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# Importance of soil micro-organisms in the formation of non-extractable pesticide residues

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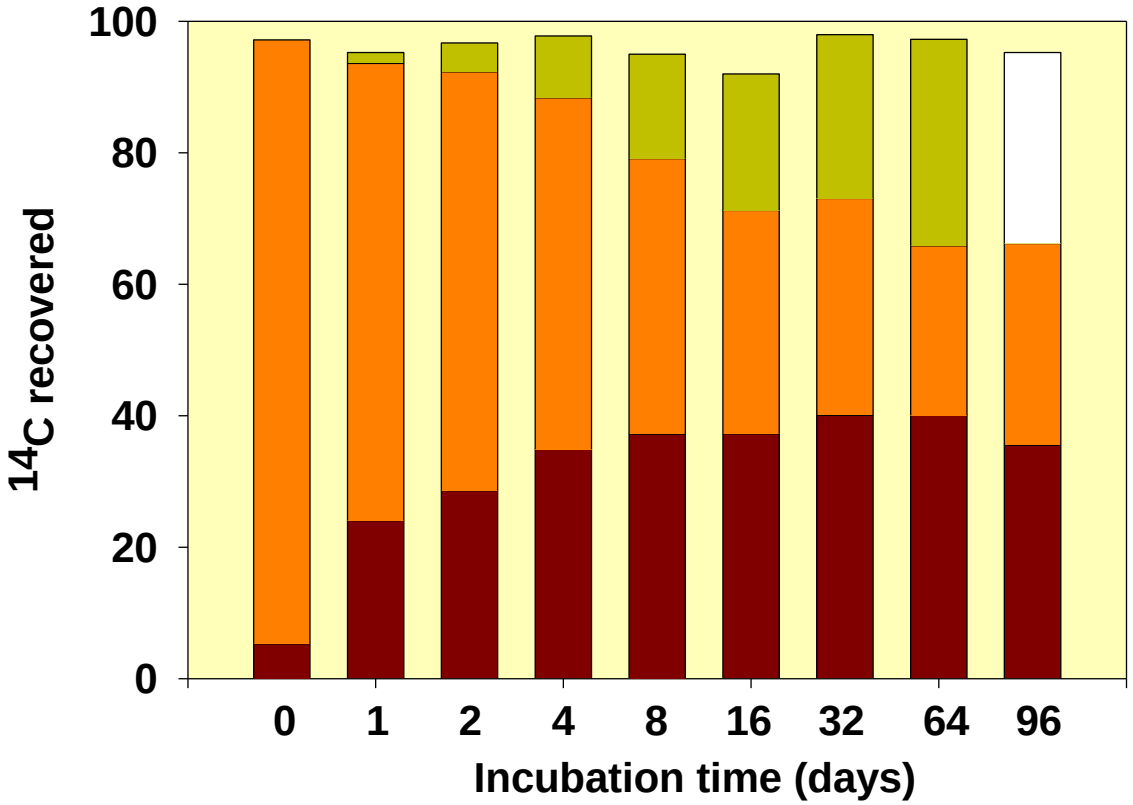
# Typical fate of organic compounds in the soil




loss = mineralised

Decreasing bioavailable fraction = extractable

Increasing non-bioavailable fraction = **non-extractable residues**

# Mechanisms for non-extractable residue formation

- Covalent binding of pesticides or metabolites with soil organic matter
- Sequestration into the three-dimensional structure of humic substances
- **Incorporation of residues into microbial biomass**

Because of agronomic, environmental and public health repercussions, a better knowledge of the mechanisms involved is needed

# Objectives

- **To quantify the role of soil microflora in pesticide stabilisation**
- **To isolate biochemical compartments of microbial biomass, which incorporate residues**
- **To identify molecular bioindicators for pesticide incorporation**

# Our approach

**Liquid cultures**

of pure strains (bacteria or fungi) in mineral media spiked with radiolabelled substrates

