The Role of Environmental Toxicants in Amyotrophic Lateral Sclerosis (ALS): Implications for Water Quality and Human Health

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Presented By

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From Water Contamination to Cellular Neurotoxicity

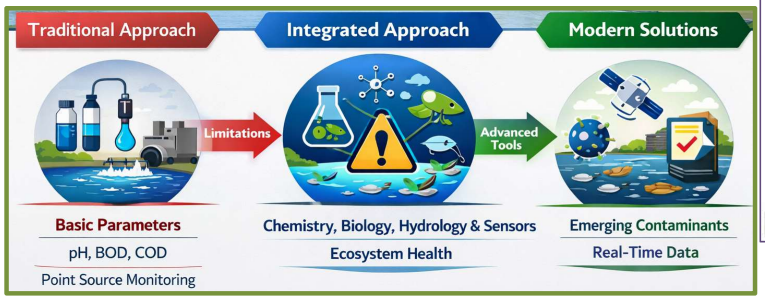
Water quality represents the physical, chemical, biological, and ecological characteristics of water that determine its suitability for human consumption, industrial applications, agriculture, and ecosystem sustainability.

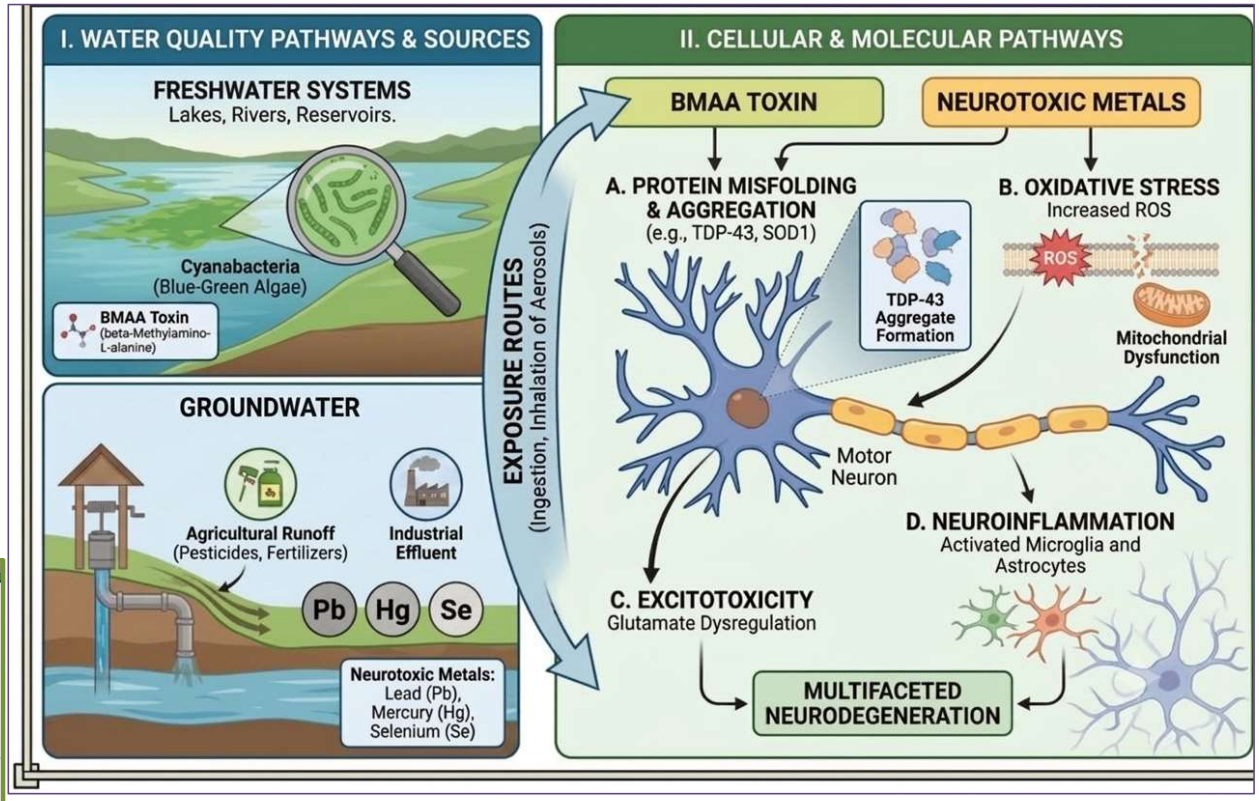
Increasing complexity of water pollution:

