MICROORGANISMS & METAL POLLUTANTS

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METAL POLLUTION

- A global concern
- Increases to toxic levels in some cases as a result of wide variety of industrial and domestic sources
- Anthropogenic emissions up to **100 fold** than that from natural sources e.g. for Pb, Cd, Va, and Zn
- It poses serious health and ecological risks. E.g. Metals, such as Al, An, As, Cd, Pb, Hg & Ag causes;

- Hypophosphatemia
  - electrolyte disturbance in which there is an abnormally low level of phosphate in the blood, leading to heart disease and liver damage
- cancer
- neurological and cardiovascular disease
- central nervous system damage
- Encephalopathy – brain injury/diseases
- Lead poisoning of children is common and leads to retardation and semi-permanent brain damage
- sensory disturbances
- Death of fish and shellfish due to methylmercury accumulation

- Metals are not as amenable to bioremediation as organics because of their toxicity.
- They are thus persistent in the environment.
- Microbes have developed unique and sometimes bizarre ways of dealing with unwanted metals, including:
  - sequestration and immobilization of metals.
  - enhancement of metal solubility in the environment.

THREE CLASSES OF METALS

- **Metals** – a class of chemical elements that form lustrous solids that are good conductors of heat and electricity. H/v, there are exceptions. E.g. Hg is a liquid

- **Metalloids** – properties intermediate between those of metals and non-metals e.g. As, B, Germanium, and Tellurium

- **Heavy metals**
  - capable of cationic-hydroxide formation,
  - specific gravity greater than 5g/ml,
  - complex formation,
  - hard-soft acids & bases,
  - Associated with eutrophication & environmental toxicity

CLASSIFICATION BASED ON BIOLOGICAL FUNCTIONS AND EFFECTS

- **The essential metals with known biological functions**
  - Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo, &W

- **The toxic metals**
  - No known biological function e.g.Ag, Cd, Sn, Au, Hg, Ti, Pb, Al, & the metalloids Ge, As, Sb, & Se

- **The nonessential, nontoxic metals with no known biological effects**
  - Rb, Cs, Sr & Ti

SOURCES OF METALS

- **Human activities**

  - Mining;
  - ore refinement;
  - nuclear processing;
  - Industrial manufacture of batteries, metal alloys, electrical components etc results in the accumulation of metals in soil

- The contaminated soil provide a metal sink from which surface waters, groundwaters, and the vadose zone become contaminated

SOURCES OF METALS

- Contaminated soil contributes to high metal concentrations in the air through **metal volatilization**
- **Industrial emissions** and **smelting activities** also cause release of substantial amount of metals to the atmosphere
- **Weathering** of parent materials containing high levels of metals
- Metal-containing sewage sludges

METAL BIOAVAILABILITY IN THE ENVIRONMENT

- **Bioavailable metals**
  - soluble, non-sorbed, & mobile
  - taken up and toxic to biological systems

- **Nonbioavailable metals**
  - precipitated, complexed, sorbed, and non-mobile

FACTORS AFFECTING BIOAVAILABILITY AND TOXICITY OF METALS TO MICROBIAL POPULATIONS

METAL CHEMISTRY

- Cationic or anionic properties of metals

- Most metals are cationic and most reactive with negatively charged surfaces (e.g. on clay minerals) and with anionic salts, such as phosphate & sulfate

- They are also attracted to negatively charged cell surfaces where they can be taken up and cause toxicity

- The size and charge of the cationic metal determines the strength of adsorption

METAL CHEMISTRY

- *Components in the soil solution also affects metal solubility- Phosphate, Sulfate & Carbonate form sparingly soluble metal-salt compounds*
- Al binds more strongly than Ca or Mg
- $Al^{3+} > Ca^{2+} = Mg^{2+} > K^{+} > Na^{+}$
- Al^{3+} has strong affinity for clay surfaces & primarily found as $Al(OH)_3$ and therefore has extremely low bioavailability

CATION EXCHANGE CAPACITY

- dependent on both organic matter and clay content of the soil
- reflects the capacity for a soil to sorb metals
- toxicity of metals within soils with high CEC (organic and clay soil) is low even at high total metal concentrations
- sandy soil with low CEC have low metal binding capacity, thus high metal toxicity

REDOX POTENTIAL

- Metal bioavailability changes in response to changing redox conditions

- Under oxidizing or aerobic conditions, metals are usually found as soluble cationic forms, e.g., Cu^{2+}, Cd^{2+}, Pb^{2+}, and Ca^{2+}

- Reduced or anaerobic conditions (found in sediments and saturated soils) often result in metal precipitation

PH

- **At high pH**, metals are predominantly found as **insoluble** metal mineral phosphates and carbonates.
- **At low pH**, they are commonly found as free ionic species or as **soluble** organometals.
- pH also affects metal sorption to soil surfaces, owing to the changes in the net charge on soil and organic particles.

PH

- **As pH increases**
  - the **electrostatic attraction** between a metal and soil constituents is **enhanced** by **increased pH-dependent CEC**
  - **Metal solubility decreases** with increase in pH, thus decreasing metal bioavailability
- As soil **pH decreases**
  - **Metal solubility increases**, thus **enhancing metal bioavailability**

METAL TOXICITY EFFECTS ON THE MICROBIAL CELL

- The toxic nature of metals results from their strong ionic nature.
- They **bind to many cellular ligands and displace native essential metals** from their normal binding sites (Fig. 17.3).
- E.g. Arsenate can replace phosphate in the cell.

METAL TOXICITY EFFECTS ON THE MICROBIAL CELL

- Metals also **disrupt**:
  - **proteins by binding to sulfhydryl (-SH) groups**
  - **nucleic acids by binding to phosphate or hydroxyl groups**
- E.g. Cd competes with cellular Zn and nonspecifically binds to DNA, inducing single-strand breaks
- Interference with **oxidative phosphorylation** and **membrane permeability** e.g. vanadate and Hg

