Microbial Ecology of Casing Soils and Food Safety Interventions to Reduce *Listeria monocytogenes* and *Salmonella* spp. Contamination of Fresh Mushrooms

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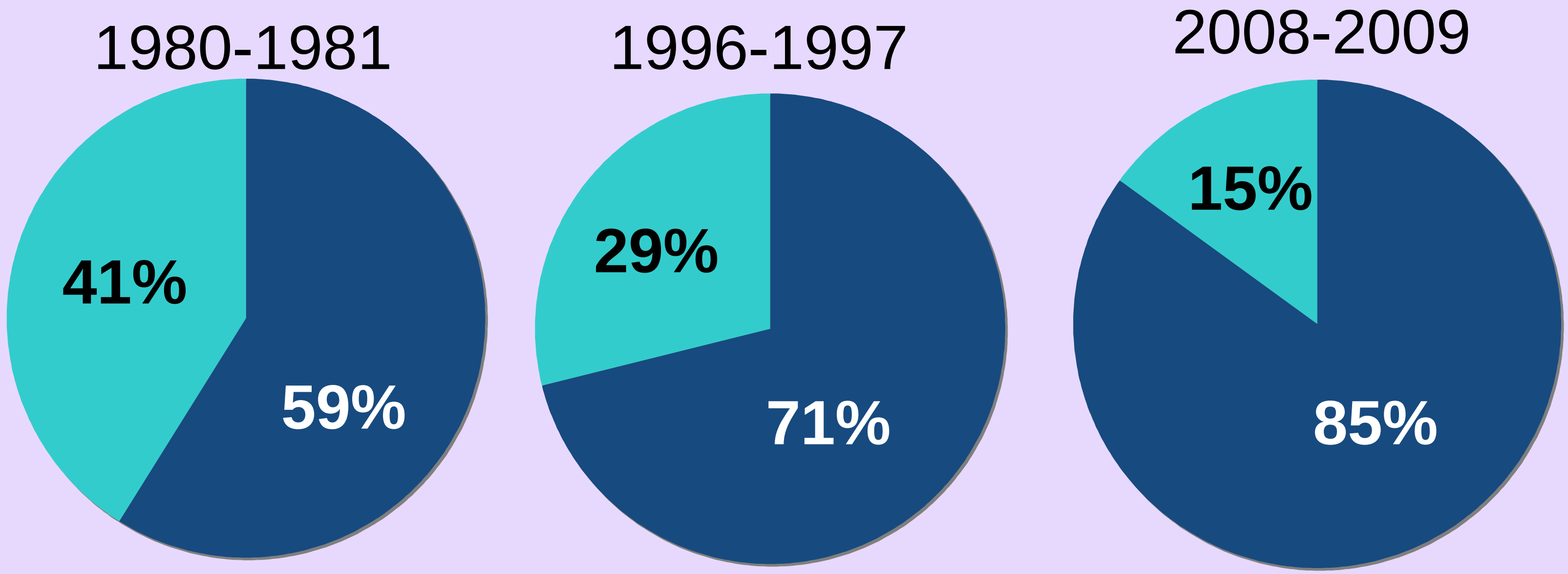
Overview

- Background and Significance
  - Food Safety
  - Casing Soil
- Hypothesis and Objectives
- Research Strategy
  - Experimental Design
  - Preliminary Data
  - Expected Results

Significance

Consumer Trends: Fresh and Processed Sales



= Fresh Market Sales (Whole and Sliced)

= Sales for Processing (Jars, Canned, Frozen)

NASS, 2009

Significance

Contamination

1988 - *L. innocua* found in 11% of supermarket mushroom samples (Heisick, et. al)

1989 - *L. monocytogenes* found in 10% of purchased mushrooms (Van Netten, et al)

1999 - *L. monocytogenes*, and *Salmonella* sp. found in 1% and 5% of mushrooms, respectively (Samadpour, et. al)

