



Risk and Toxicology Research Efforts

NM Produced Water Research Consortium - Year-end Meeting

December 1, 2021

Las Cruces, New Mexico



“SEIZE THE OPPORTUNITIES”

Risk and Toxicology Committee Goals

Work with the Consortium to:

- Determine what's in the raw and treated water
- Assess treatment options
- Use best available toxicological methods to assess human and environmental health risks
- Share information
- Engage stakeholders throughout the process



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Risk and Toxicology Committee Collaboration

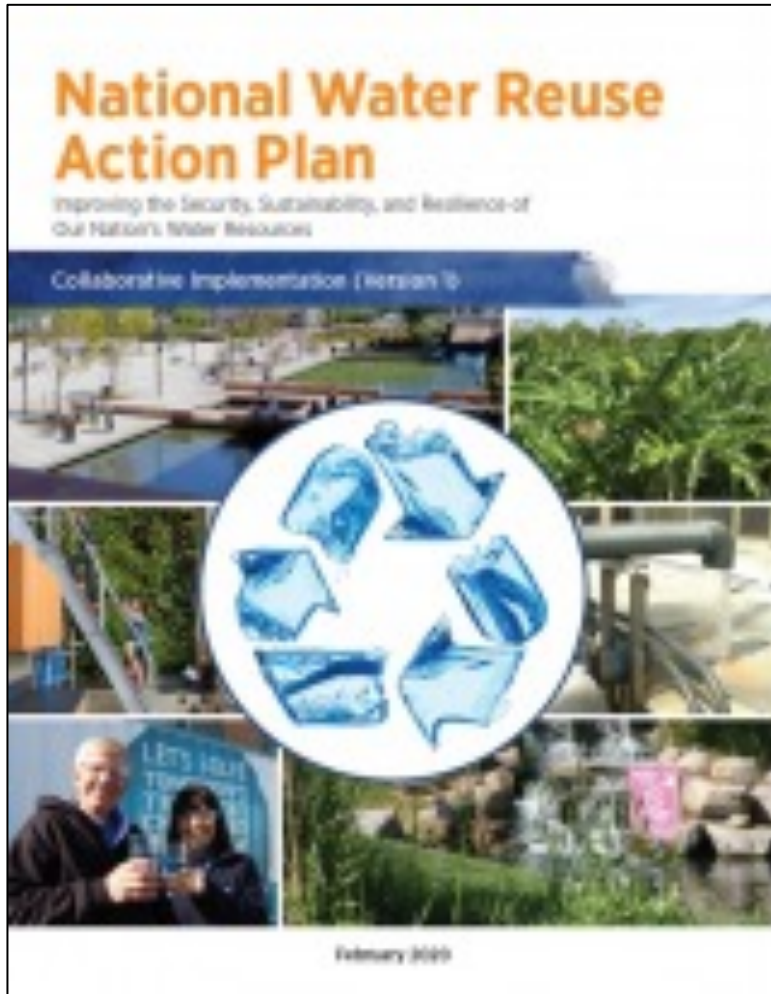
Within the Consortium

- Water Analysis Committee
- Treatment Testing Committee
- Socio-economic Environmental Cost Benefit Analysis Committee
- Fit-for-purpose Applications Working Group

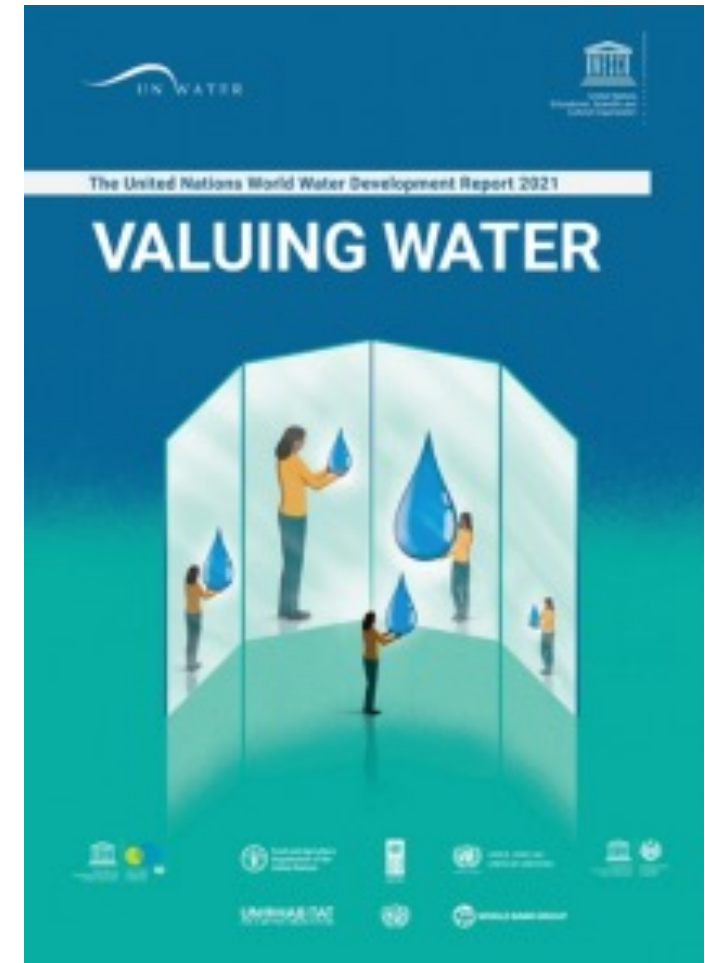
Nationally

- Environmental Protection Agency (EPA)
 - EPA WRAP program
 - Regional (6 & 8) Applied Research Effort (RARE) on produced water to support the WRAP
 - human cell-line testing,
 - advanced toxicological testing and evaluation, and
 - innovative whole effluent toxicity testing with zebra fish
- Universities and Industry
 - Agricultural analysis of soils and crops in green houses
 - Innovative Environmental Risk Assessment Analysis
 - Fate and transport modeling

2020 EPA National Water Reuse Action Plan



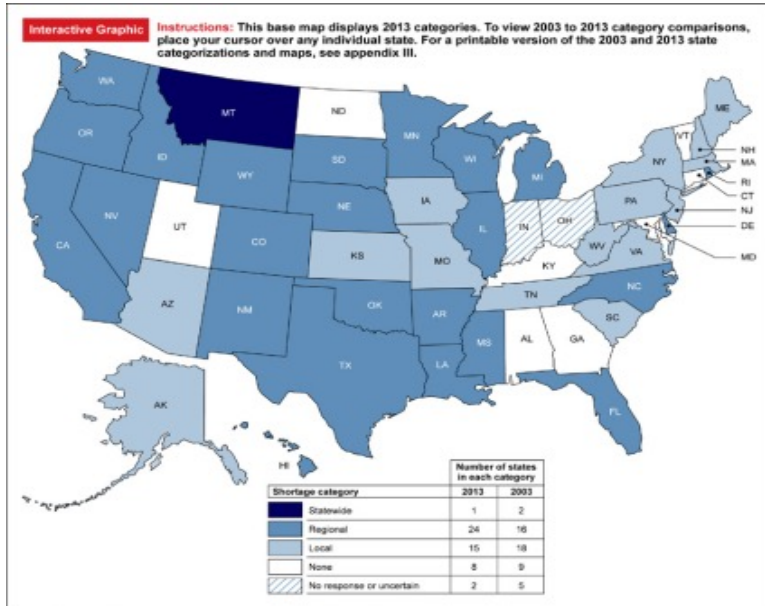
- Focus on fit-for-purpose treatment and reuse of waste water to support sustainable development
- In five major areas:
 - Thermo-electric cooling water
 - Agricultural waste water
 - Municipal waste water
 - Produced water
 - Storm water
- Two of the United Nations' Sustainable Development Goals identify **water reuse as key to a more sustainable future.**