**Biochemical fractionating of the biomass**

after incorporation of the substrate

- glucose (easily incorporated)
- herbicides

in lipids, polysaccharides, proteins

**Counting of the radioactivity**

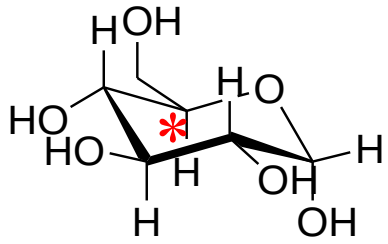
# Experimental models

## Microbial strains

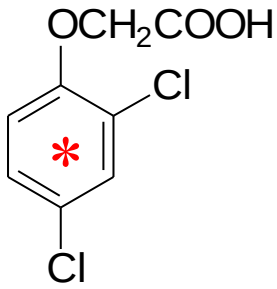
> *Alcaligenes xylosoxidans*, bacteria GRAM-
> *Fusarium solani,* fungus Ascomyceta
> *Trametes versicolor,* fungus Basidiomyceta

## Substrates

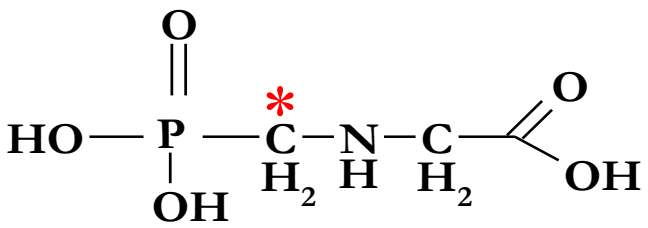
glucose




2,4-D



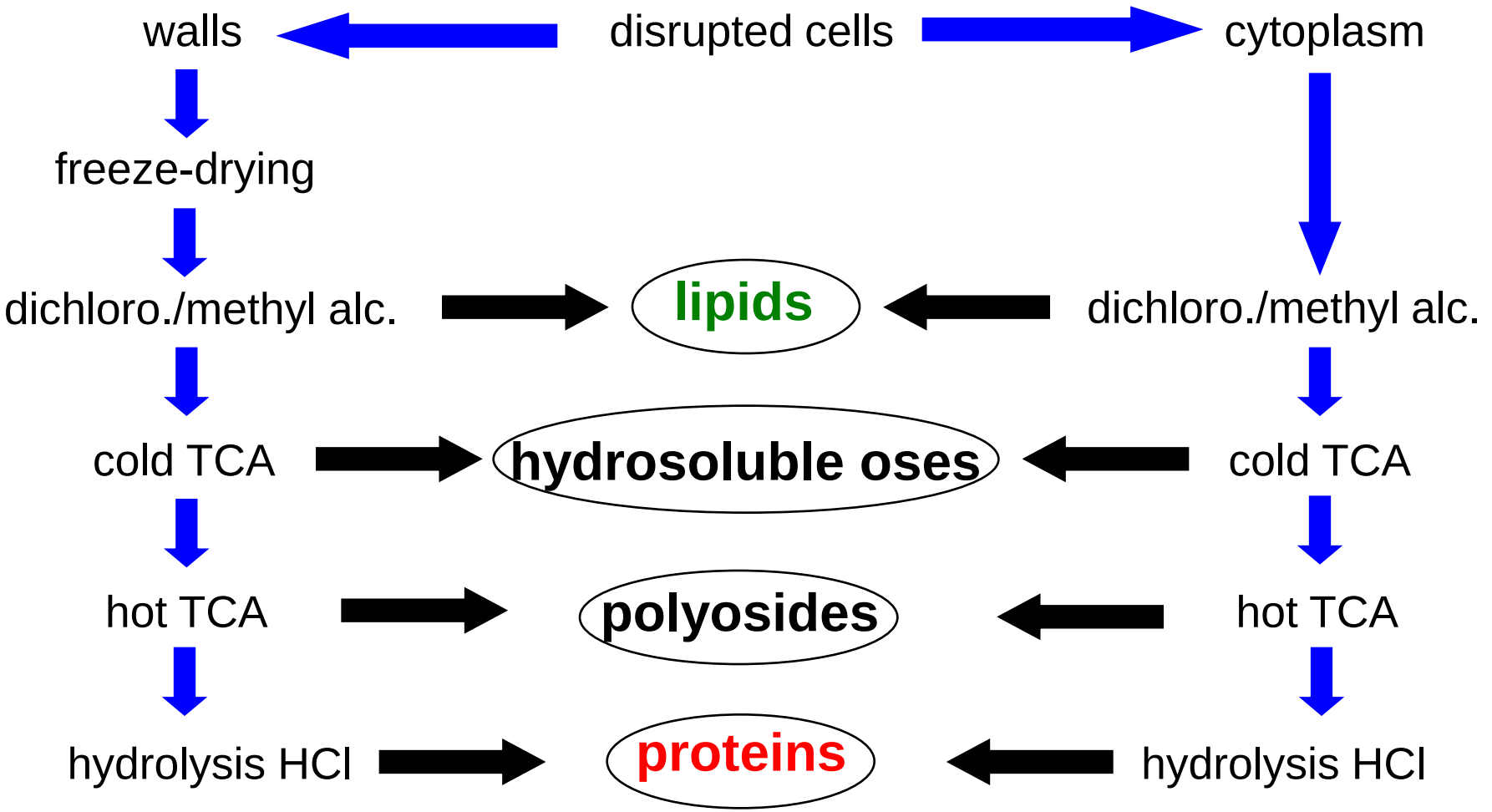

glyphosate



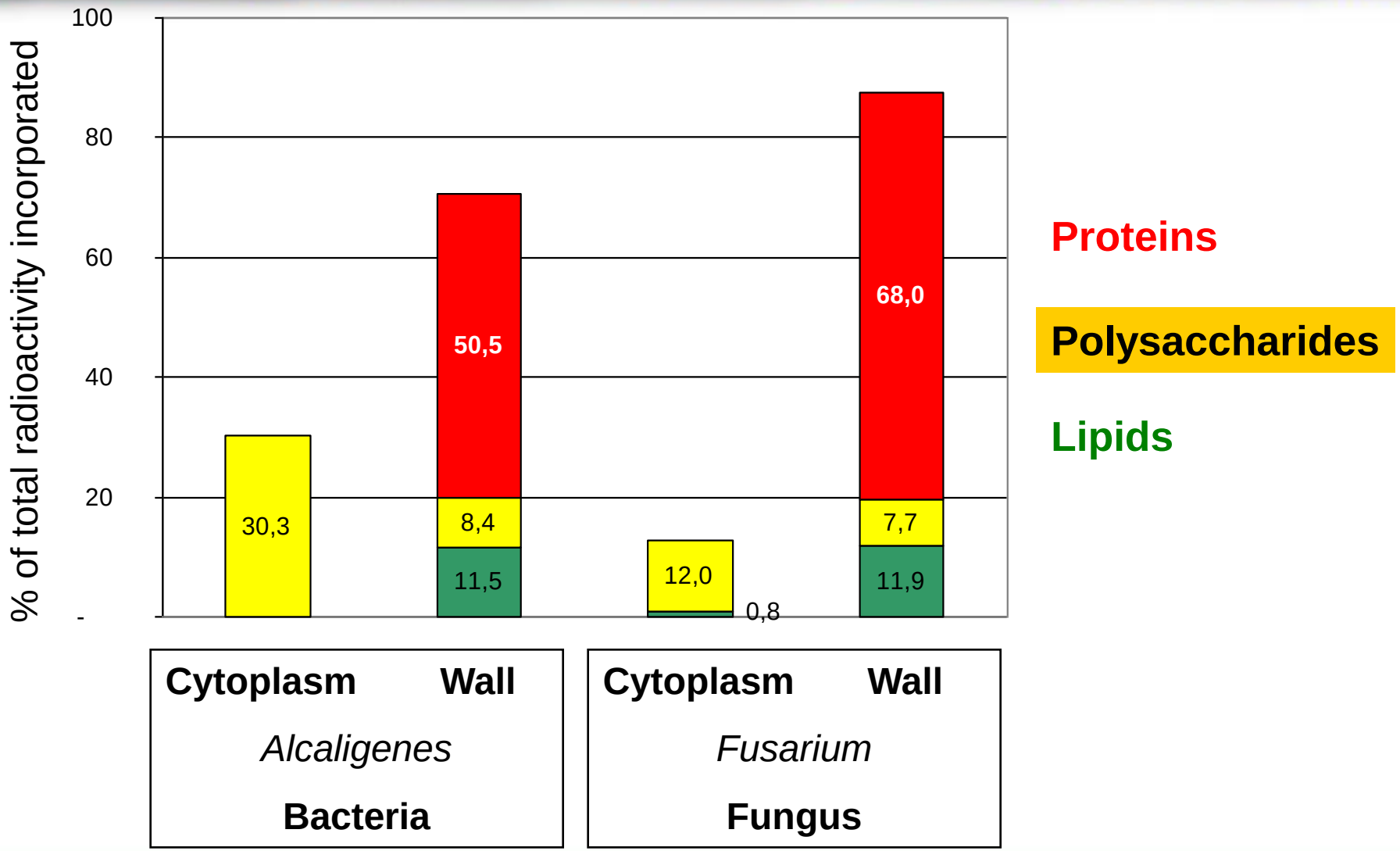
# Characteristics of biochemical compartments