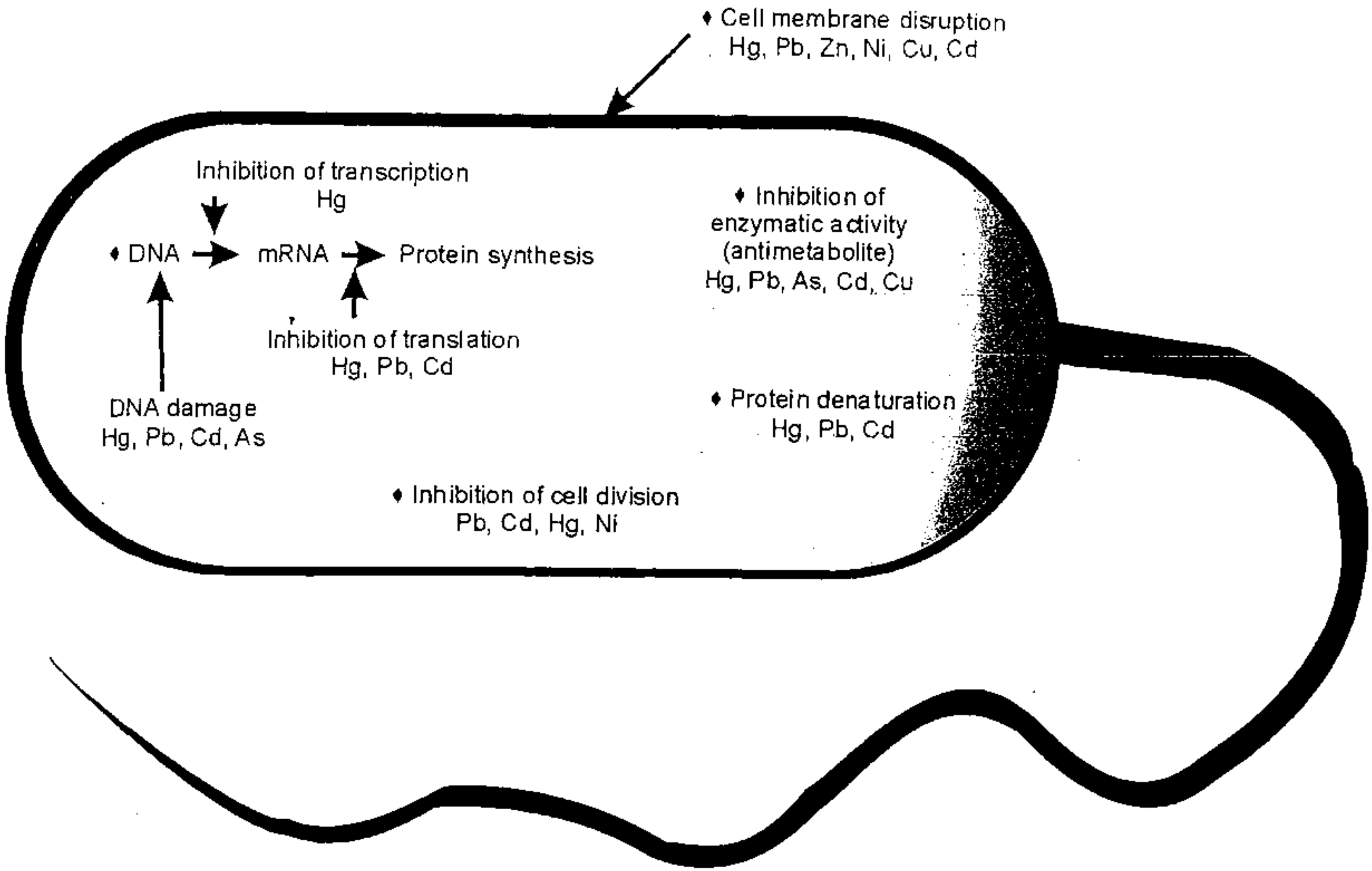


FIGURE 17.3 Summary of the various toxic influences of metals on the microbial cell demonstrating the ubiquity of metal toxicity. Metal toxicity generally inhibits cell division and metabolism. As a result of this ubiquity, microorganisms have to develop "global" mechanisms of resistance that protect the entire cell from metal toxicity.

METAL TOXICITY EFFECTS ON THE MICROBIAL CELL

- The various systems used to transport metals into cell is illustrated in Fig. 17.4
- Toxic metals can cross membranes, via diffusion or via pathways designed for other essential metals
- e.g. Cd^{2+} transport occurs via the Mn^{2+} active transport system in *S. aureus*