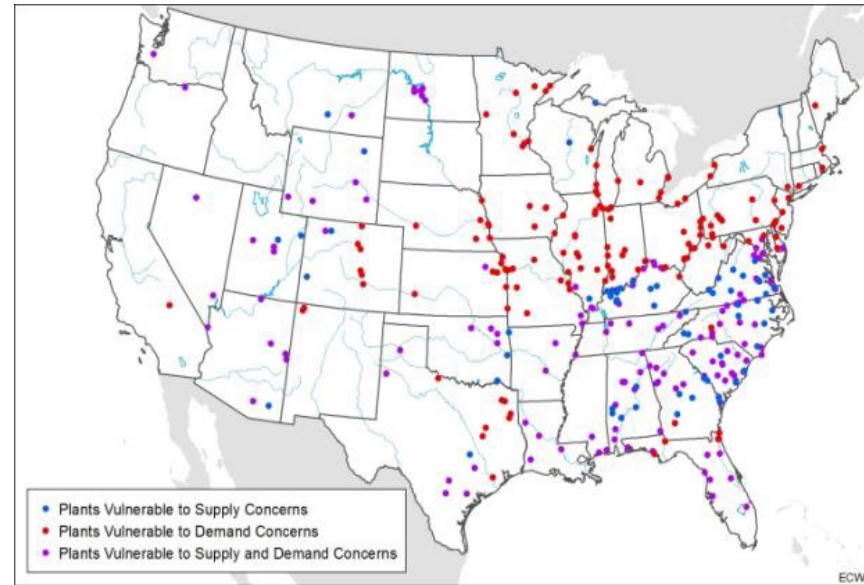
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Water Stress Driving EPA Produced Water Reuse Program

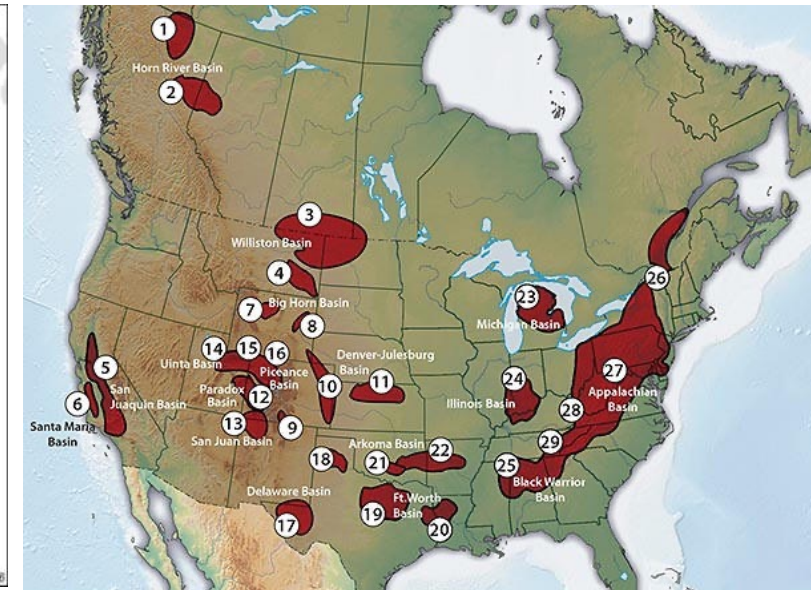
Produced water occurs in many water stressed basins across the nation



State Water Manager Identified Stress



Electric Power Identified Water Stress



Oil and Natural Gas Production Areas

Consortium is collaborating with the EPA to support produced water research and outreach

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Consortium Risk and Toxicology Analysis Approach

- Complete chemical analysis of produced water and treated produced water on a recurring basis. (NMSU/EPA)
- Evaluation of Treated Produced Water by:
 - Recurring Whole Effluent Toxicity Testing: (NMSU/EPA)
 - Human Cell Line Testing: (EPA)
 - Soil and Plant toxicology testing on Greenhouse plants from NMSU and Texas A&M using treated produced water (NMSU/EPA)
- Use of innovative environmental risk assessment models and approaches to improve risk analysis and user liabilities
- Fate and transport modeling of constituents in the environment
- Continuous monitoring for evidence of soil, plant, or terrestrial or aquatic toxicities over time.

1. Spectroscopy analysis for Chemical Identification:



Citation: Journal: WATER, 2021,13,183: A Critical Review of Analytical Methods for the Comprehensive Characterization of Produced Water

- Different technologies can be used to identify different types of chemicals in produced water.
 - ICP-OES: Inductively Coupled Plasma-optical emission Spectrometry
 - ICP-MS: Inductively Coupled plasma-mass Spectrometry
 - GC-MS: Gas Chromatography-Mass Spectrometry
 - LC-MS: Liquid Chromatography-Mass Spectrometry
 - SSEM/EDX: Scanning Electron Microscopy/Energy Dispersive X-ray
- Testing on NM produced water since Jan 2021

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2. Whole Effluent Toxicity Testing

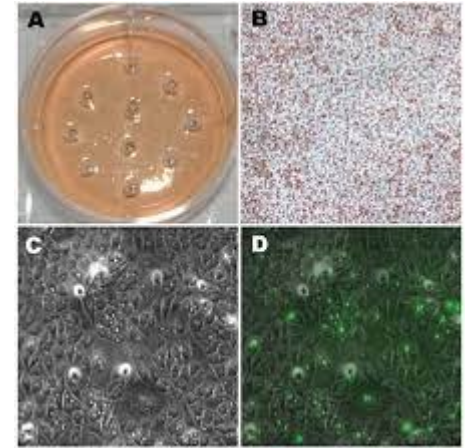
- WET tests measures effluent effects on specific test organisms' ability to survive, grow and reproduce
- Addition of Zebra Fish/Zebra Fish Embryo (Fish Embryo Acute Toxicity (FET) Test: OECD 236)
 - Strong human genetic similarities
 - Highly sensitive to environmental toxins
 - Rapid toxicity testing
 - Analysis of toxins impact to aquaculture species
- Availability of treated produced water will drive testing
- NMSU testing to start in 2022 after updating labs in 2021



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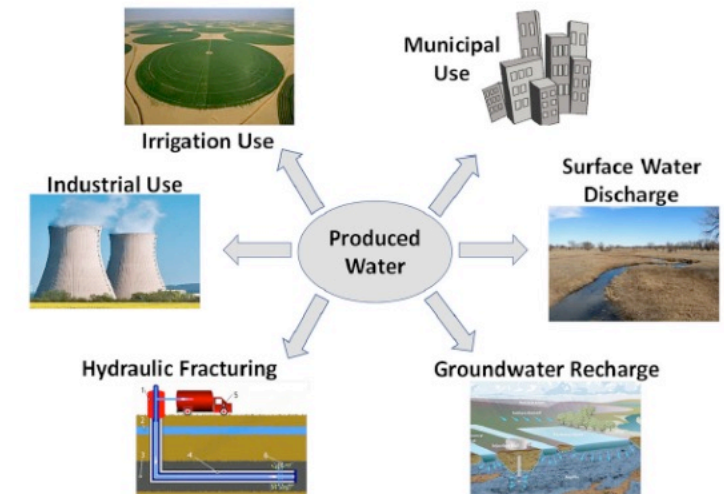
3. Human Cell Line Testing

- EPA will assess the toxicity of Produced and Treated Produced Water on Human Cells.
 - female breast cancer cells due to their high endocrine sensitivity.
 - human liver cells due to their function of toxin removal.
 - In both cell lines all genes will be evaluated for any alterations or disturbances.
- Started collaboration with EPA in July 2021 with produced water and treated produced water from ten sites in NM
- Initial data availability by March 2022



4. Greenhouse Evaluation for Soil and Plant Toxicity with Treated Produced Water

- Greenhouses at NMSU, Texas Tech, and Texas A&M, and agricultural extension services locations are being used and considered.
- Alfalfa, grasses, cotton and other crops will be studied.
- Analysis for contaminants in roots, stems, leaves, fruit, and soils will be obtained over multiple years to assess for bioaccumulation over time.
- Evaluation to include emerging contaminants.
- Data will also support
 - Socio-Economic cost/benefit analysis in 2021
 - Fate and transport modeling and analysis beginning in 2022.