| | Localization and amount | Main properties | Specific extraction |
|---|---|---|---|
| **Lipids** | Membranes, wall (10-15 %) | **Hydrophobicity** Affinity to (+/-) non-polar organic solvents | **Dichloromethane Dichloro. / Methyl alcohol** |
| **Polysac-charides** | Wall, mucigels (30-40 %) | **Neutral compounds** Hydrosoluble after acidic hydrolysis | **Hydrolysis with sulfuric or trichloroacetic acid** |
| **Proteins** | Membranes, cytoplasm (40-50 %) | **Amphoteric compounds** Precipitate, hydrolysis | **After precipitation hydrolysis with hydrochloric acid** |

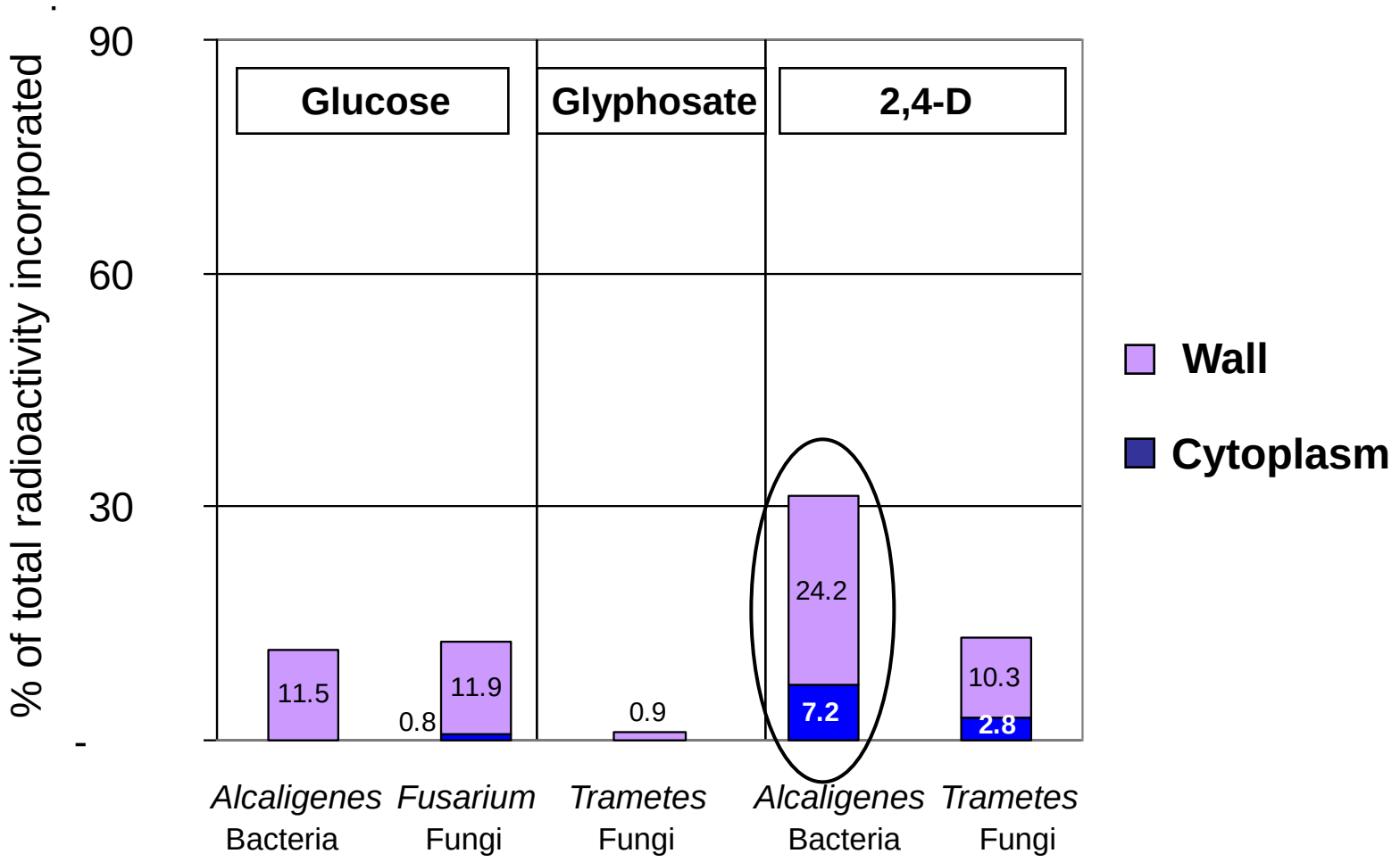
# Biomass fractionation



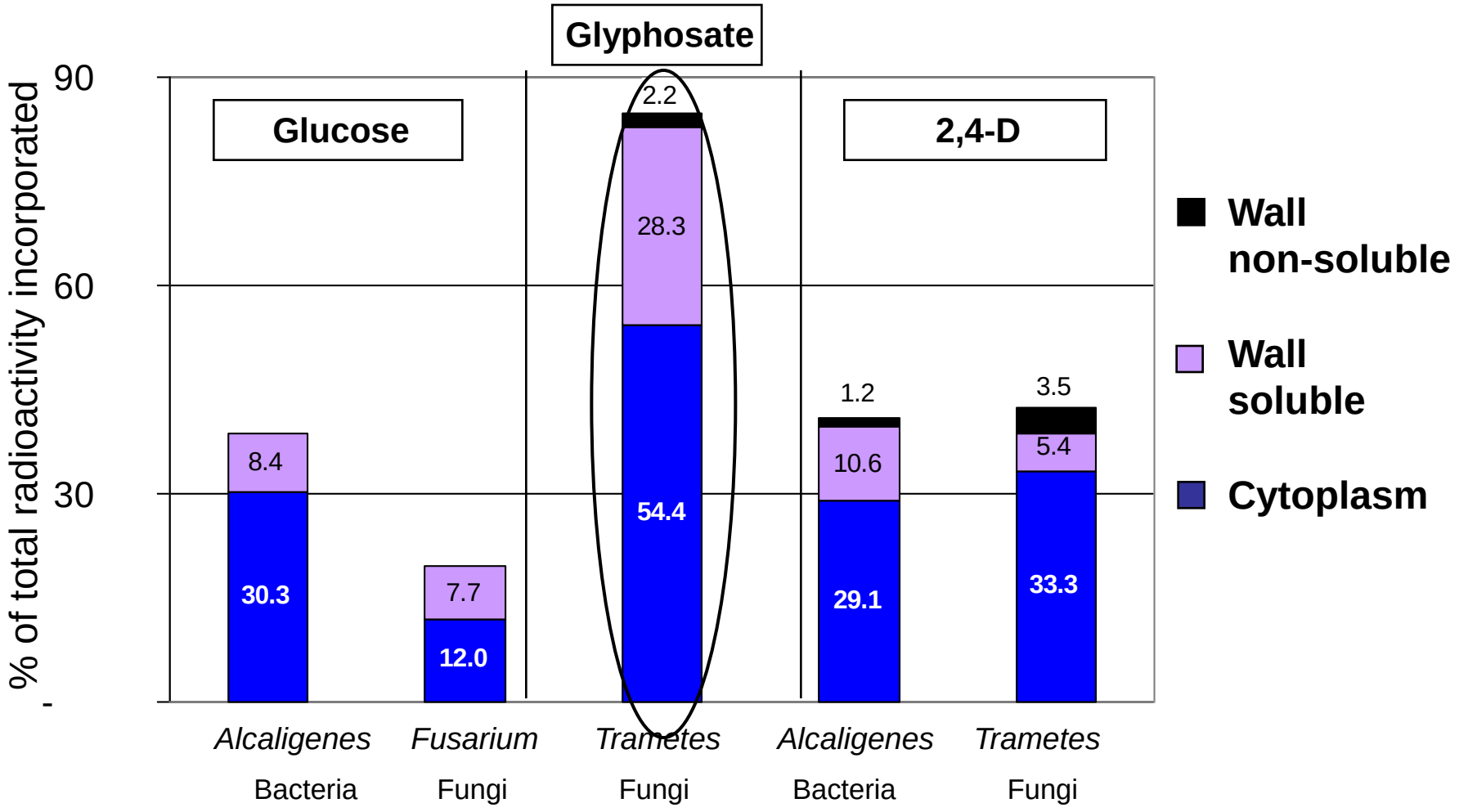
# Distribution of carbon from $^{14}$C-glucose