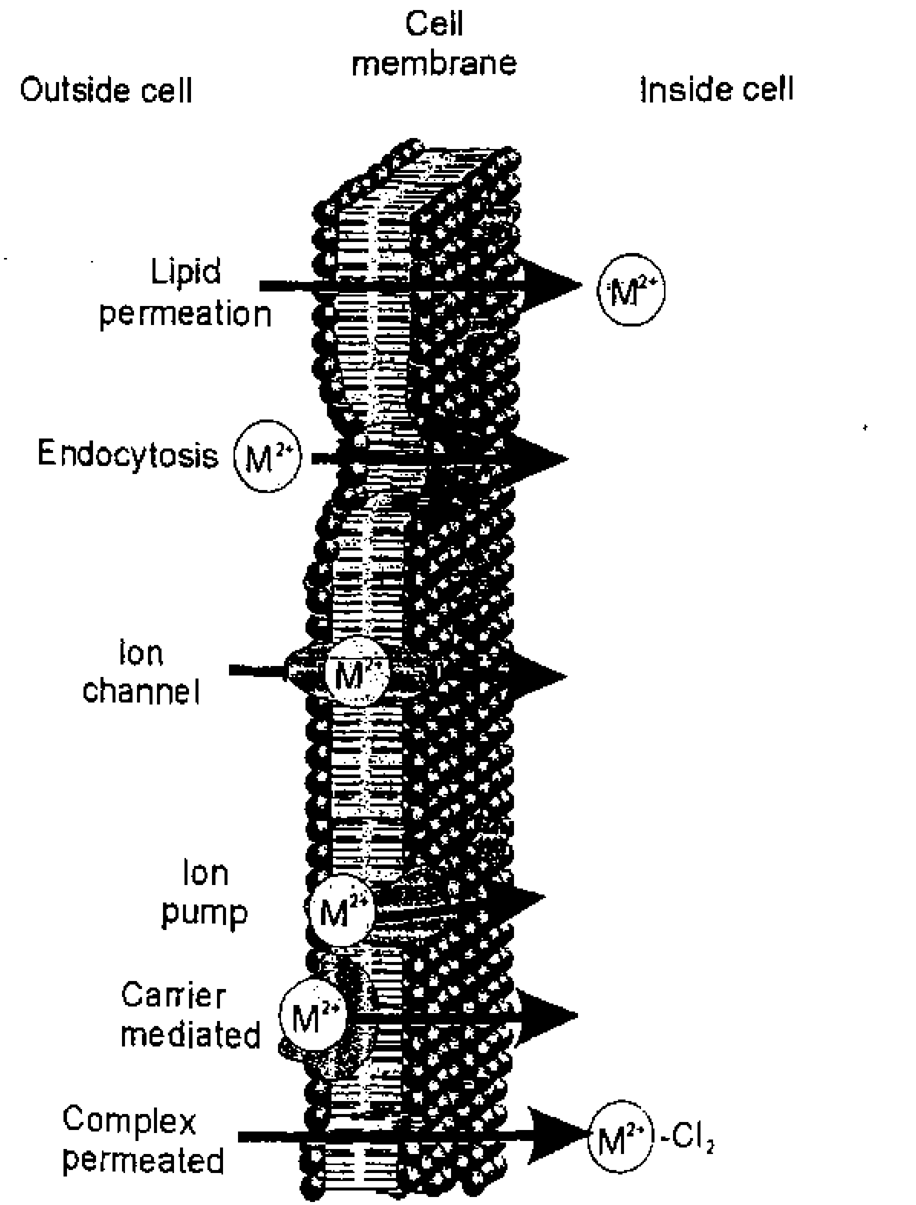


FIGURE 17.4 Mechanisms of metal (M) flux across the microbial cell membrane. (Adapted from Simkiss and Taylor, 1989.)

OUTCOME OF METAL-MICROBE INTERACTIONS

- Decreased microbial growth
- Abnormal morphological changes
- Inhibition of biochemical processes in individual cells
- Decrease in overall community numbers and diversity
- Possible symbiotic relationships between metal resistant and metal sensitive populations
- Conferment of resistance to metal sensitive population

MECHANISMS OF MICROBIAL METAL RESISTANCE & DETOXIFICATION

- Microorganisms are believed to have evolved metal resistance as a result of their exposure to toxic metals
- Some resistance mechanisms in MOS are **plasmid encoded** and tend to be **specific** for a particular metal
- **Others are general** conferring resistance to a variety of metals. These include resistance mechanisms that are:
  - general and do not require metal stress;
  - dependent on a specific metal for activation;
  - general and are activated by metal stress

GENERAL MECHANISMS OF METAL RESISTANCE

PRODUCTION OF EXTRACELLULAR MATERIALS

Polysaccharides

- **immobilizes the metal & prevents its entry** into the cell. E.g. Cd, Pb, Zn & Fe bind to **anionic cell surfaces**
- **Algal surfaces** contain carboxylic, amino, thio, hydroxo and hydroxy-carboxylic groups that strongly bind metals
- **Phosphoryl groups** and **phospholipids** in the outer membrane of **bacterial LPS** strongly interact with cationic metals

PRODUCTION OF EXTRACELLULAR MATERIALS

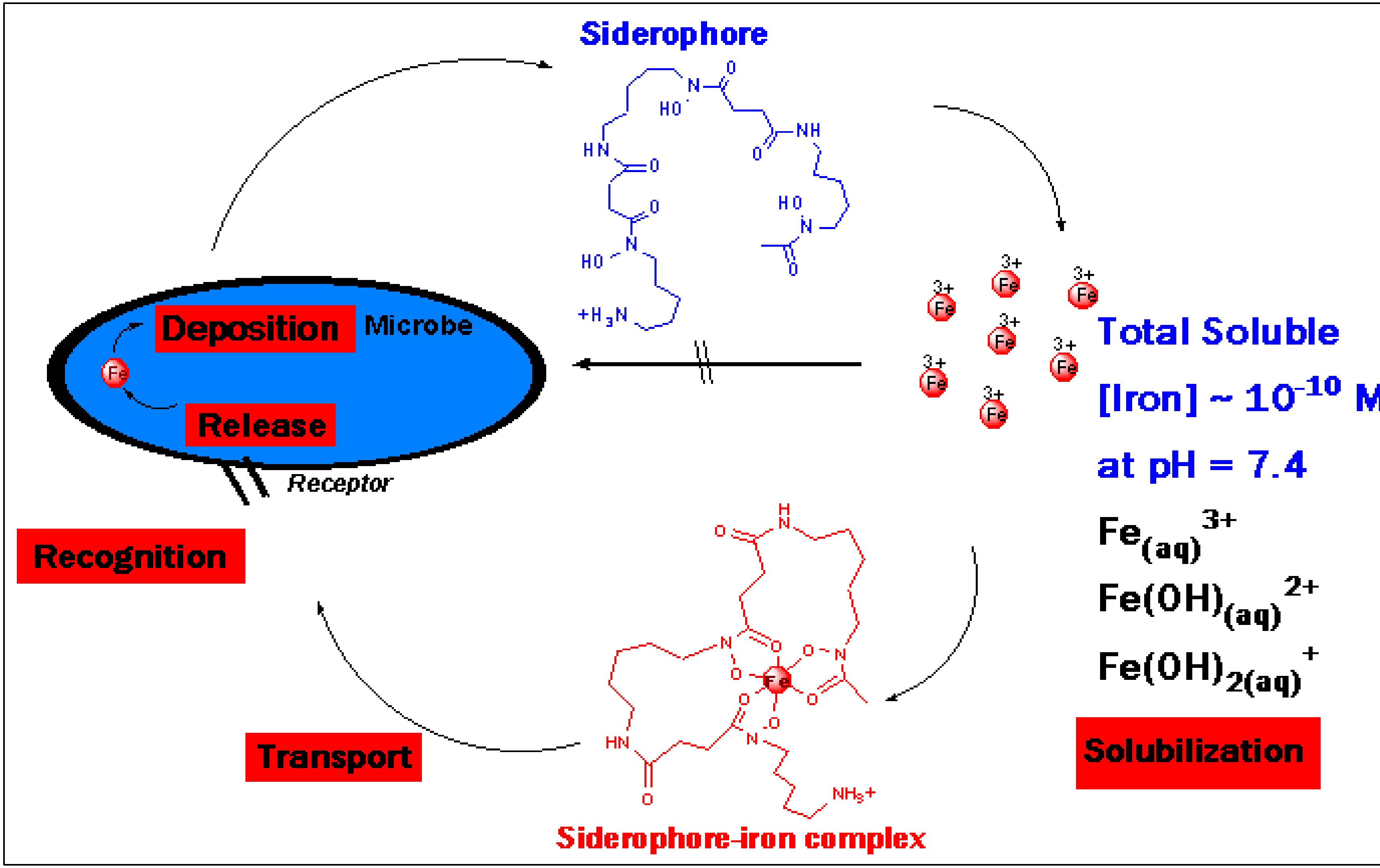
Biosurfactant

- Able to complex metals such as Cd, Pb, & Zn
- Biosurfactant complexation can increase the apparent solubility of metals, however, the biosurfactant-complexed metal is not toxic to cells