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Risk and Toxicology Research Efforts

Risk and Toxicology Assessment for Produced Water Treatment and Reuse in the Permian Basin

Civil Engineering: Yanyan Zhang, Pei Xu

Plant and Environmental Sciences: Manoj Shukla, Kenneth Carroll

Chemical Analysis and Instrumentation Laboratory: Tanner Schaub, Robert Young

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What We Have Done:

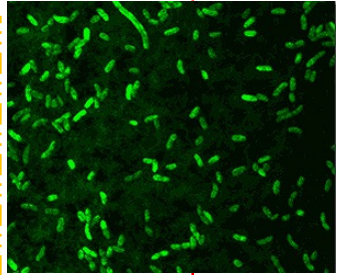
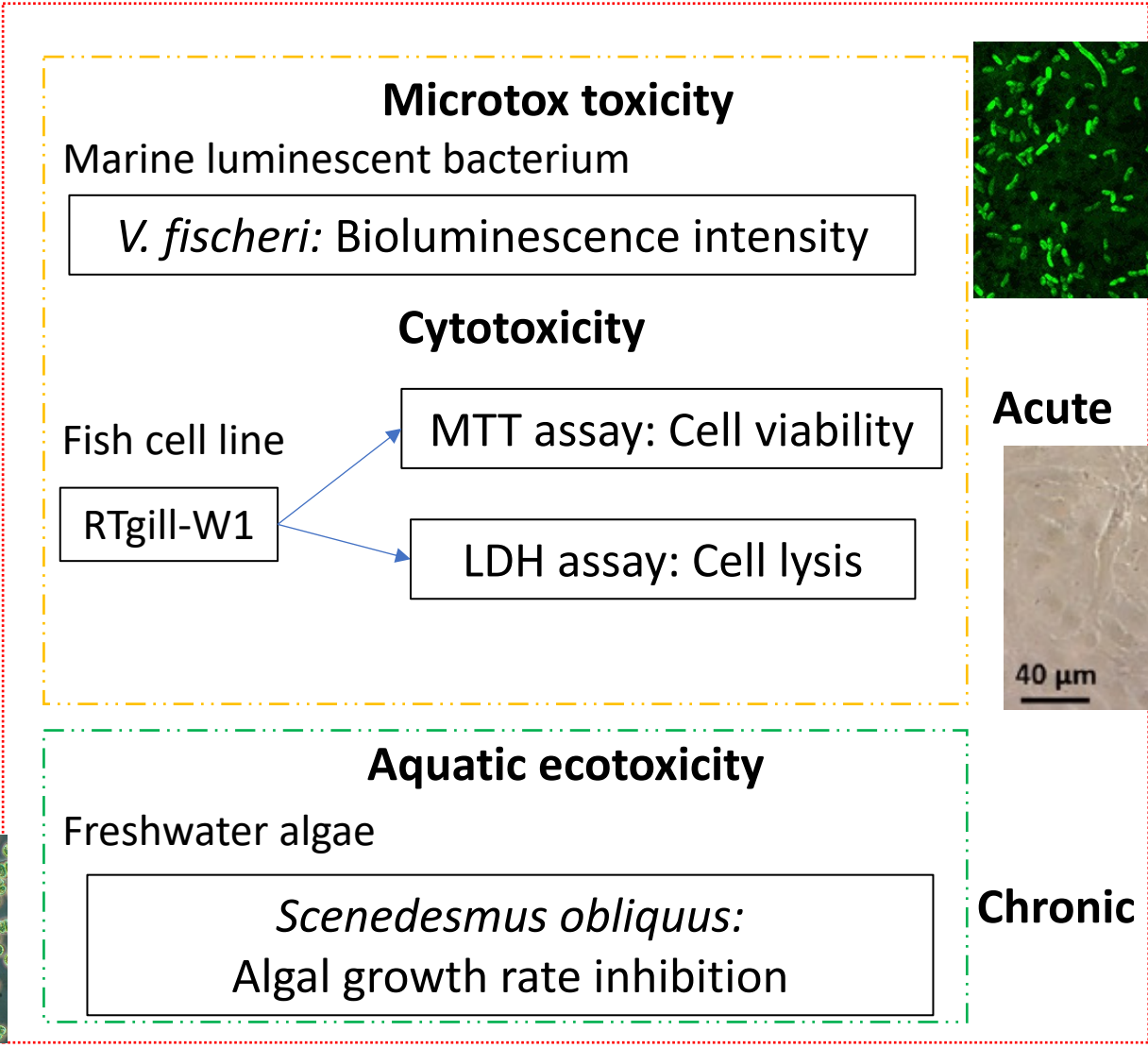
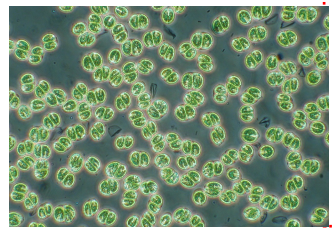
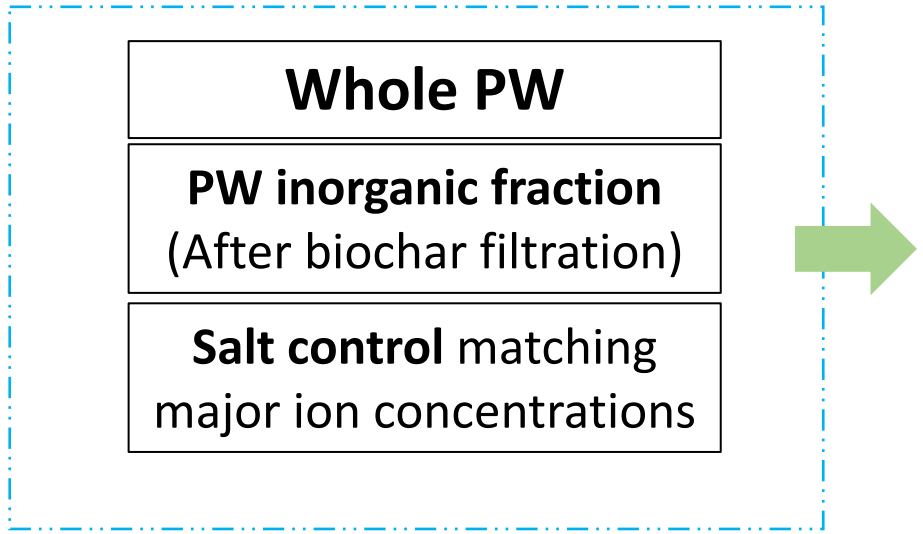
Evaluating the Toxicity of Raw Produced Water in the Permian Basin

- Objectives
 - Assist in determining spill mitigation strategies by considering the dilution factors
 - Understand which fractions of PW contaminants are toxic and help to identify treatment technologies