2001 - *Salmonella* sp. isolated from mushrooms, casing, and compost in Northern Ireland (Meikle)

Significance

Recalls - *Listeria monocytogenes*

- 2003 - Georgia Department of Agriculture (FDA)
- 2006 - Ohio Department of Agriculture (FDA)
- 2008 - Canadian Food Inspection Agency (CFIA)

Background

Food Safety in Mushroom Production

Growing Environment

People

Substrate

Casing

Background

Food Safety in Mushroom Production

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Food Safety in Mushroom Production

Growing Environment

People

Substrate

Casing

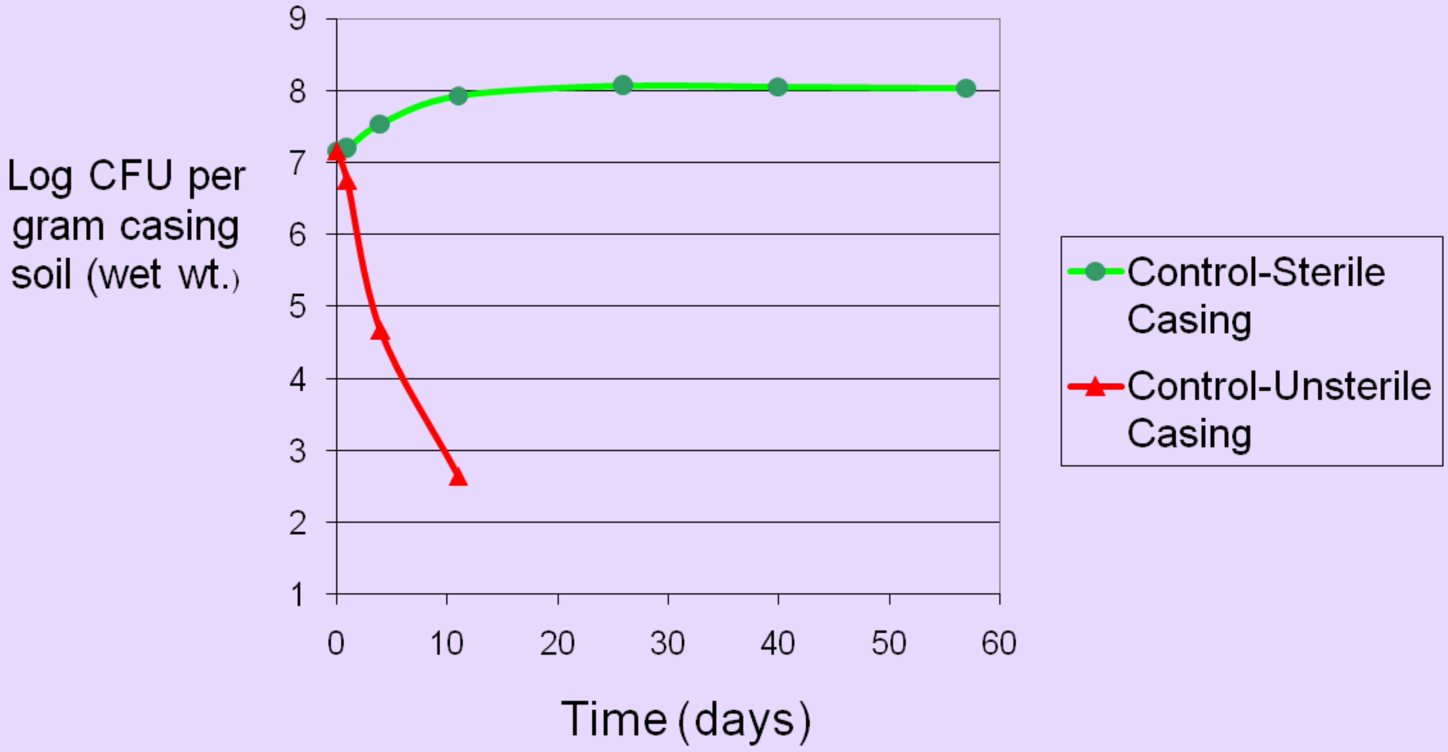
Chikthimmah, 2006

Mushroom Casing Layer

- Peat moss, $CaCO_3$ (lime), and water
- Two main types: light and dark
- Use of dark peat in the industry
  - Heavier texture
  - Higher water-holding capacity