PRODUCTION OF EXTRACELLULAR MATERIALS

Siderophore

- Natural **iron binding compounds** that chelate ferric ions (which form insoluble colloidal hydroxides at neutral pH and are then inaccessible) and are then taken up together with the metal ion.
- Iron-complexing, low molecular weight organic compounds
- Concentrates iron in the environments where concentration is low and facilitates its transport into the cell
- Siderophore may interact with other metals that have chemistry similar to that of iron. E.g. Al, Cr, Ga



Siderophore Mediated Iron Bioavailability

METAL-DEPEDENT MECHANISMS OF RESISTANCE

Metallothionein or similar protein (MLP) production

- Metal binding or sequestration by MLP can result in cellular accumulations visible as electron dense areas within the cell matrix

- Form highly insoluble compounds (with solubility far below that of the corresponding hydroxides)

PLASMID-ENCODED ENERGY-DEPENDENT METAL EFFLUX SYSTEMS

- Some efflux systems involve **ATPases**, and others are **chemiosmotic ion/proton pumps**
- Effectively pump toxic ions that have entered the cell back out of the cell via **active transport** (ATPase) pump) or **diffusion** (chemiosmotic ion/proton pump)
- Arsenate, chromium & Cd are most commonly associated with efflux resistance
- In a number of bacteria, **arsenate** resistance involves the **enzymatic reduction of arsenate to arsenite followed by a plasmid-mediated arsenite efflux**
- **Cadmium resistance** is another example of **efflux-based resistance**

PLASMID-ENCODED ENERGY-DEPENDENT METAL EFFLUX SYSTEMS

- **Gram-positive** bacteria use an **ATP-mediated efflux pump**
- **Gram-negative** bacteria use **chemiosmotic anti-porter pumps**
- The plasmid-borne ***cadA*** gene encodes a cadmium specific ATPase in several bacterial genera, including *Staphylococcus, Pseudomonas, Bacillus* & *Escherichia*
- *czc* operon is responsible for Cd resistance in *Alkaligenes eutrophus* CH34
- Cd resistance operon (*ncc*) has recently been identified in *Alcaligenes xylosoxidans*.

METHYLATION OF METALS

- A **metal dependent mechanism** of resistance because only certain metals are involved
- Methylation generally **increases metal toxicity** as a result of **increased lipophilicity**, thus **increased permeation** across cell membranes
- **Metal volatilization (MV)** facilitates metal diffusion away from the cell, thus decreasing metal toxicity

METHYLATION OF METALS

- MV has been observed with Pb, Hg, Sn, Se & As. E.g. Hg^{2+} is readily oxidized to the volatile and very toxic forms
- **Methylmercury** and **dimethylmercury**, can then diffuse away from the cell.
- *Hg resistance may involve the enzymatic reduction of Hg2+ to elemental mercury (Hg0) in both G+ve and G-ve bacteria*

METHYLATION OF METALS

- Two additional pathways of Hg resistance involve;
  - the detoxification of organo-mercurial compounds via cleavage of C-Hg bonds by an **organo-mercurial lyase (MerB)**
  - Reduction of Hg^{2+} to Hg^{0} by a flavin adenine dinucleotide (FAD)-containing, NADPH-dependent **mercuric reductase (MerA)**

- Specific to inorganic mercury;
  - the **MerP protein** in the periplasmic space shuttles Hg^{2+} to the membrane-bound **MerT protein**,
  - which releases Hg^{2+} to the cytoplasm
  - Once in the cytoplasm, Hg^{2+} is reduced to Hg^{0} by **mercuric reductase**

