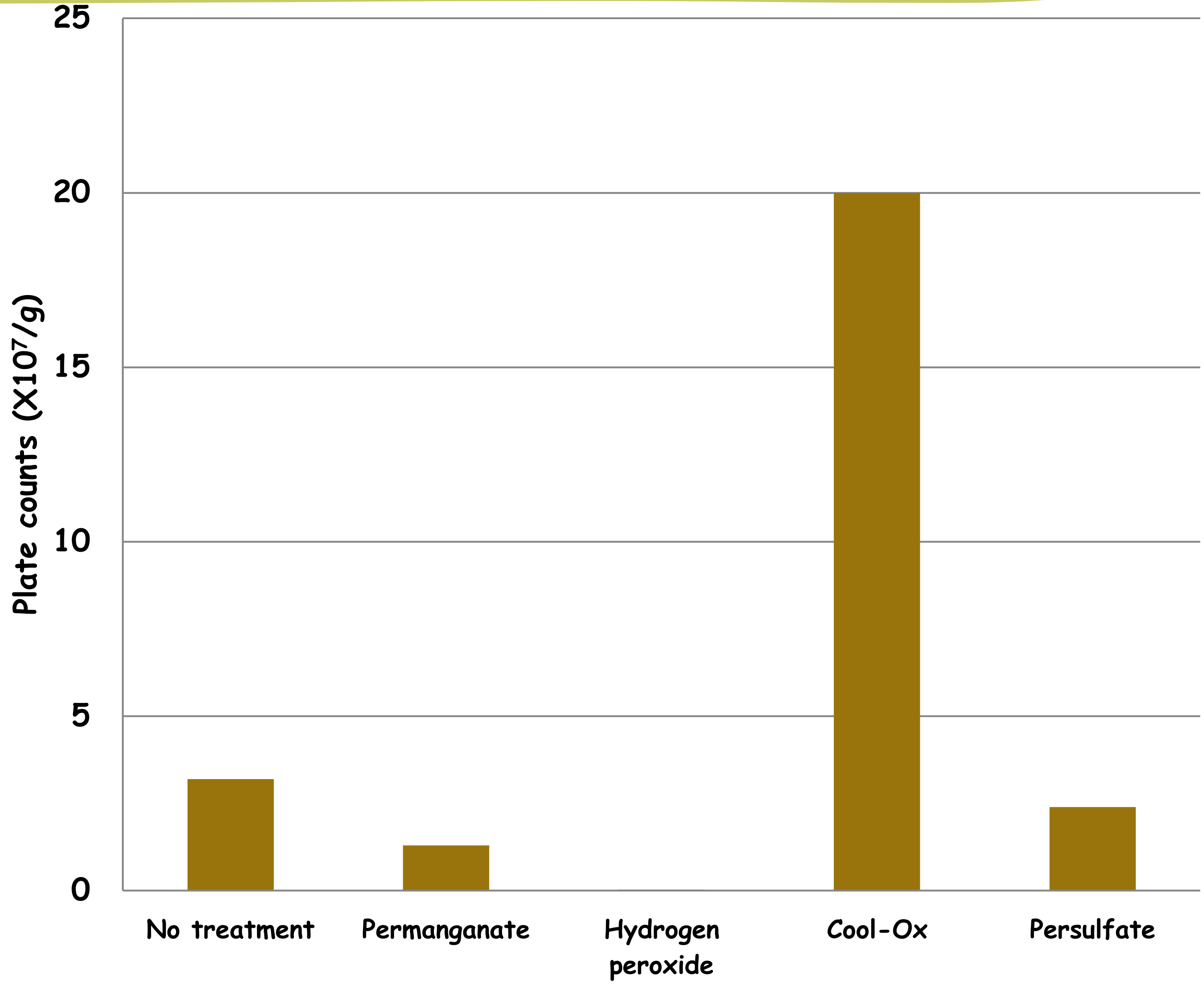


FIGURE 1

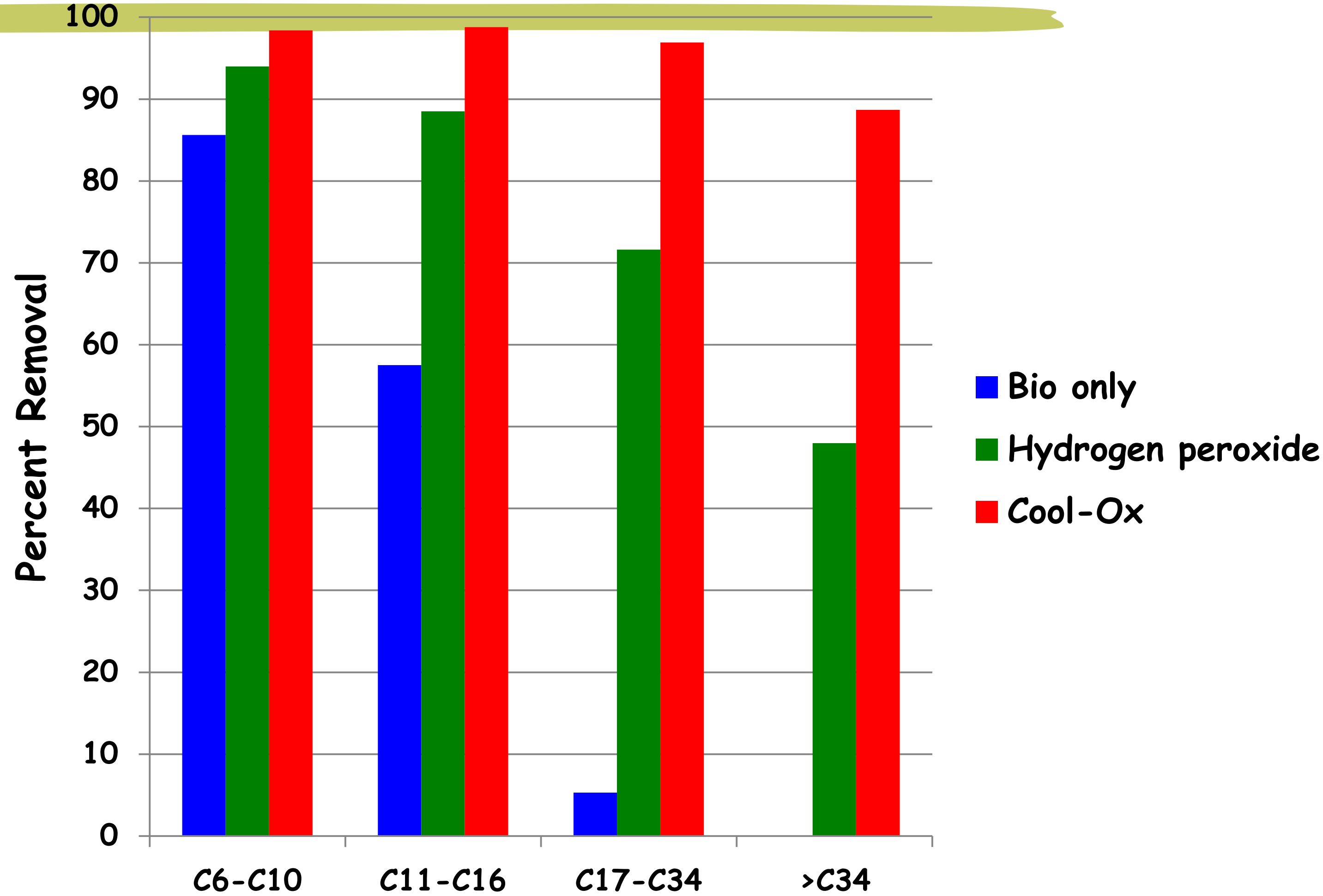
PCE Concentrations With Time In The Slurry Reactors.