- ✓ Nutrients (N, P compounds)
- ✓ Heavy metals
- ✓ Organic micropollutants
- ✓ Microplastics
- ✓ Pathogens
- ✓ Emerging contaminants

Need for Advanced Instrumentation

- ✓ High-frequency monitoring
- ✓ In-situ measurements
- ✓ Multi-parameter analysis
- ✓ Real-time decision support





Introduction to ALS & The Etiology Dilemma

- **What is ALS?** A progressive, fatal neurodegenerative disease targeting motor neurons in the brainstem and spinal cord.
- **The Clinical Reality:** Median survival is only **2 to 4 years** post-diagnosis.
- **Etiology Gap:**
  - Only **5% to 10%** of cases are familial (genetic).
  - **90% to 95%** of cases are sporadic (non-familial).
- **The Core Hypothesis:** The **gene-time-environment hypothesis** suggests that environmental toxicant exposure triggers neurodegeneration in genetically vulnerable individuals.



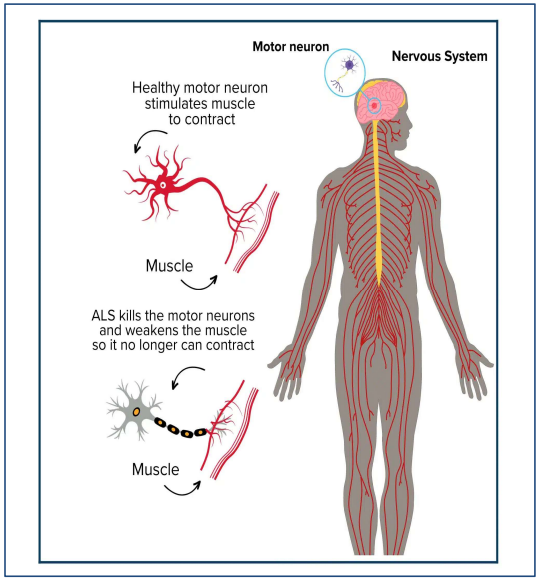
With 90–95% of ALS cases being sporadic, environmental toxicants and lifestyle exposures are critical contributors, making water quality and pollution control central to prevention strategies.

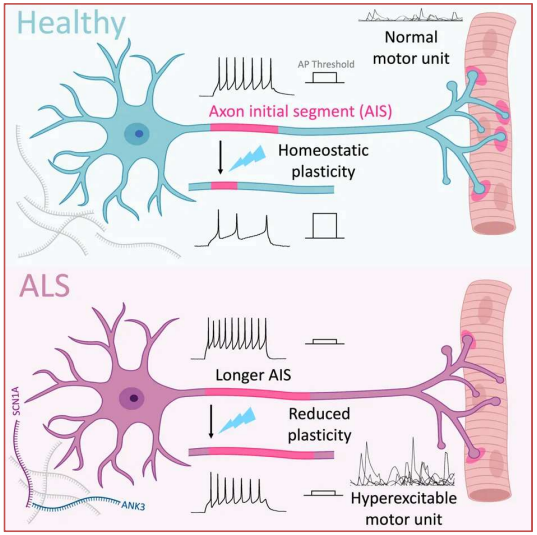
Water Contamination: A Hidden ALS Trigger

- **The Raw Water Hypothesis:** Chronic exposure to raw or untreated water sources is strongly tied to geographic ALS clusters globally.
- **Statistical Risk:** A pilot case-control study found that **raw water exposure** yielded an **Odds Ratio (OR) of 4.74** for developing ALS.
- **Proximity to Waterbodies:** Living within 2 miles of waterbodies prone to toxic blooms significantly increases residential risk profile.