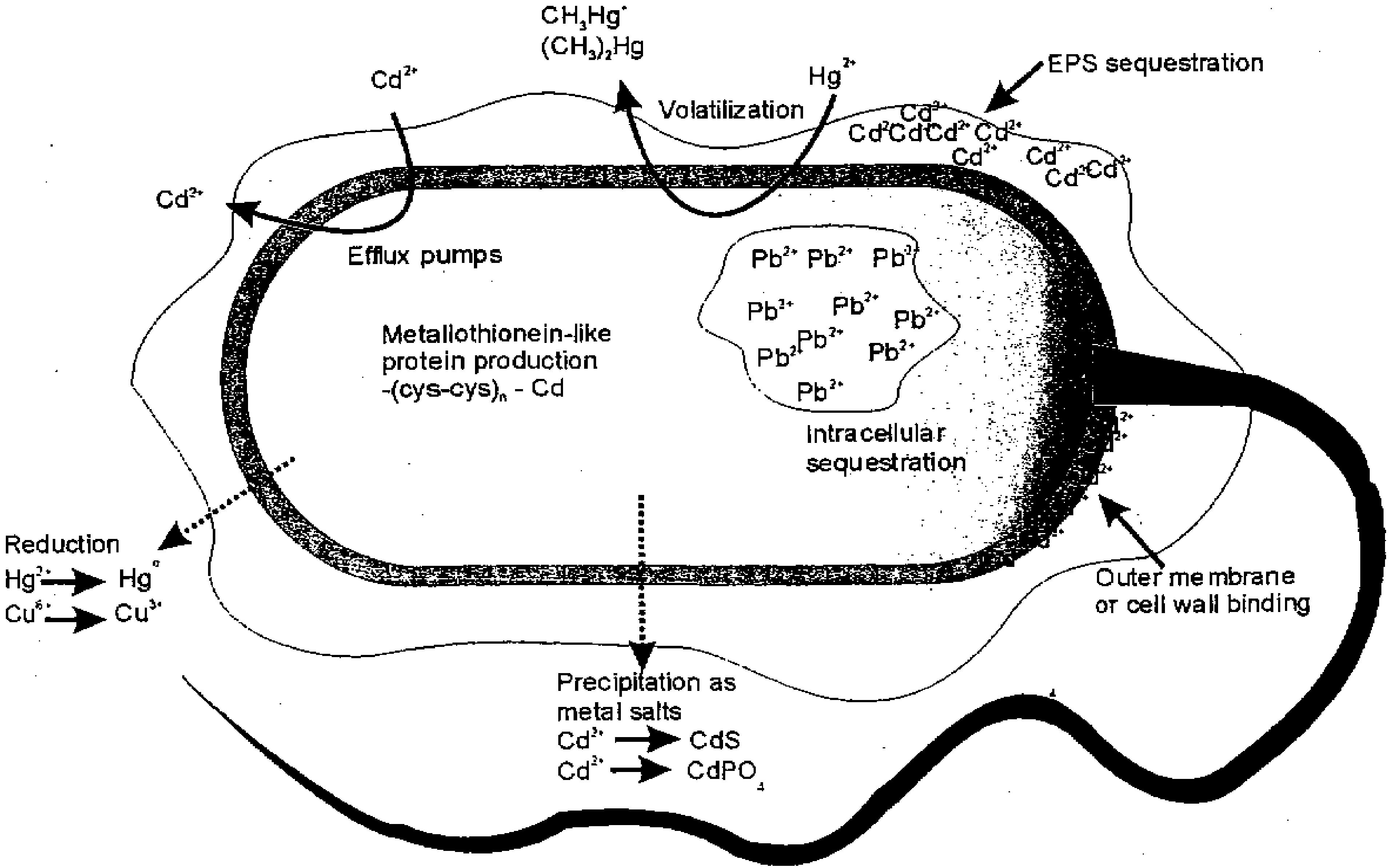


FIGURE 17.5 In response to metal toxicity, many microorganisms have developed unique mechanisms to resist and detoxify harmful metals. These mechanisms of resistance may be intracellular or extracellular and may be specific to a particular metal, or a general mechanism able to interact with a variety of metals.

ADVERSE EFFECTS OF MICROBIAL METAL TRANSFORMATIONS

Acid mine drainage (AMD) formation

- Metals are found in a reduced state e.g. pyrite (FeS_2)
- When exposed to O_2, **large production of acid** occur due to **auto-oxidation** and **microbial oxidation** of iron & sulfur
- As a result, acid facilitates metal solubilization, resulting in a metal-rich leachate, AMD
- AMD is highly toxic to plants & animals, often resulting in widespread fish kills

ADVERSE EFFECTS OF MICROBIAL METAL TRANSFORMATIONS

Microbially induced corrosion of metal pipes

- Fuel & storage tanks are susceptible
- Acid producing bacteria or fungi and/or attack by SO_4 reducing bacteria causes corrosion
- H_2SO_4 production by *Thiobacillus* spp & other sulfur oxidizers cause acid corrosion of **ferrous metals**
- For nonferrous metals, the production of **organic acids** is important. E.g. by fungus, ***Cladosporium resinae***

MICROBIAL METHYLATION OF METALS

- Involves the **transfer of methyl groups (CH_3) to metals** & metalloids, e.g. Hg, As & Se
- **Increases metal mobility** because organo-metals are volatile
- **Increases metal toxicity**
- Organo-metals are more **lipophilic** than the metal species
- Results in potential **bioaccumulation & biomagnification in food webs**

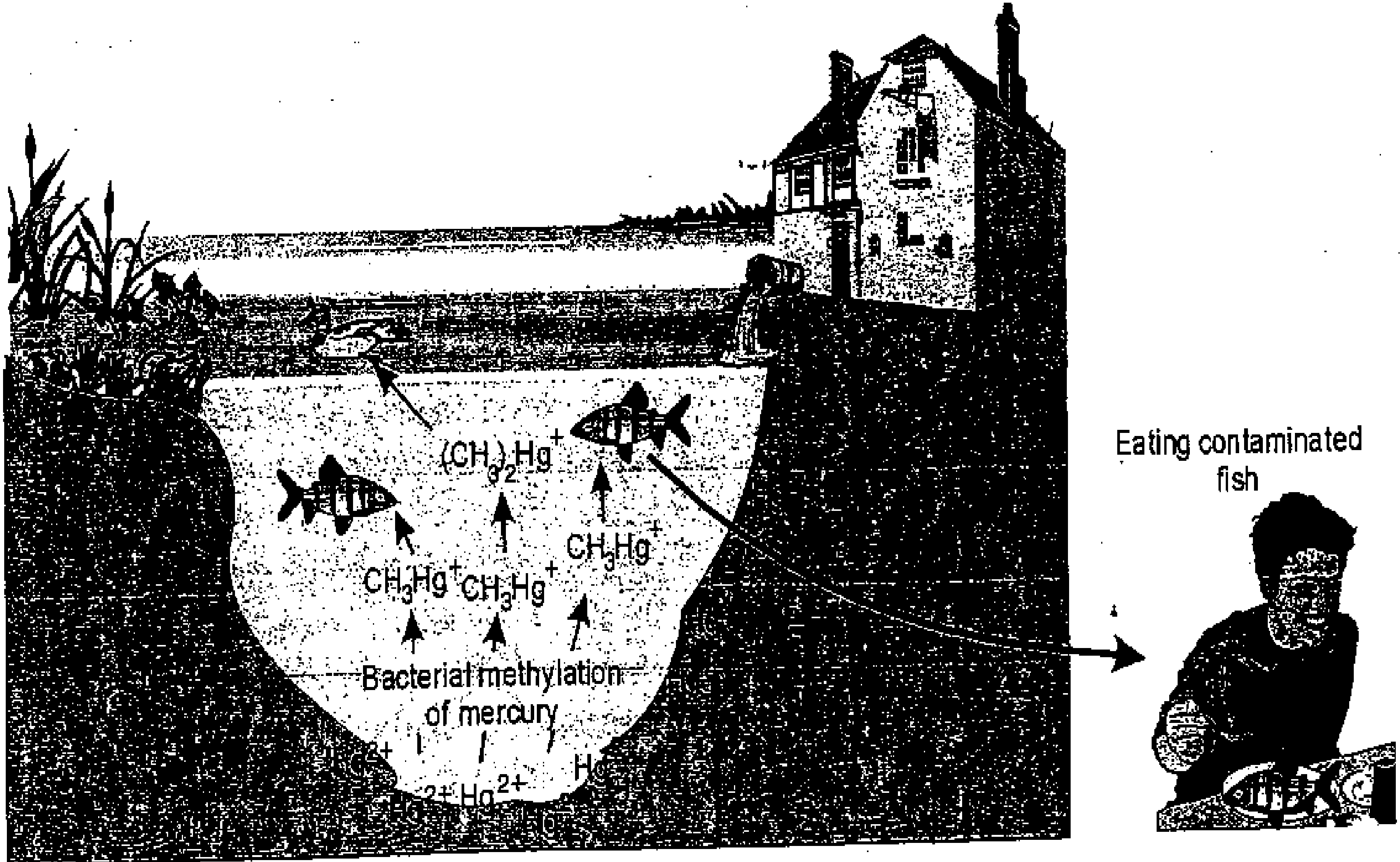


FIGURE 17.12 Schematic demonstrating the potential for the bioaccumulation of mercury as a result of mercury methylation. Once in the food chain, methylmercury poses serious health risks to the human population.