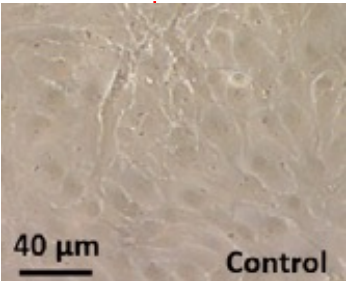
Methodology

In vitro acute and chronic toxicity assays

Different constituents in PW



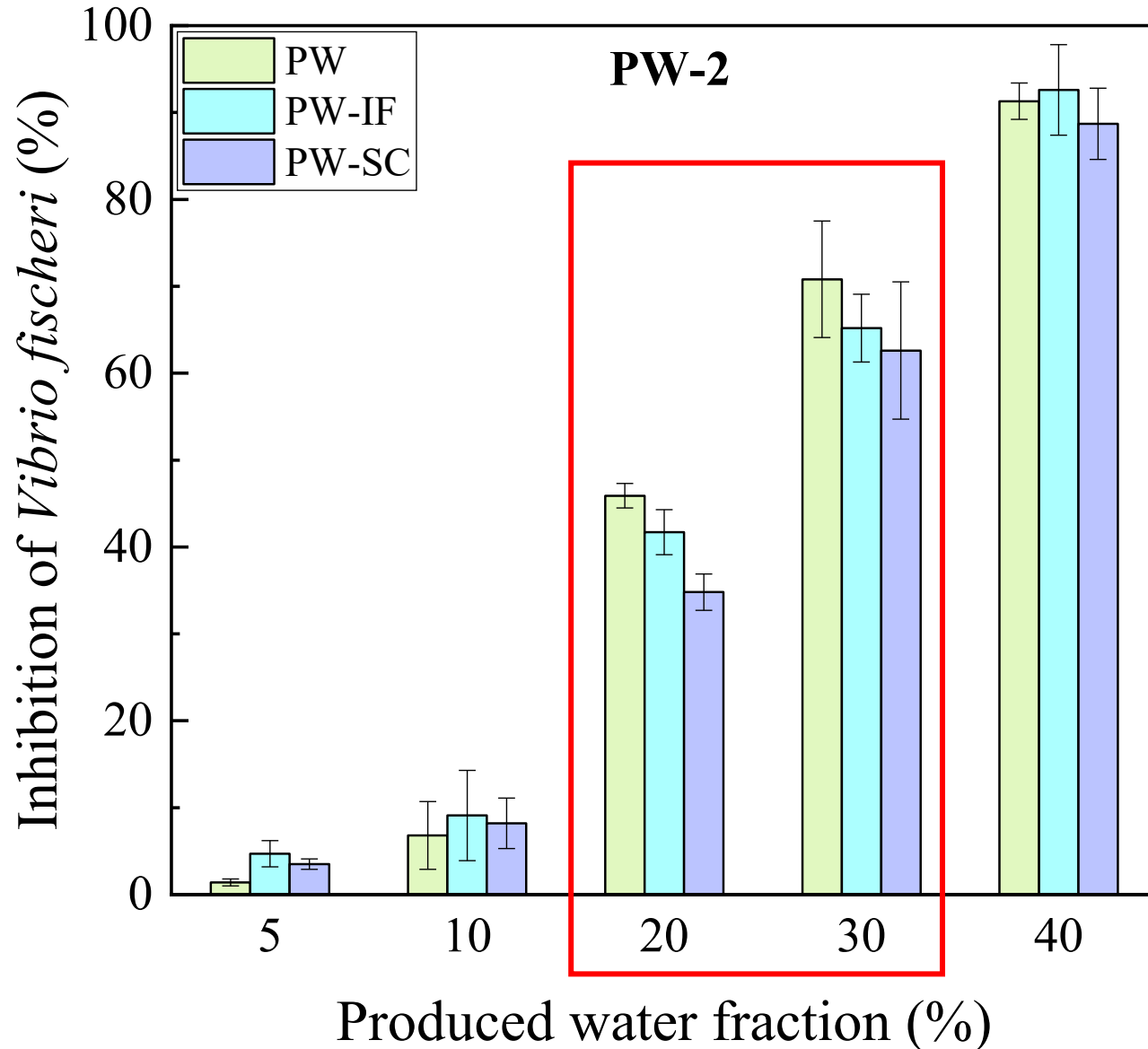
Acute



Chronic

Results

Microtox[®] toxicity



PW: Whole PW

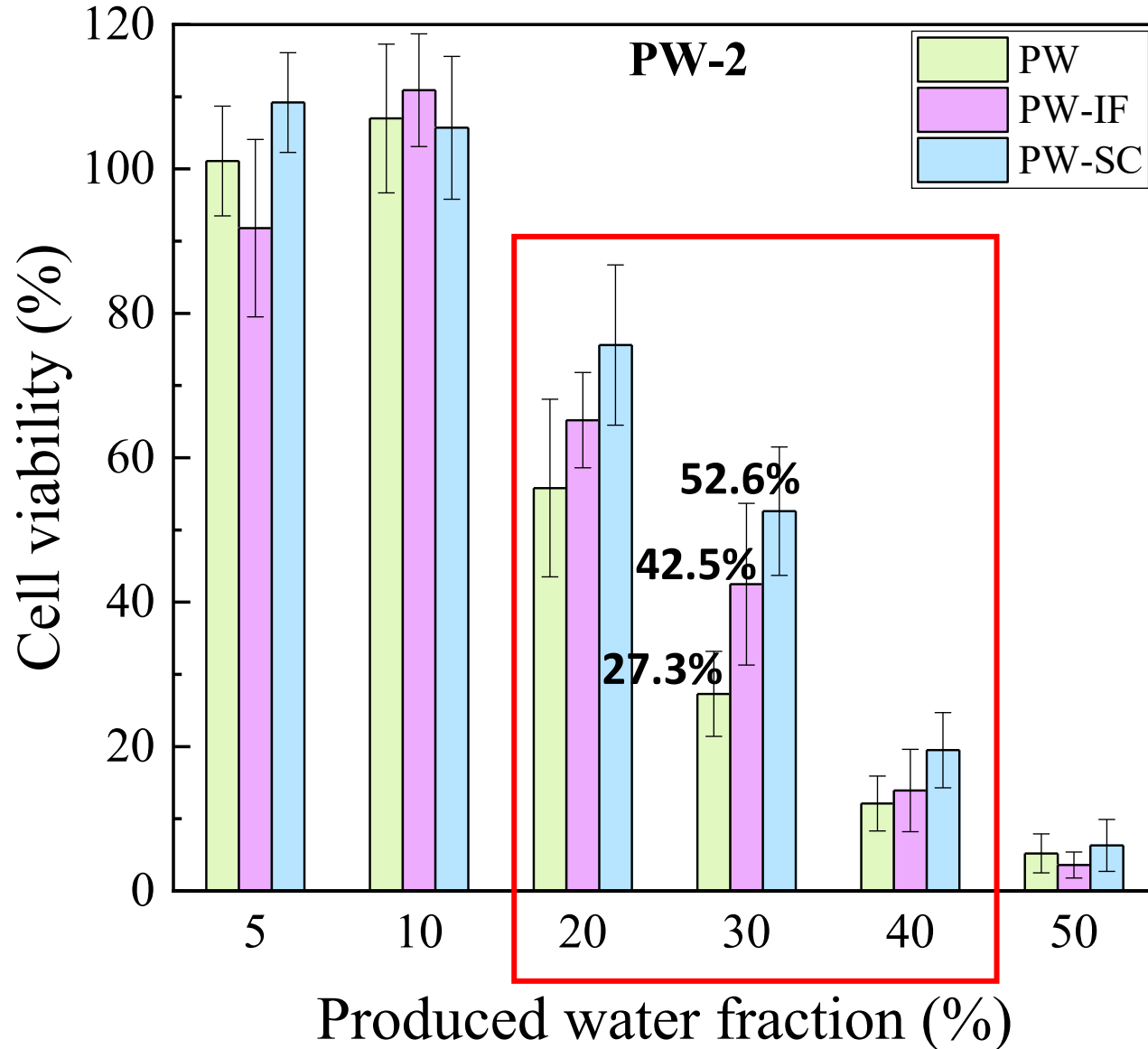
PW-IF: PW inorganic fraction

PW-SC: PW salt control

- Enhanced toxicity was probably attributed to the **increased salinity** in PW.
- **Organics** were partially responsible for the acute toxicity.
- High salinity was **the predominant toxicological driver**.