An **odds ratio (OR) of 4.5** means that individuals exposed to poor water quality are **4.74 times more likely** to develop Amyotrophic Lateral Sclerosis (ALS) compared to those who were not exposed to poor water quality. In medical research, an OR greater than 1.0 indicates that an exposure is linked to a higher risk of an outcome.

Cause Type	Examples	Contribution
Genetic	SOD1, TARDBP, FUS.	~10% of cases
Environmental	Heavy metals, pesticides, cyanobacteria.	Major role in sporadic ALS
Lifestyle/Occupational	Smoking, military service, head injuries.	Increases risk significantly



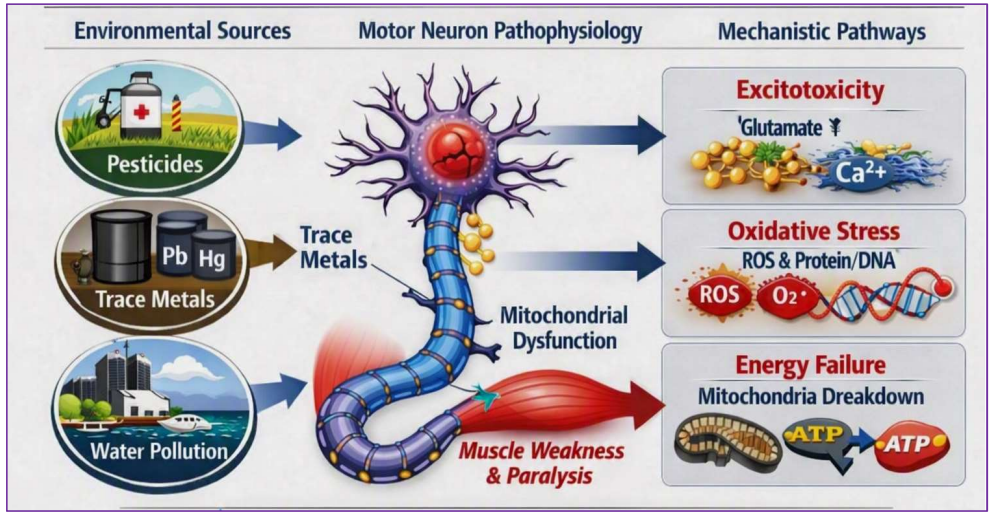


References:
Stipa et al . Is Chronic Exposure to Raw Water a Possible Risk Factor for Amyotrophic Lateral Sclerosis? A Pilot Case-Control Study. Brain Sci. 2021, 11, 193.
Peter et al. Fungal-contaminated grass and well water and sporadic amyotrophic lateral sclerosis, Neural Regen Res, 2019, 14(9):1490-1493.
Angeline et al .Environmental and occupational exposures and amyotrophic lateral sclerosis (ALS) in New England, Neurodegener Dis, 2017, 17(2-3):110–116.

How Toxins Kill Motor Neurons?

Motor neurons are the nerve cells that control our muscles. They are very sensitive because they use a lot of energy and handle strong signals.

- **Excitotoxicity**: Some toxins overstimulate motor neurons with too much glutamate, causing calcium overload and cell death.
- **Oxidative stress**: Heavy metals, pesticides, and cyanotoxins generate harmful molecules that damage neurons.
- **Mitochondrial damage**: Toxins weaken the cell's energy supply, making motor neurons more vulnerable.
- **Astrocyte toxicity**: In ALS, support cells (astrocytes) can release toxic factors that kill nearby motor neurons.