Effect of chemical oxidation of soil bacteria

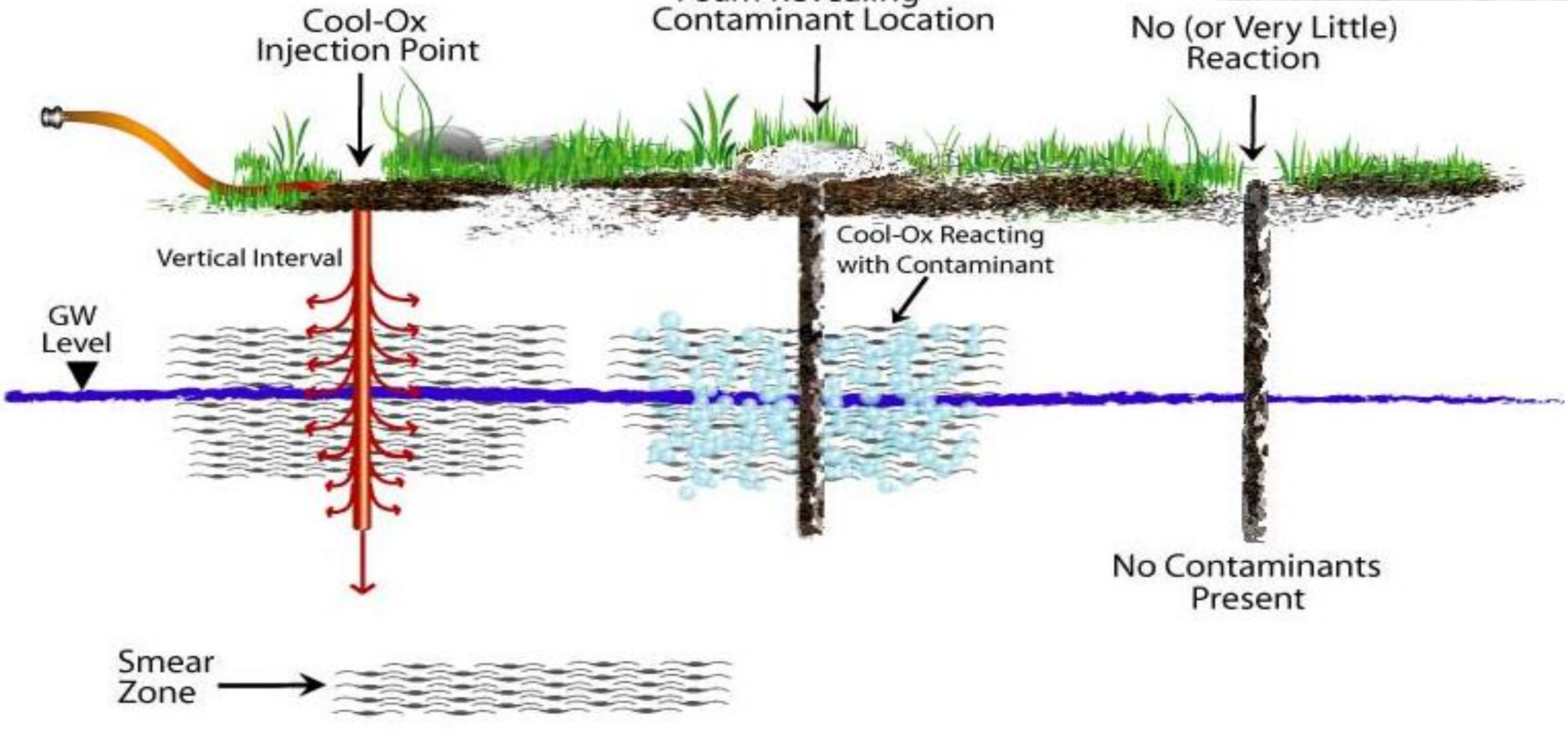


Removal of hydrocarbon fractions



Finding the contamination

Cool-Ox Technology As A Site Characterization Tool



Hydro-Dart application of Cool-Ox reagent

Excavation with surface application of Cool-Ox reagent

Deep delivery of Cool-Ox reagents with intense mixing

Surface treatment of highly weathered hydrocarbon

Surface application of Cool-Ox followed by bioremediation

Moderate pH (8) of Cool-Ox treatment does not interfere with bioremediation and does not promote corrosion