Results

Cytotoxicity toxicity---MTT viability assay



PW: produced water

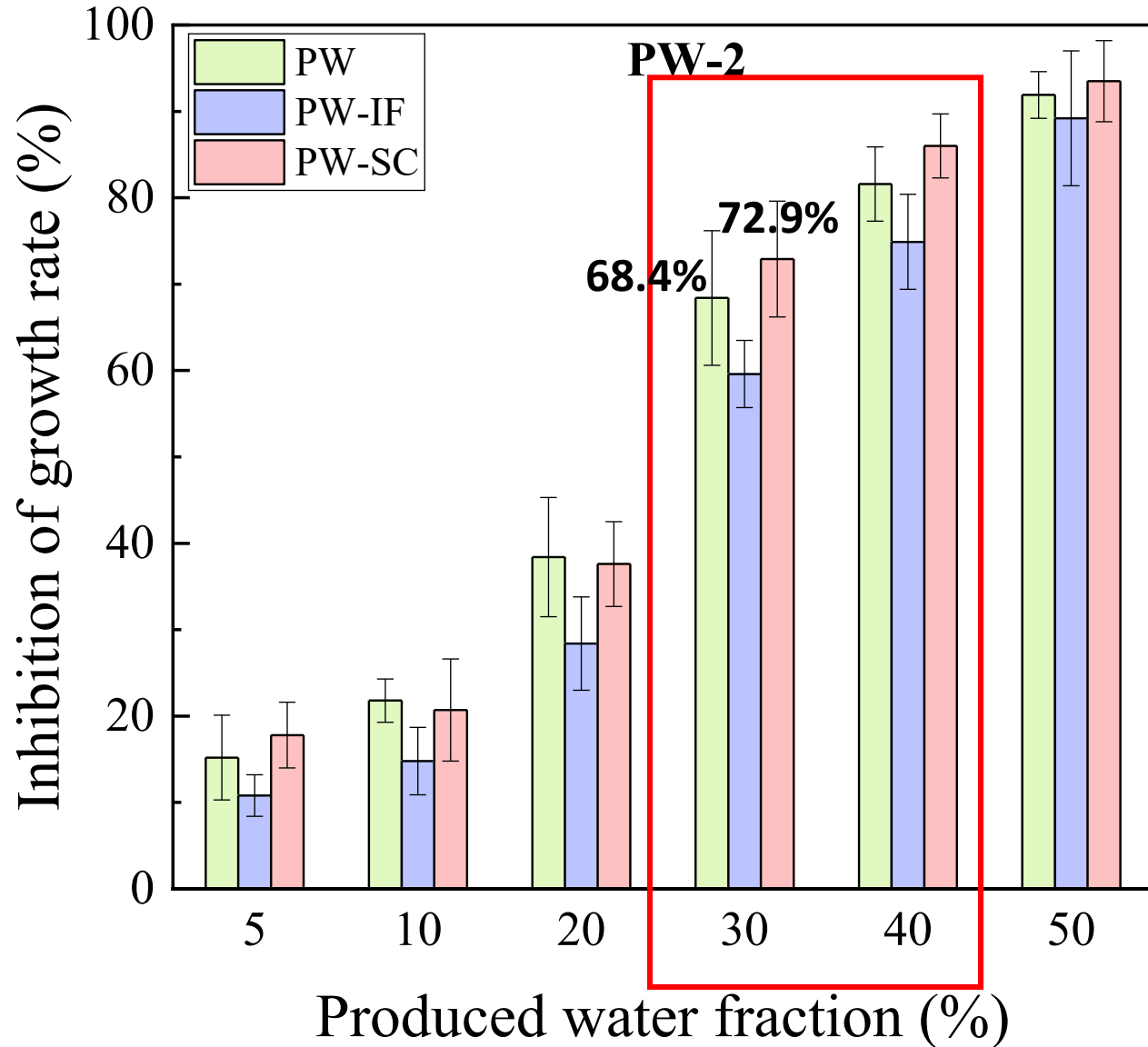
PW-IF: produced water inorganic fraction

PW-SC: produced water salt control

- Lower cell viability reflects the higher cytotoxicity.
- Cell viability : **PW < PW-IF < PW-SC.**
- **Organic** fraction caused a **stronger lethal effect** on RTgill-W1.

Results

Aquatic ecotoxicity-Freshwater Algae



PW: produced water

PW-IF: produced water inorganic fraction

PW-SC: produced water salt control

○ Dose-response relationship

○ Aquatic ecotoxicity: **PW-SC > PW**.

○ High **ammonium** can **promote the algal growth**.

Results

Comparison of EC_{50}

EC_{50} ---the concentration that results in 50% of bioluminescence inhibition/mortality/growth rate inhibition

Organisms	Toxicity assay	Exposure time	EC_{50}		
			PW-1	PW-2	PW-3
<i>Vibrio fischeri</i>	Microtox®	15 min	25.9%	22.7%	21.6%
RTgill-W1	MTT	48 h	26.5%	23.1%	20.3%
	LDH	24 h	33.5%	31.2%	27.5%
<i>S. obliquus</i>	Growth inhibition	7 d	27.9%	25.3%	11.5%

Generally,
Higher TOC, TDS and NH_4^+
↓
Lower EC_{50} (higher toxicity)

Water Quality Results

	PW-1	PW-2	PW-3
TOC (mg/L)	52.1	72.5	139.7
TDS (g/L)	160.4	172.2	219.6
NH_4^+ (mg/L)	483.4	654.4	879.3

Methodology

Identify the contaminant groups with toxicity

In vitro acute and chronic toxicity assays

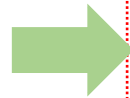
Different treatment technologies

Biochar filtration (**Organic removal**)

Air stripping (**Ammonium removal**)

Chemical precipitation with Na_2CO_3
(**Heavy metal removal**)

Combination of three treatments
(**Removal of organics, ammonium
and heavy metals**)