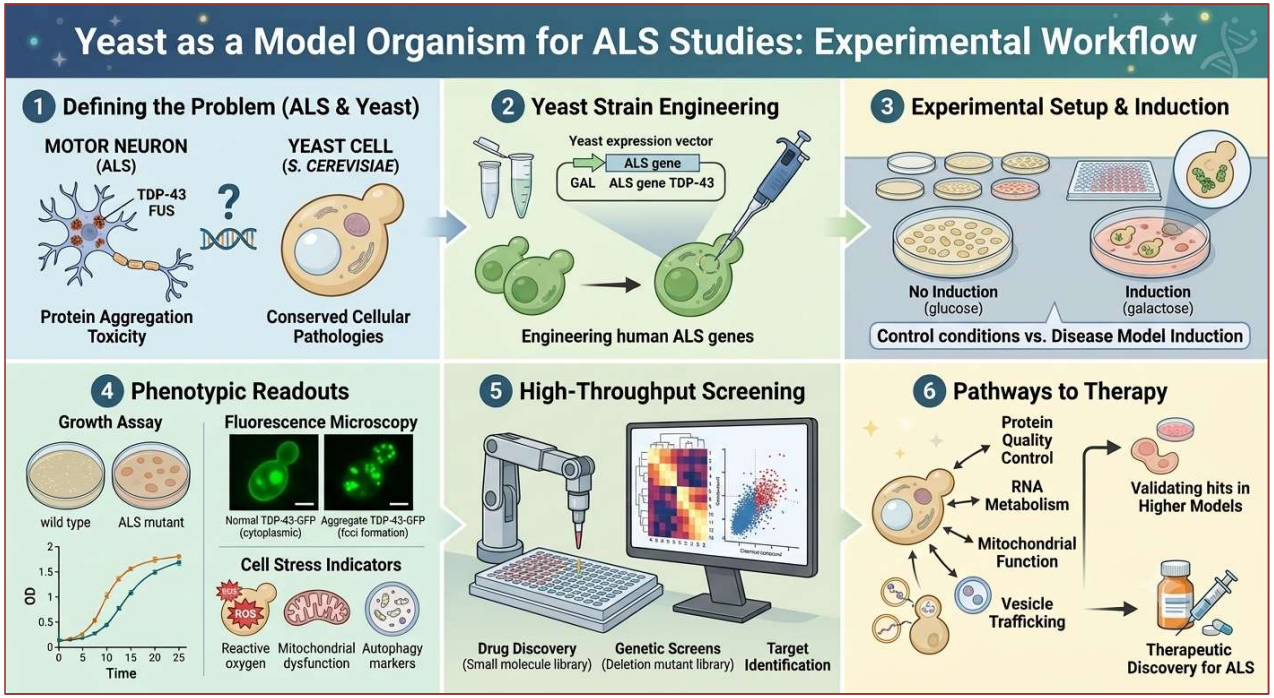


⚠ Why this matters?: Motor neurons are especially sensitive because they use lots of energy and handle strong signals. When toxins disrupt their balance, they die, leading to muscle weakness and paralysis seen in ALS.

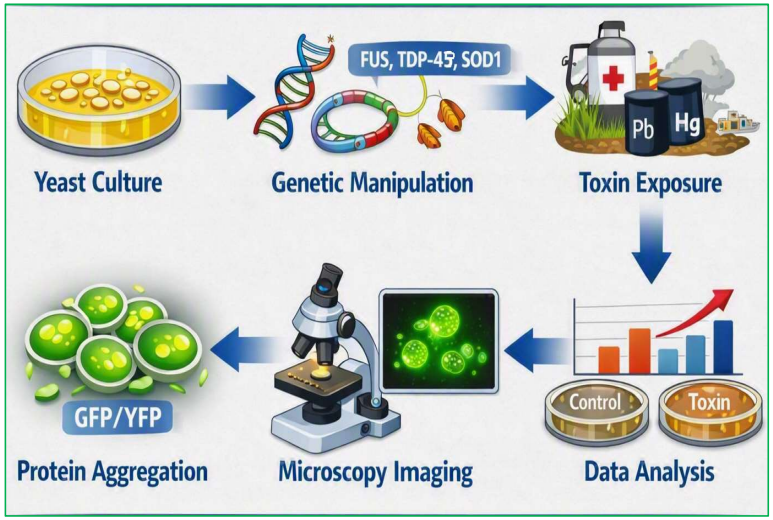
Gene	Function
C9 or f72	Controls cell transport
SOD1	Breaks down harmful oxygen
TDP-43	Handles RNA/DNA
FUS	Works with RNA transport