Peatmoss.com, Hoitink, and Fahy 1986

L. Monocytogenes in Light Peat



Chikthimmah, 2006

Background

Dark Peat Casing Soil

- Plant Pathology research has shown more plant disease with use of dark peat
  - More decomposed organic matter
  - Lower levels of indigenous microflora

Hoitink and Fahy, 1986

Hypothesis

Hypothesis

Commercial dark peats contain lower levels of indigenous microflora compared to light peat.

Objectives

1. Determine the levels of indigenous microflora in dark peat compared to light peat
2. Determine the fate of human pathogens in casing soil held under commercial growing conditions
3. Determine the fate of human pathogens in casing soil held under commercial growing conditions in a growing system (colonized with *Agaricus bisporus*)
4. Determine the effect of supplementing irrigation water with sanitizers on pathogen reduction
5. Evaluate if there is a hurdle effect with a certain light:dark peat ratio combined with irrigation water supplementation

Objective 1 - Experimental Design (No Agaricus)

Microbiology of Light and Dark Peat

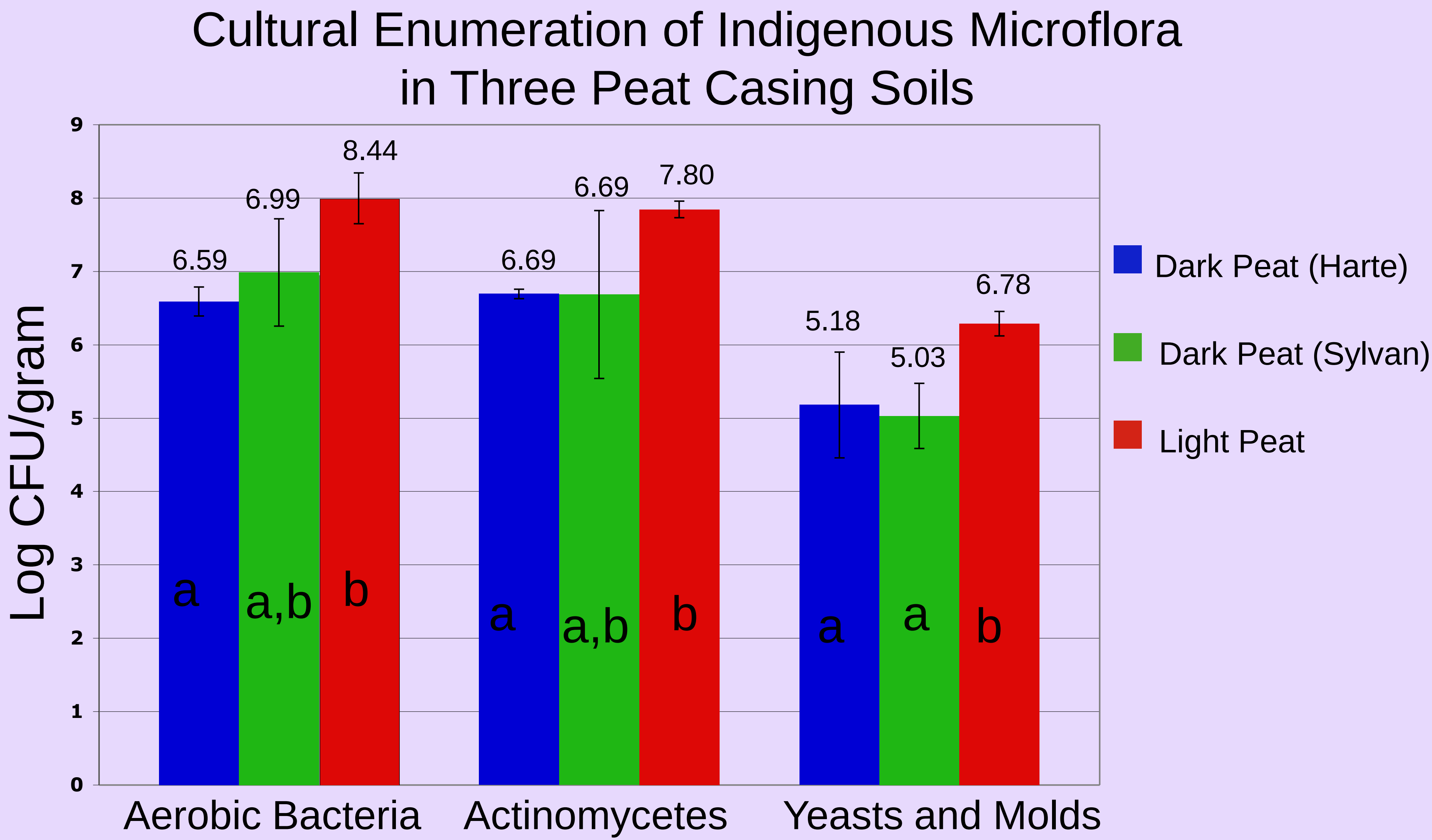
Light Peat | Sylvan Dark Peat | Harte Dark Peat