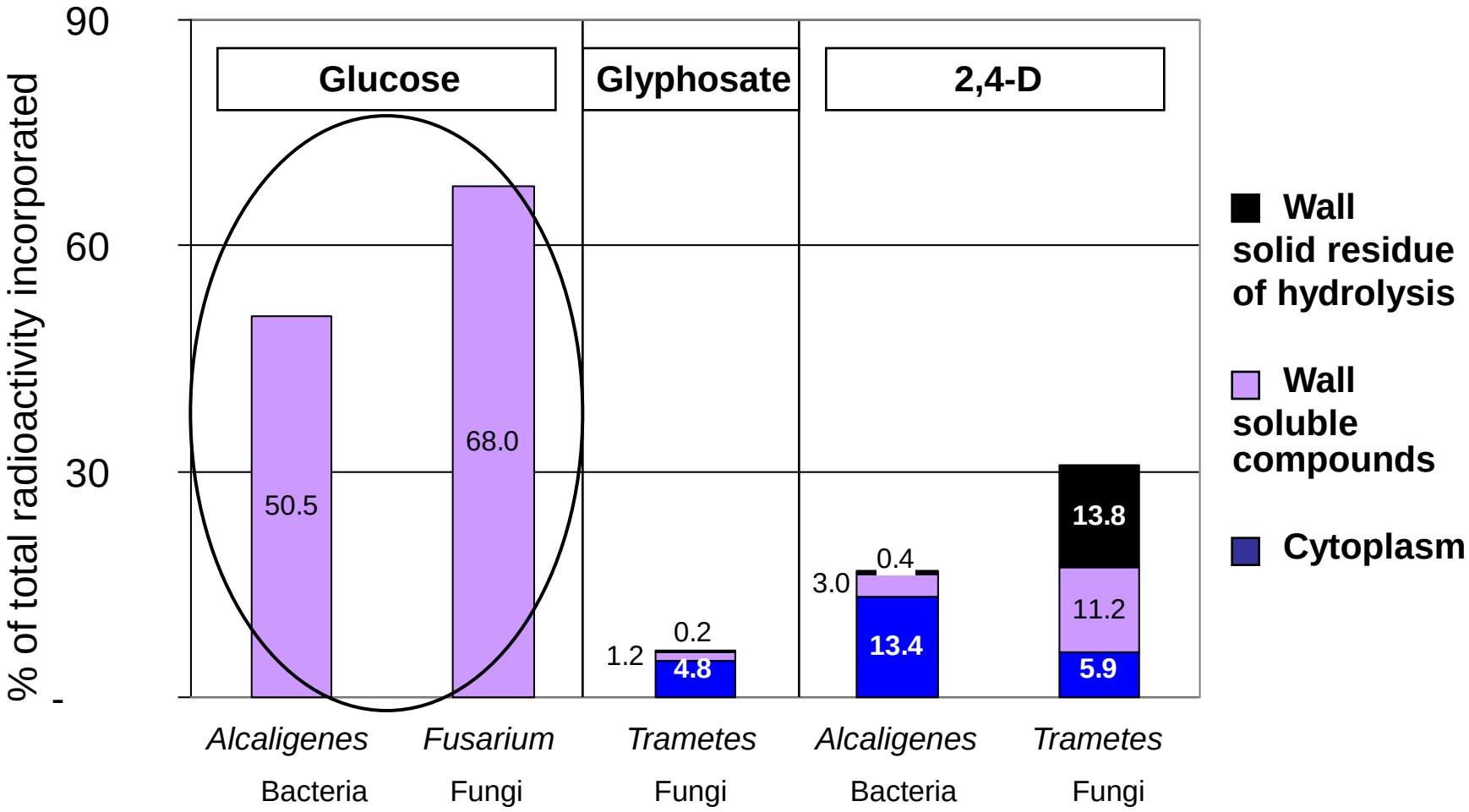
# Distribution of $^{14}$carbon in **lipids**