Microtox toxicity

V. fischeri: Bioluminescence intensity

Cytotoxicity

MTT assay: Cell viability

LDH assay: Cell lysis

RTgill-W1

Acute

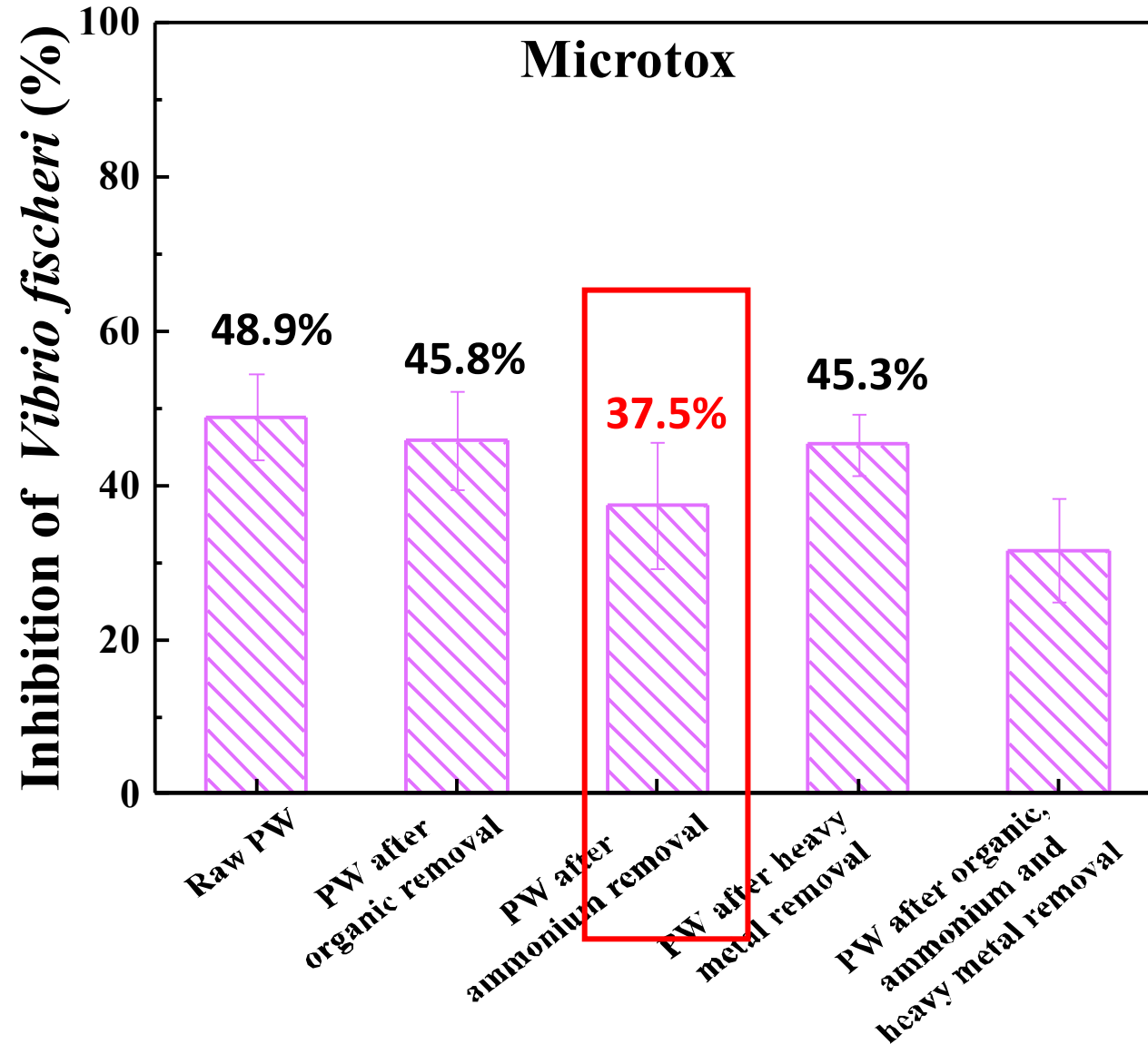
Aquatic ecotoxicity

Scenedesmus obliquus:
Algal growth rate inhibition

Chronic

Results

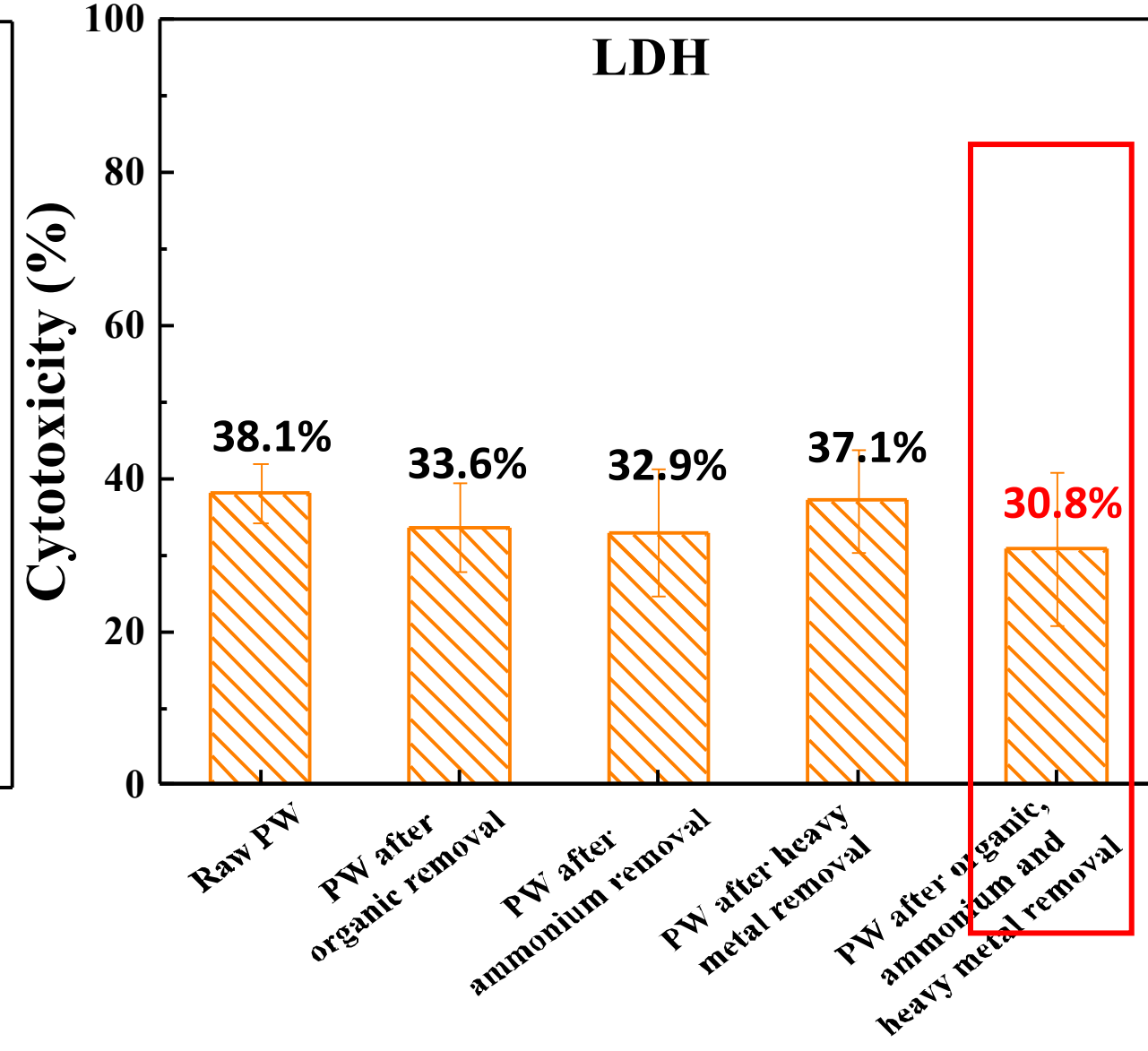
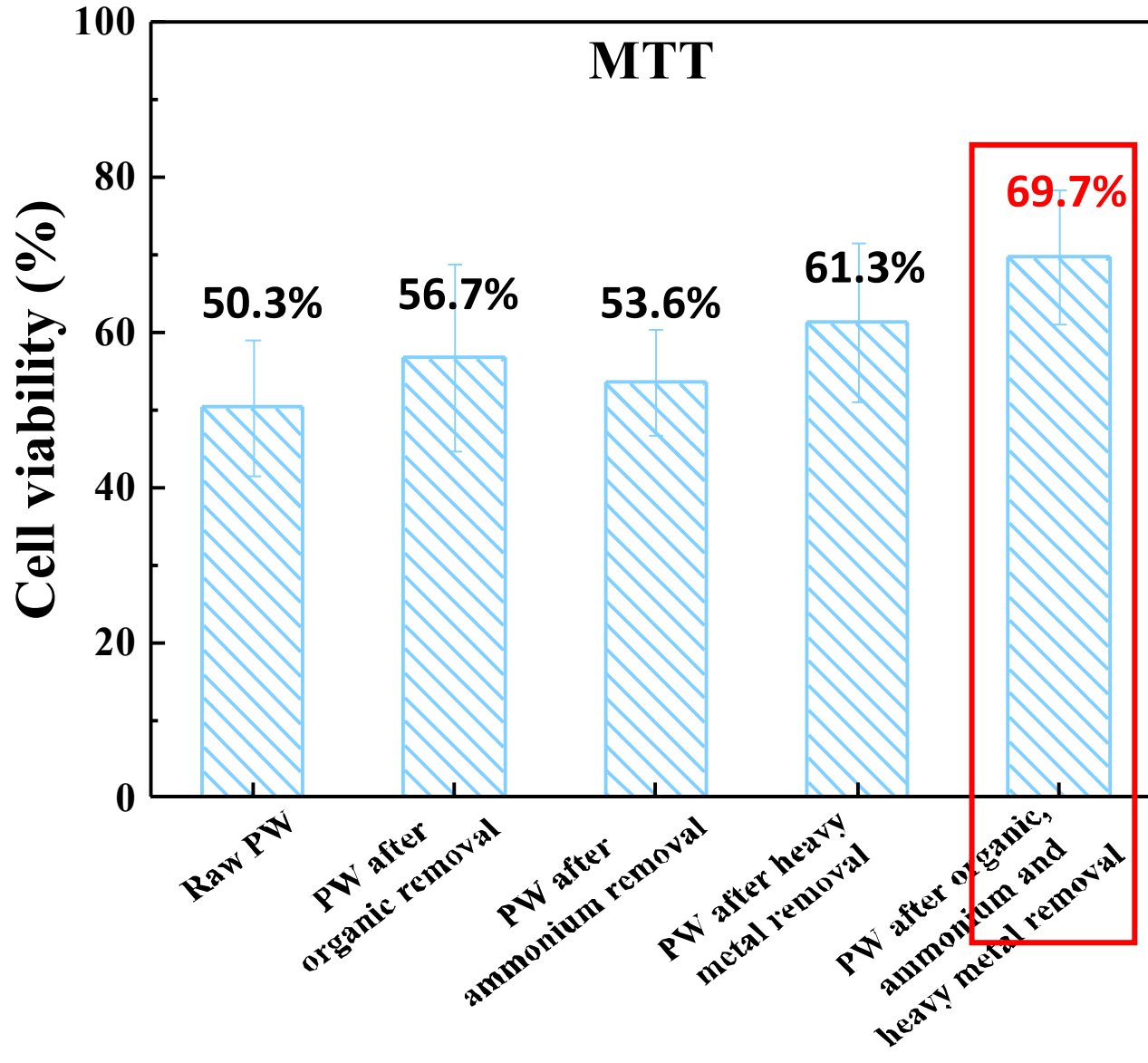
Effect of different pretreatments on the toxicity of PW