MICROBIAL METHYLATION OF METALS

- **The best studied of the metals that are methylated is Hg**
- Methylmercury compounds are highly **lipophilic** & **neurotoxic**
- In Minimata bay (Japan) chemical processing plant release Hg-containing effluents
  - results in serious illness in people consuming fish with elevated levels of Hg
- Arsenic is also methylated by some fungi such as ***Scopulariopsis brevicaulis*** to mono-, di-, & trimethylarsenes;
  - highly toxic forms of arsenic

THE BENEFITS OF METAL-MICROBIAL INTERACTIONS

Reduction of metal waste and bioavailability

- Microbially catalysed oxidation of minerals is used in the **commercial recovery** of Cu, uranium & gold from low grade

- **Controlled leaching** or metal recovery is done using;
  - heap leaching;
  - vat leaching or
  - in-place leaching

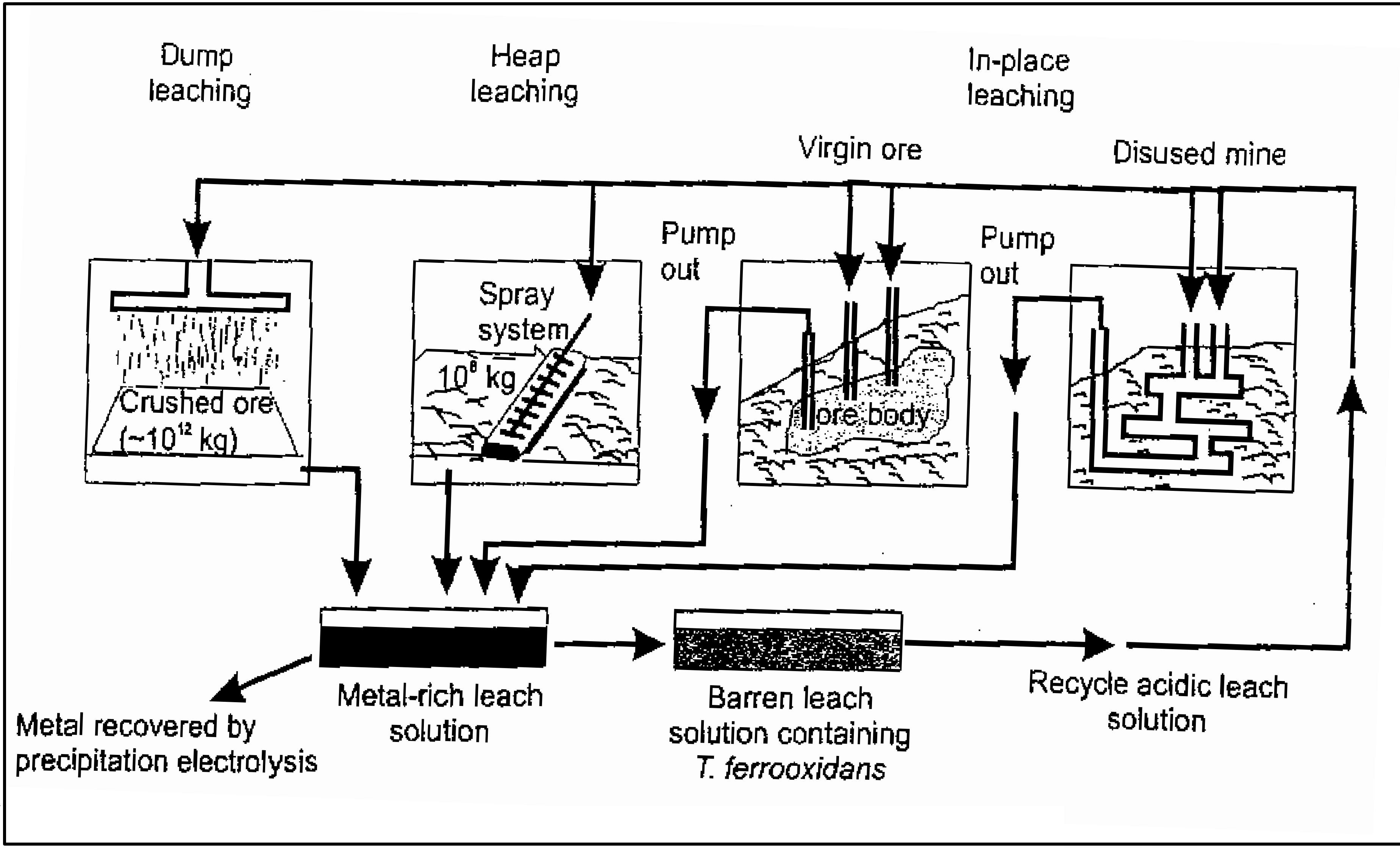


FIGURE 17.13 Various applications of micrometallurgy in the recovery of metals from crushed ores. Metals can be recovered from ores either in place, in heaps, or in dump leaching. In each method, an acidic leach solution containing *Thiobacillus ferrooxidans* is flushed through the ore, leaching out the metal to be recovered via precipitation or electrolysis.

PHYSICAL/CHEMICAL METHODS OF METAL REMEDIATION

METHODS AIMED AT PREVENTING MOVEMENT OF METALS TO THE IMMEDIATE SURROUNDINGS

- **immobilization** – Reduces metal solubility
- **pH alteration (metal solubility decreases with increasing pH)**
- **addition of organic matter** – electrostatic attraction between metals and organic matter

METHODS AIMED AT PHYSICALLY REMOVING OR DESTROYING THE CONTAMINATION

- **Physical Excavation** – e.g. of sediments (dredging)
- **Soil washing techniques** – washing with acidic solutions or chelating agents (e.g. EDTA) solubilizes metals
- **Incineration** of soils – Thermal treatment involving combustion at high temperature

INNOVATIVE MICROBIAL APPROACHES IN THE REMEDIATION OF METAL CONTAMINATED SOIL

Goals

- immobilize the metal *in situ* to reduce metal bioavailability and mobility
  - impossible to know if the metals will remain immobilized indefinitely
- remove the metal from the soil
  - difficult due to the heterogeneous nature of soil & is expensive