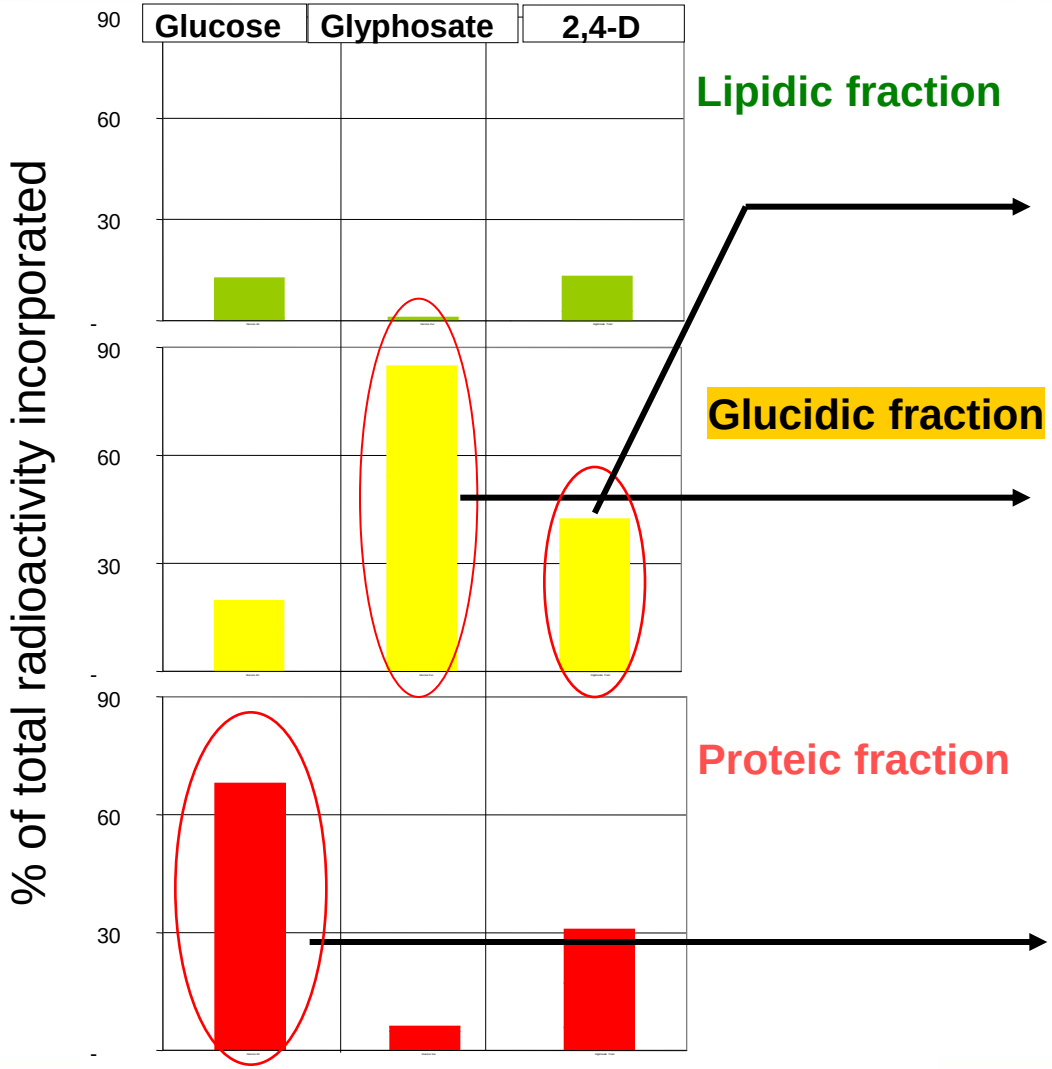
# Distribution of $^{14}$carbon in **glucids**



# Distribution of $^{14}$carbon in **proteins**



# Distribution of $^{14}$carbon in **fungi**




**2,4-D** :
Accumulation in the cytoplasm (glucidic form)

**Glyphosate** :
Accumulation in cytoplasm (glucidic form)

**Glucose** :
Accumulation in wall (proteic form)

# Conclusions

- **Development of a method allowing the biochemical fractionation of microbial constituents**

- **Carbon incorporation in the biochemical compartment depends on:**
  **- the substrate**
  **- the strain**

- **No possible generalisation of metabolic pathways for pesticide incorporation in the microbial biomass**

# Perpectives

- **To confirm the molecular nature of biochemical extracts**
- **To identify markers of pesticide incorporation**
- **To search and quantify these markers in complex matrix (soils, organic wastes…)**
- **To identify regulation factors of the biological incorporation of pesticides (microbial diversity, availability of organic subtract…)**

***Many thanks for your attention !***