Yeast as a Model Organism for ALS Studies

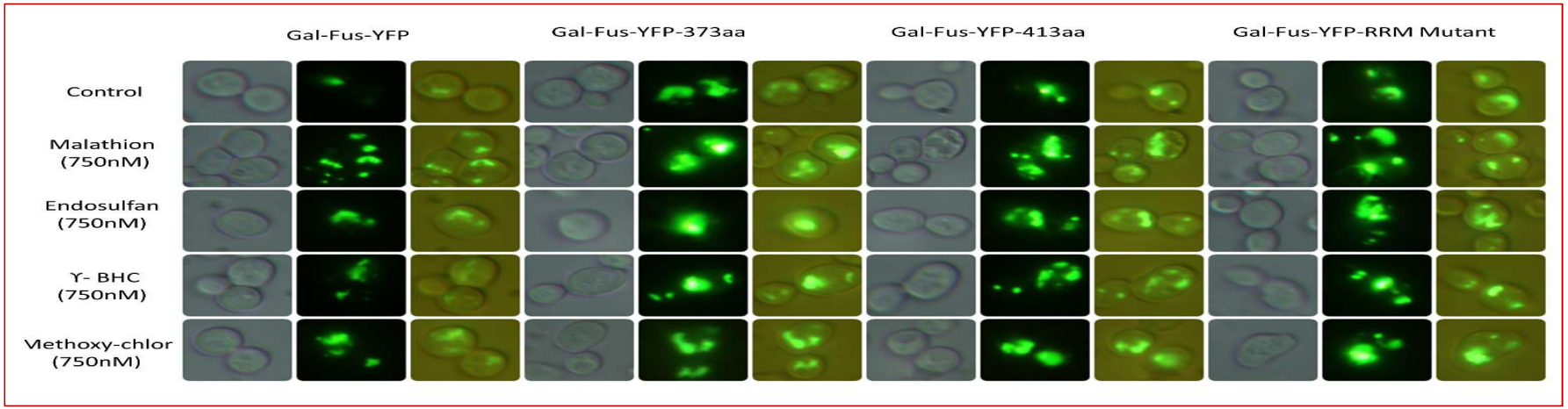
- **Basic cell structure** → Yeast has a nucleus and mitochondria, just like human cells.
- **Shared pathways** → Protein folding, stress response, and energy metabolism are similar to humans.
- **Easy to study** → Genes can be added, removed, or modified quickly.
- **Fast growth** → Experiments finish in days, not weeks.
- **Safe and low-cost** → Non-animal, inexpensive, and ethical.



Experimental Workflow: Toxin Exposure



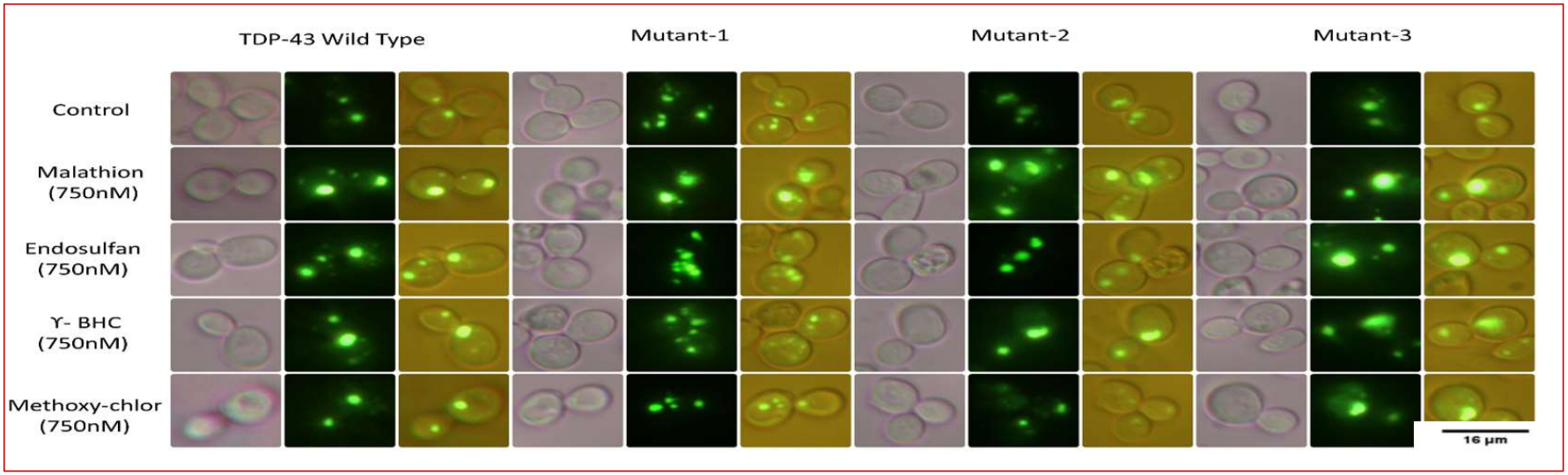
Fluorescence images of FUS constructs in yeast under Pesticide exposure