METHODS FOR MICROBIAL REMEDIATION OF METAL-CONTAMINATED SOIL

- Microbial leaching
- Microbial surfactants & polysaccharides
- Microbially induced metal volatilization
- Microbial immobilization and complexation
- Metal sequestration – relies on the ability of some microorganisms to produce metal-complexing polymers, such as siderophore

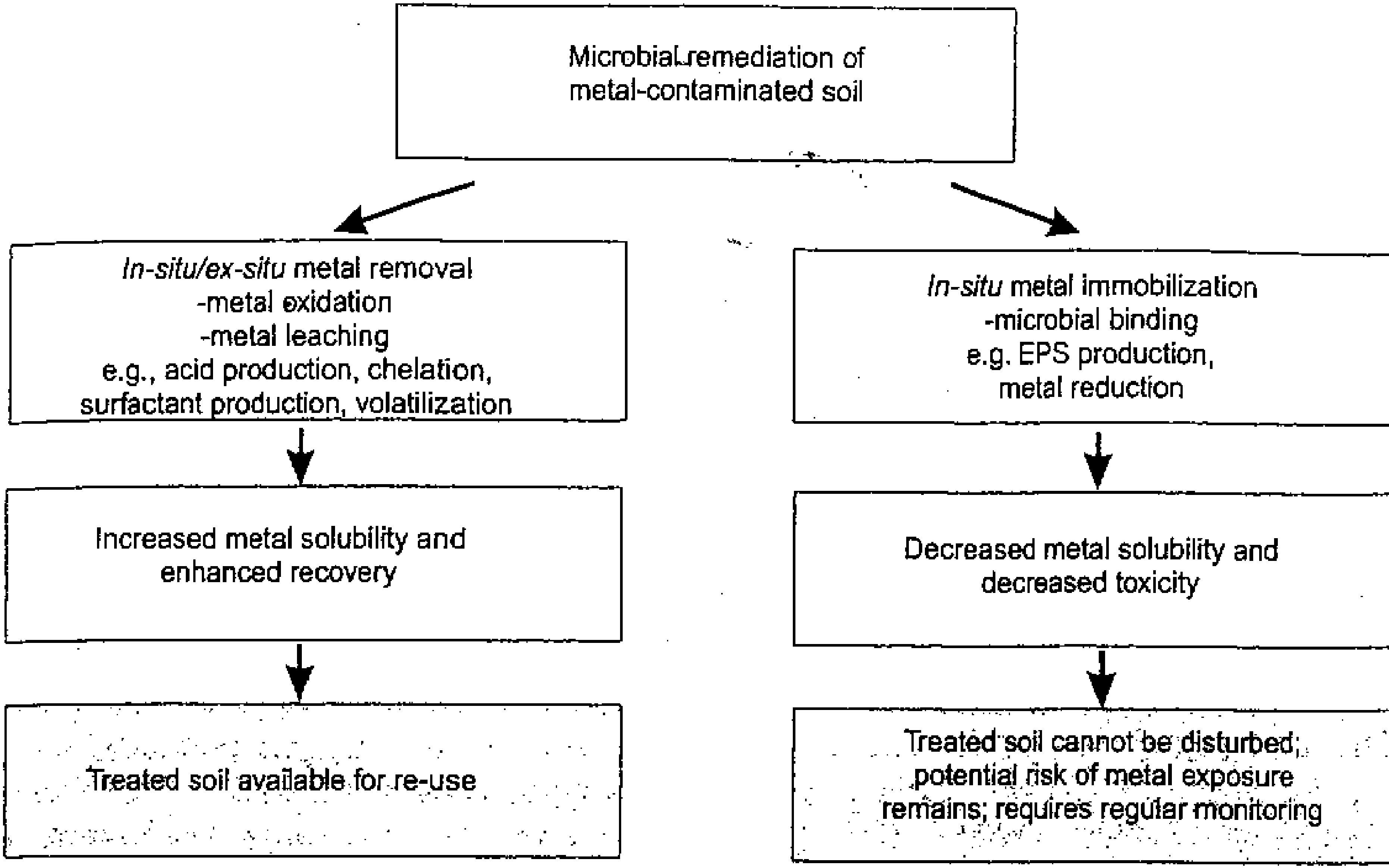


FIGURE 17.14 Microbial metal remediation in metal-contaminated soils relies on either metal removal or, more commonly, metal immobilization. Metal removal is ideal because following treatment the soil is available for reuse. In metal immobilization, soil reuse is limited because of the continued potential risk of exposure.

INNOVATIVE MICROBIAL APPROACHES IN THE REMEDIATION OF METAL-CONTAMINATED AQUATIC SYSTEMS

- Based on the ability of microorganisms to complex and precipitate metals
- Results in both detoxification and removal from the water column
- (Fig. 17.15)