○ **Ammonium** was one of the **main contributors** to the Microtox toxicity.

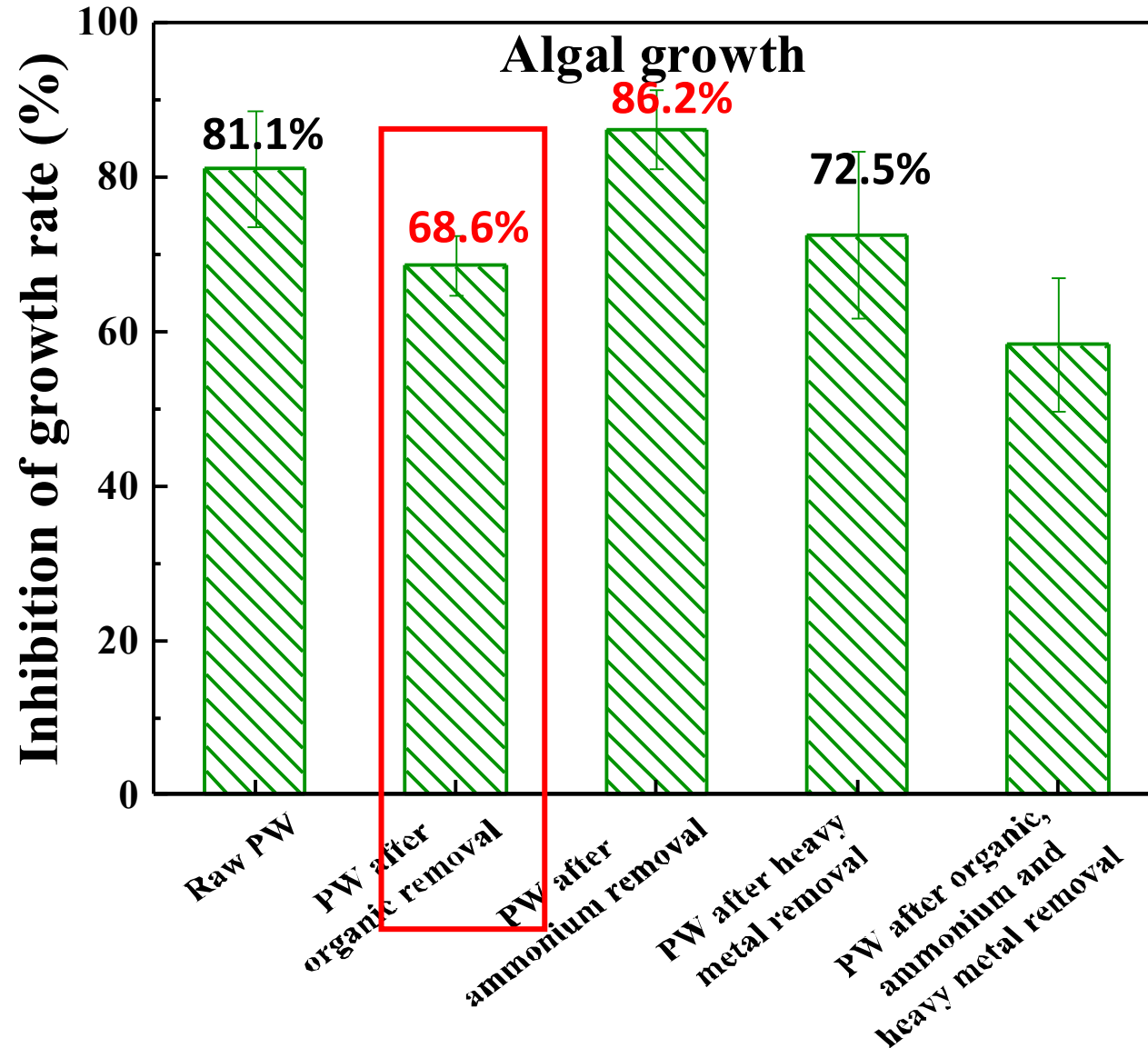
Results

Effect of different pretreatments on the toxicity of PW



Results

Effect of different pretreatments on the toxicity of PW

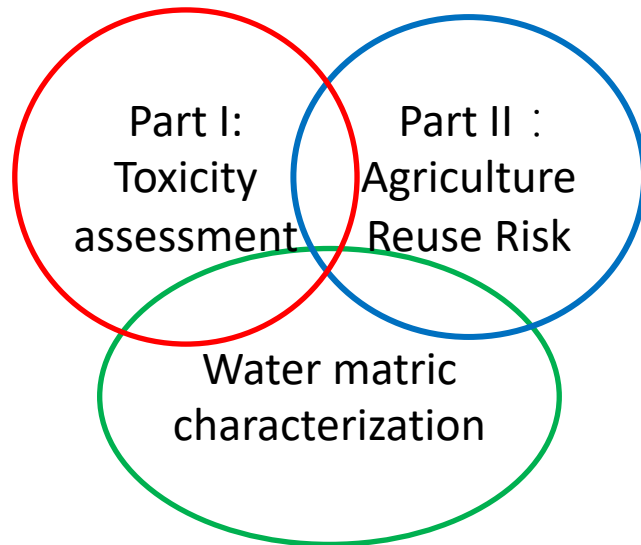


- In addition to salinity, **organics have the most significant impact.**

Major Findings from Raw PW Toxicity Assays

- High salinity was the predominant toxicological driver in PW.
- Organic contaminants had an important impact on the toxicity of PW.
- Heavy metals and ammonium in PW also contribute to toxicity.
- Strong correlations were found between chemical components and toxicity results.
- Toxicity assays should be selected based on the target compounds in PW.