Construct	Description	Observed Pattern
Gal-Fus-YFP	Full-length FUS tagged with YFP	Control shows diffuse fluorescence; pesticide treatments induce small aggregates.
Gal-Fus-YFP-373aa	Truncated FUS (373 amino acids)	Aggregation increases under all pesticide exposures, suggesting stress-induced condensation.
Gal-Fus-YFP-413aa	Slightly longer truncation (413 amino acids)	Strong cytoplasmic foci formation, especially with Endosulfan and Malathion.
Gal-Fus-YFP-RRM Mutant	RNA recognition motif mutant	Aggregates appear less distinct, implying reduced RNA-binding–driven condensation.

Treatment	Effect on Fluorescence	Possible Mechanism
Control	Smooth, diffuse green signal	Normal soluble FUS distribution
Malathion	Bright puncta, strong cytoplasmic aggregation	Severe stress response; condensation of FUS granules
Endosulfan	Produces large, bright aggregates	Strong neurotoxic stress response.
γ-BHC	Moderate aggregation	Likely affects protein folding.
Methoxy-chlor	Fewer, smaller aggregates	Mild stress; limited condensation

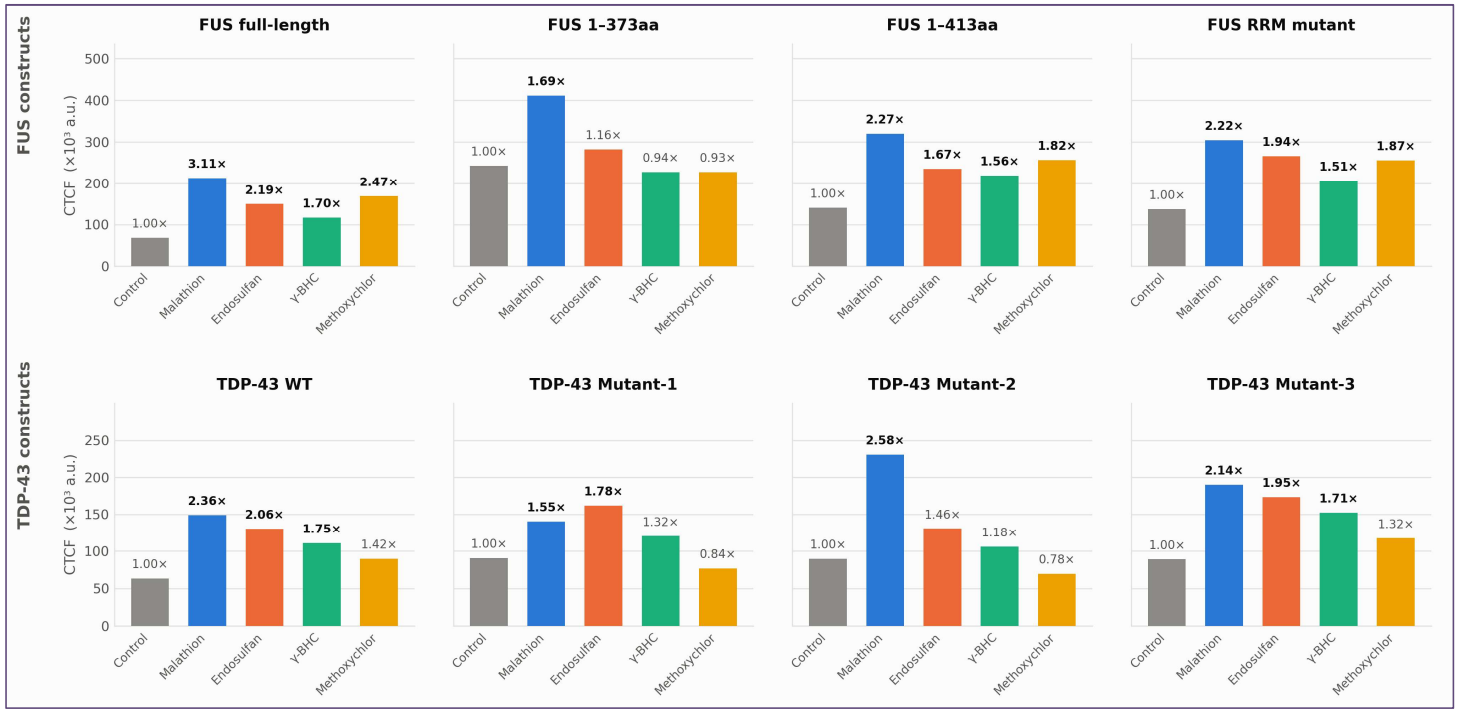
Fluorescence images of TDP-43 constructs in yeast under Pesticide exposure