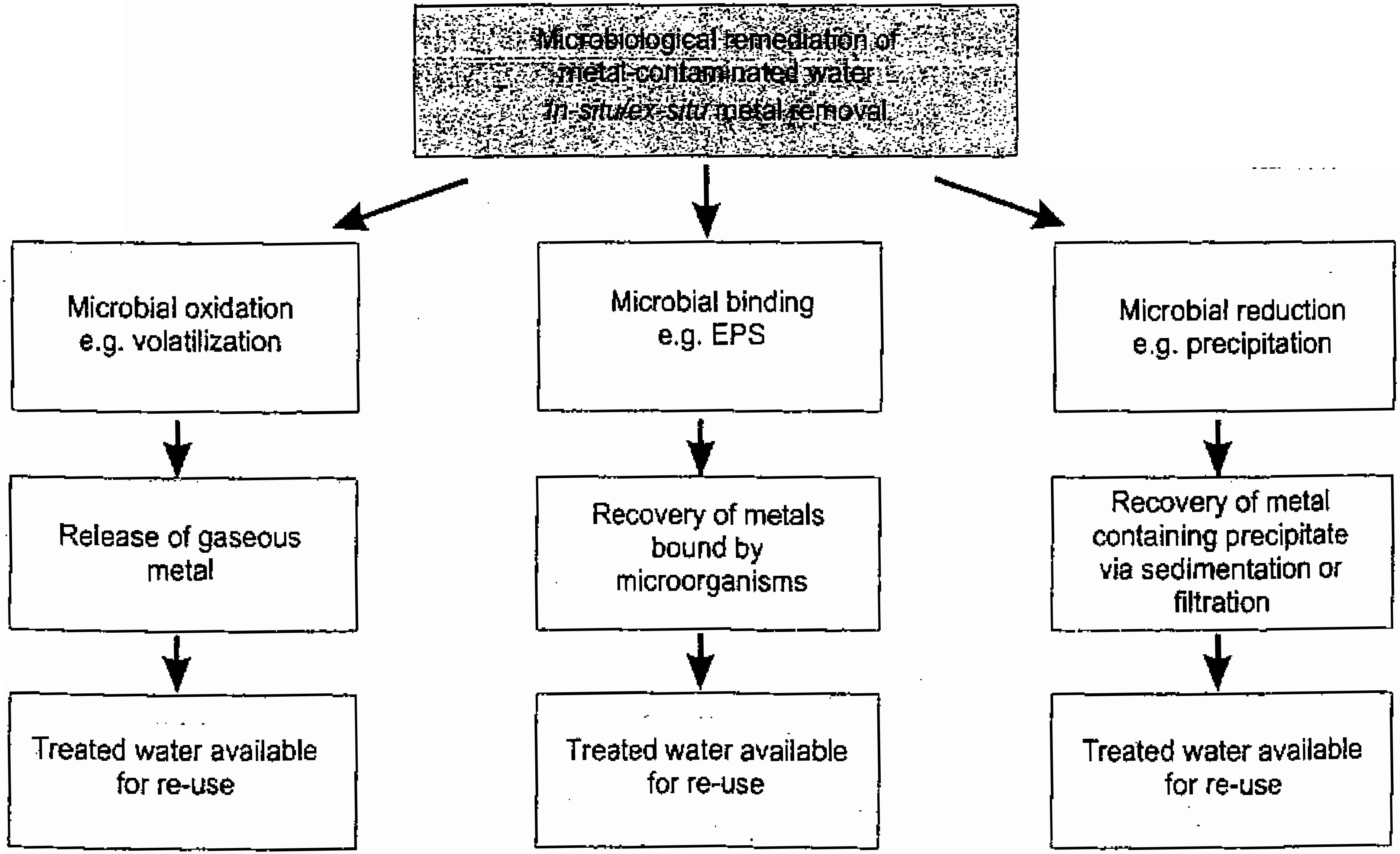


FIGURE 17.15 Microbial metal remediation approaches for metal-contaminated waters. In each method, the treated water is safe to release into the environment. Both metals and microorganisms can easily be recovered during treatment for proper disposal.

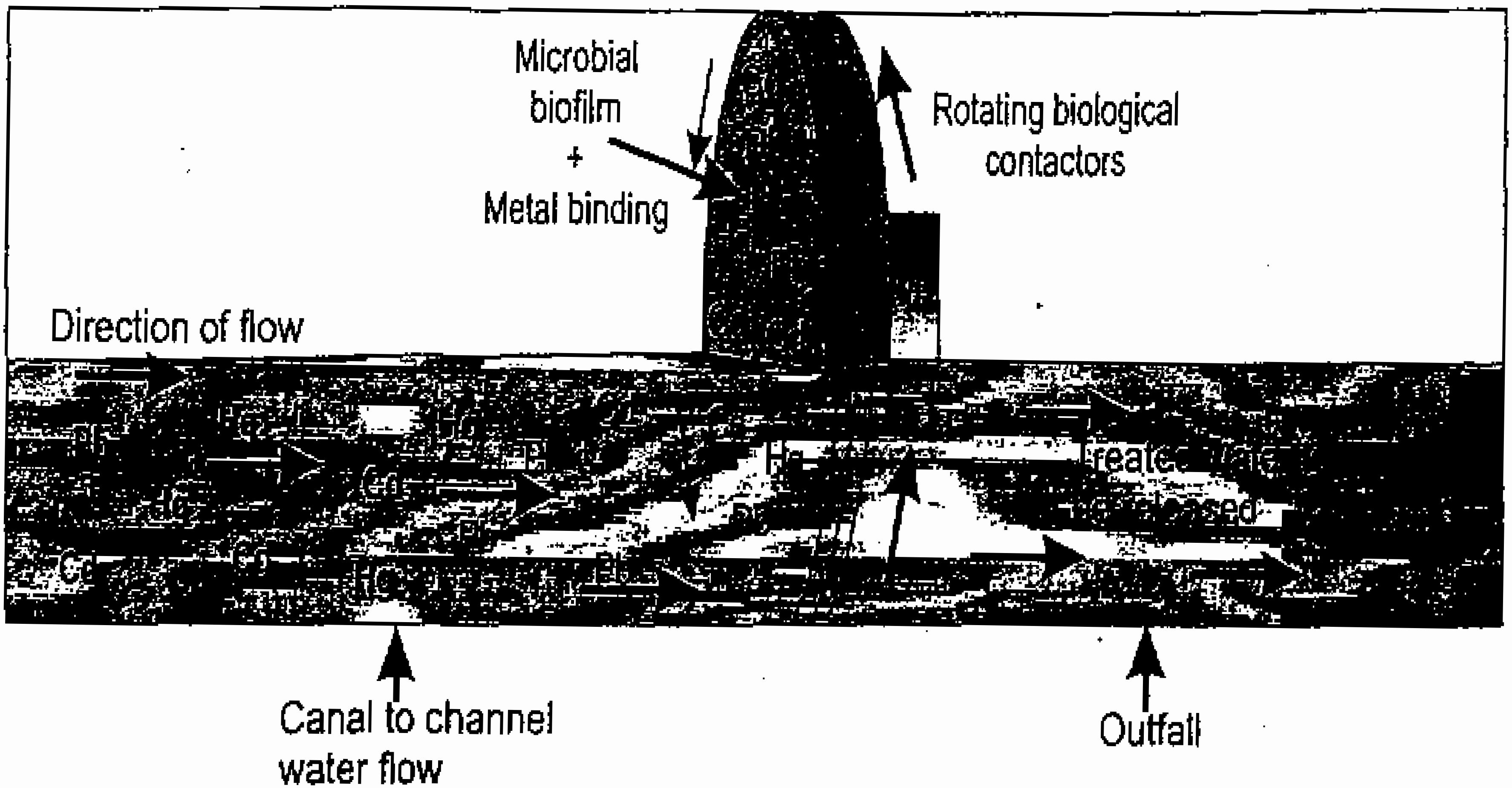


FIGURE 17.16 Schematic demonstrating how microbial biofilms are used in removing metals from contaminated wastestreams. The biofilm located on the rotating drum accumulates metals as the water passes through the drum. The treated water can be safely released. The biofilm may either be viable or nonviable. When viable, the biofilm rarely needs to be replaced; however, non-living biofilms need to be replaced periodically for their metal removal efficiency will decrease with time.