Research Plan for 2022 and beyond



Toxicity assessment

- Evaluate the existing treatment processes based on toxicological behaviors of **treated water**
- Use Toxicity Identification Evaluation to understand the correlation between chemical compositions and toxicity of **treated PW**

Risk of agriculture reuse

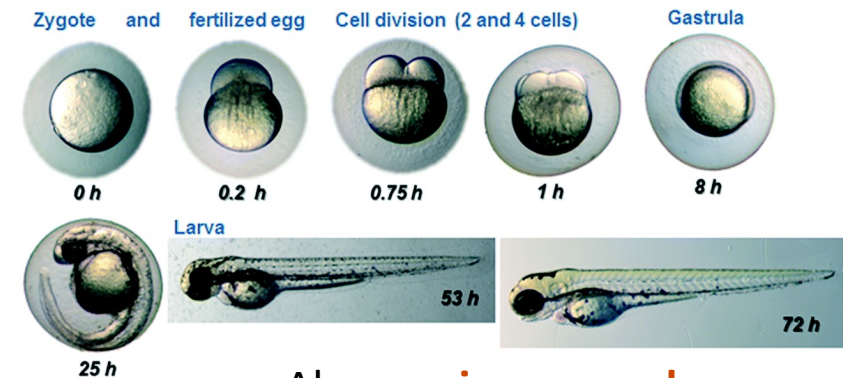
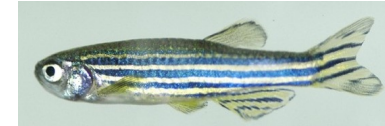
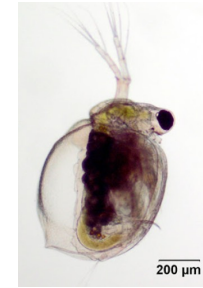
- Evaluate the feasibility of using **treated PW** for agricultural irrigation
- Identify the effect of using **treated PW** on germination and growth of plants.
- Determine the accumulation patterns of various ions and organic hydrocarbons in soil and crops

Treated Produced Water

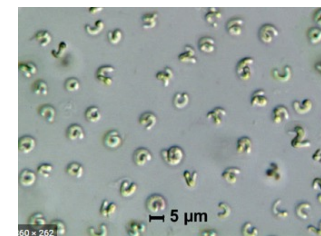
- Treatment processes
 - Electrocoagulation
 - Filtration/biofiltration
 - Solar distillation
 - Membrane distillation
 - Other pilot scale tests

Toxicity Assays

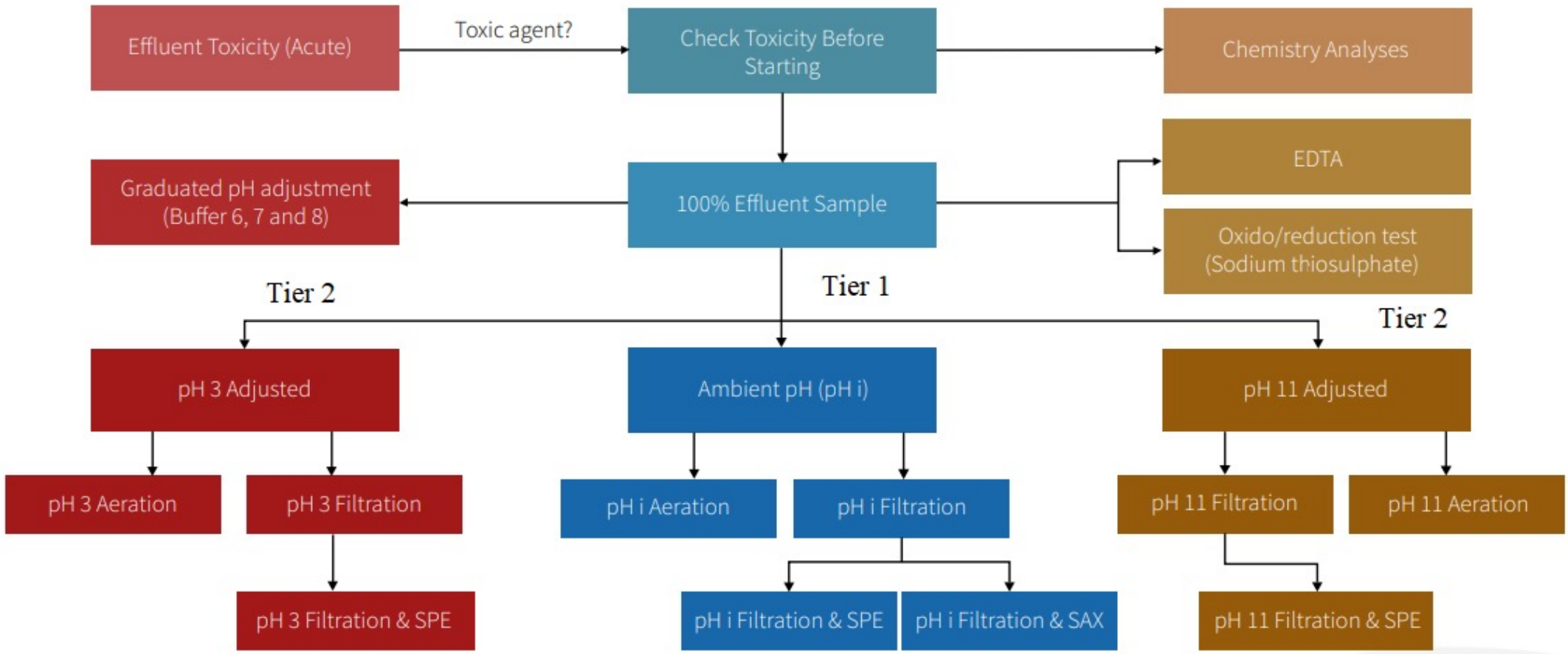
- Acute toxicity test with *Daphnid*
 - Based on immobilization of *Ceriodaphnia dubia*
 - EPA-821-R-02-012, Method 2002.0
- Fish Embryo Acute Toxicity (FET) Test
 - Based on the development of fish embryos
 - OECD, Test No. 236
- Chronic toxicity with *Daphnid*
 - Based on survivals & reproduction of *Ceriodaphnia dubia*
 - EPA-821-R-02-013, Method 1002.0
- Chronic toxicity with freshwater green algae
 - Chronic toxicity towards *Selenastrum capricornutum*
 - Algal growth
 - EPA-821-R-02-013, Method 1003.0



Algae: **primary producers**
Invertebrates: **primary consumers**
Fish: **secondary consumers**



Toxicity Identification Evaluation



Agriculture Reuse Risk

- The Crops

- two food crops chile and guar
- two forage crops triticale and alfalfa.
- The soil is from farms in the Permian Basin

- Irrigation Water

- Tap water as baseline
- Treated PWs

- Germination Experiments (Phase I)

- Seeds will be placed on soil moistened with various treated PW
- Record: Germination rate (#seeds/day), percent germination, mean germination time, seedling length (root +shoot), seedling dry weight (root +shoot)

Agriculture Reuse Risk

- Plant growth (Phase II)

- The seeds without adverse germination effects will be planted in cylindrical PVC pots
- Growth Record: Mean relative growth (MRGR) rate, relative length, relative weight, relative leaf area, crop yields, chlorophyll content, leaf temperature

- Analyses

- Plant: Ions, metals, organics
- Leachate: Volume, electrical conductivity, ions, metals, organics
- Soil: Ions, metals, BTEX, gasoline and diesel range hydrocarbons, microbiome

Thank you!

- Your comments and suggestions are appreciated!
- Contact:
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