Variant	Description	Observed Pattern
TDP-43 Wild Type	Normal RNA-binding protein	Control shows diffuse fluorescence; pesticides induce cytoplasmic puncta and aggregates.
Mutant-1	Likely N-terminal or RNA-binding domain alteration	Aggregation increases under all pesticide exposures, suggesting stress-induced misfolding.
Mutant-2	Possibly C-terminal truncation	Strong cytoplasmic foci formation, especially with Endosulfan and γ-BHC.
Mutant-3	Aggregation-prone variant	Large, bright inclusions even under control conditions, intensified by pesticide stress.

Treatment	Effect on Fluorescence	Possible Mechanism
Control	Diffuse green fluorescence, mostly nuclear	Normal soluble distribution, no stress granules.
Malathion	Wild type bright cytoplasmic puncta; mutants weaker	Induces oxidative stress and protein misfolding.
Endosulfan	Wild type dense puncta; mutants faint/moderate	High toxicity → mislocalization and aggregation.
γ-BHC	Moderate aggregation	Moderate stress → partial granule assembly; mutants unstable.
Methoxy-chlor	Wild type mild puncta; mutants diffuse/faint.	Suggests shared mechanism of stress-induced aggregation.

Relative fluorescence intensity of FUS and TDP-43 constructs in yeast under pesticide exposure



Inference

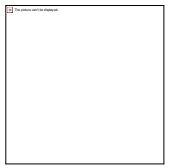
- **Malathion** consistently triggers the highest fluorescence across constructs, indicating potent **oxidative and proteotoxic stress**.
- **Endosulfan** and **Methoxychlor** also elevate aggregation.
- **γ-BHC** shows moderate effects, suggesting differential toxicity mechanisms.
- The **mutant constructs** exhibit variable sensitivity, highlighting how **structural domains modulate stress-induced phase separation**.

CONCLUSIONS

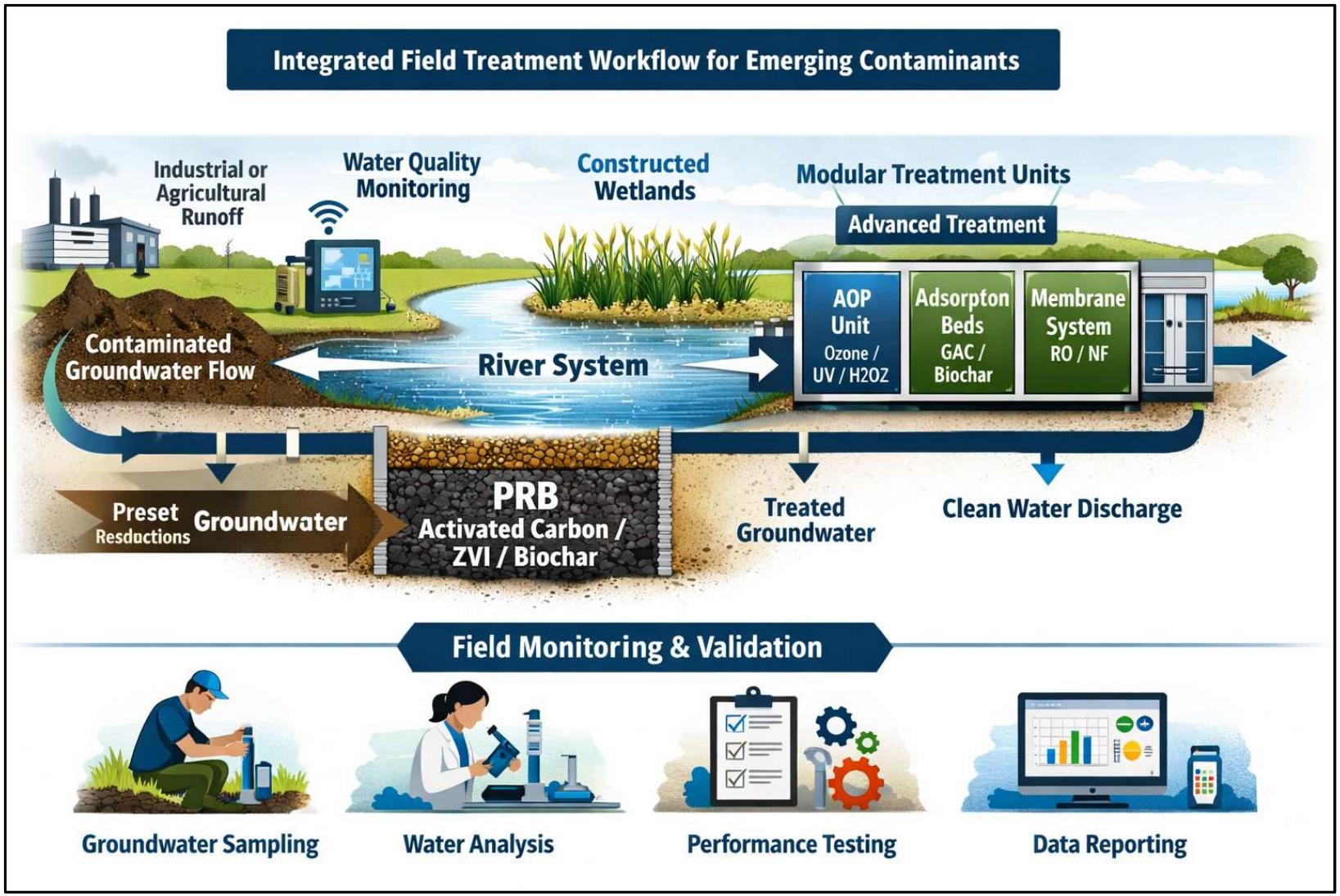
- Control cells show diffuse or faint fluorescence, meaning the proteins not clumped.
- Pesticide-treated cells display fewer, larger, and brighter foci - a hallmark of protein aggregation.
- Malathion and Endosulfan cause the strongest aggregation in both FUS and TDP-43 constructs.
- Chemical exposure acts as a trigger for misfolding and condensation, highlighting environmental contributions to proteinopathy.

Pesticide stress promotes ALS-linked protein aggregation.

Future Directions: Screening broader environmental toxins and uncovering stress pathways in ALS proteinopathy.



Integrating Environmental chemistry, Toxicology, and Disease biology to map neurotoxic risks and protect public health


Integrated Field Treatment Workflow for Emerging Contaminants
Industrial or Agricultural Runoff
Water Quality Monitoring
Constructed Wetlands
Modular Treatment Units
Advanced Treatment
AOP Unit
Ozone / UV / H2O2
Adsorpton Beds
GAC / Biochar
Membrane System
RO / NF
Contaminated Groundwater Flow
River System
Preset Resductions
Groundwater
PRB
Activated Carbon / ZVI / Biochar
Treated Groundwater
Clean Water Discharge
Field Monitoring & Validation
Groundwater Sampling
Water Analysis
Performance Testing
Data Reporting

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