Combined with Buffered Peptone Water

Plated and enumerated

PCA (Aerobic bacteria) | AIA (Actinomycetes) | DRBC (Yeasts and molds)

Objective 1 – Results (No Agaricus)



Error bars represent the standard deviation from the mean from 3 separate trials. Columns with different letters represent a significant difference at $\alpha = 0.05$ for each category.

Objective 1 – Conclusions (No Agaricus)

- There is a significantly lower amount of aerobic bacteria, actinomycetes, and yeasts and molds in some dark peat than in the light peat.

- Will this lower level of indigenous microflora have an effect on the survival of *L. monocytogenes* and *Salmonella* introduced into the soil?

Objective 2 - Experimental Design (No Agaricus)

Dark Peat (Harte)

Dark Peat (Sylvan)

Light Peat

Inoculate with *Listeria* and *Salmonella*

Incubate at 22 ºC for 28 days

Sample and enumerate *Listeria* and *Salmonella* levels over time

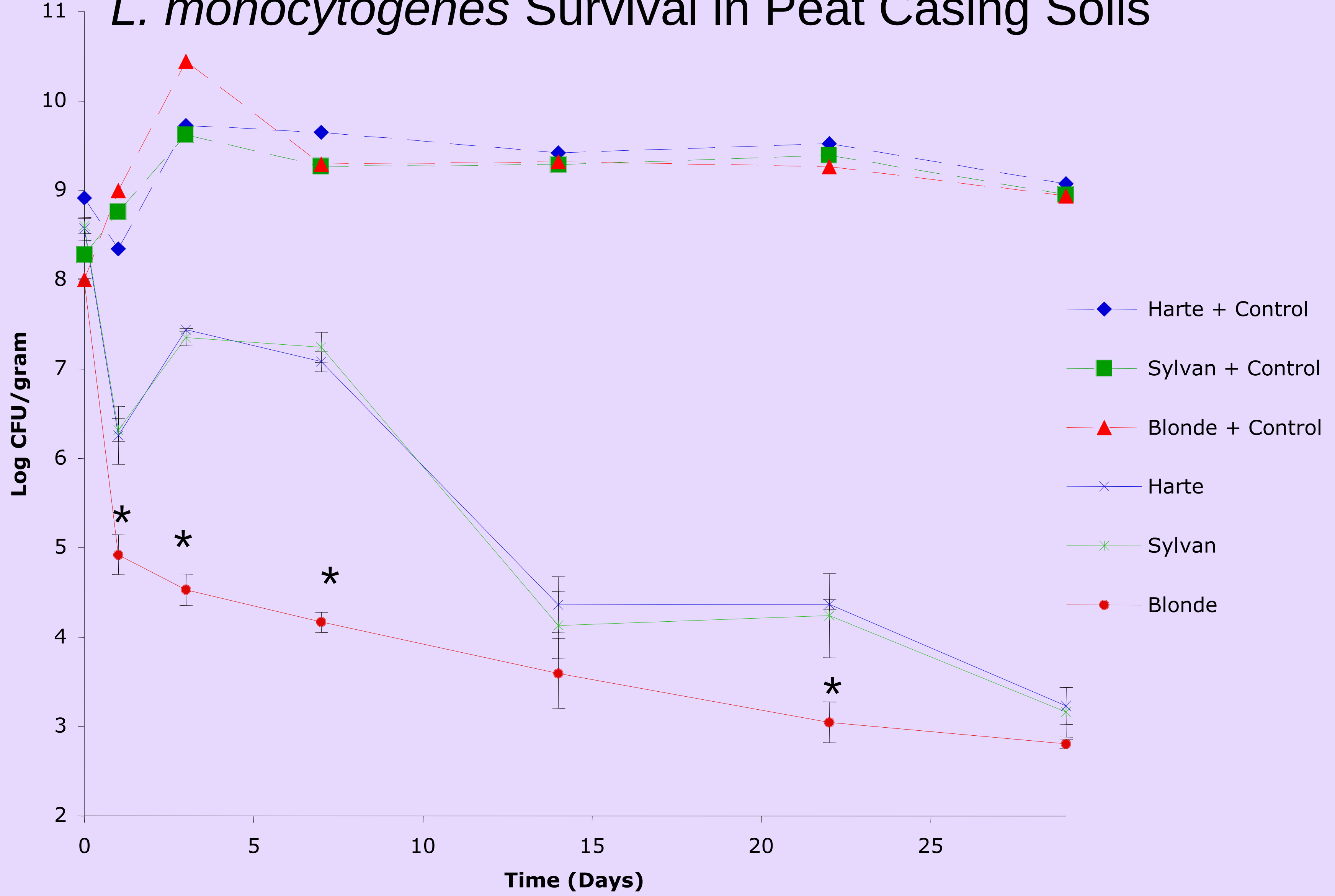
Objective 2 - Experimental Design

Challenge Study

Survival of *L. monocytogenes* and *Salmonella* spp. in light and dark peat

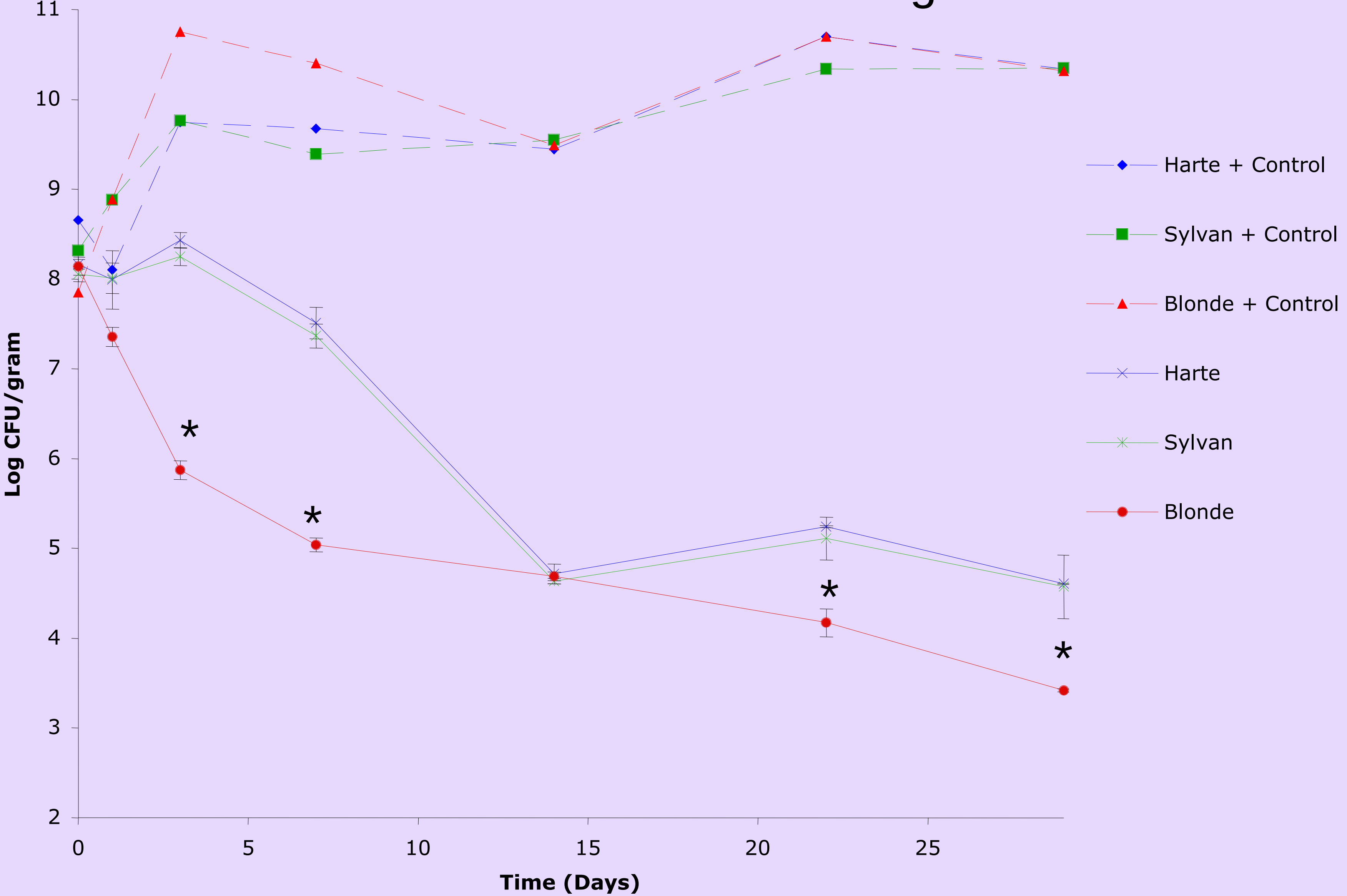
Positive Controls	All three peats autoclaved separately & inoculated with cocktail of *Salmonella* and *Listeria*
Negative Controls	All three peats inoculated with 0.75% saline
Samples	All three peats inoculated with cocktail of *Salmonella* and *Listeria*

L. monocytogenes Survival in Peat Casing Soils



Error bars represent the standard deviation from the mean of three trials. An asterisk (*) denotes a significant difference (α=0.05) between the blonde peat and both dark peats.

Salmonella Survival in Peat Casing Soils



Error bars represent the standard deviation from the mean of three trials. An asterisk (*) denotes a significant difference (α=0.05) between the blonde peat and both dark peats.

Objective 2 – Conclusion (No Agaricus)

Listeria and *Salmonella* both die off more rapidly in light peat compared to dark peat.

Challenge Study - Colonized with *Agaricus bisporus*

Objective 3 - Experimental Design (With Agaricus)

Mix casing soils in three ratios with CAC:

- 0% Dark Peat, 100% Light Peat 1.5% CAC
- 20% Dark Peat, 80% Light Peat 1.5% CAC
- 40% Dark Peat, 60% Light Peat 1.5% CAC

Inoculate with pathogen cocktail

Add casing to spawned compost in deli containers

Grow in temperature/humidity controlled chamber

Cultivate mushrooms, sampling periodically and enumerating *Salmonella* and *Listeria* over time (soil + mushrooms)

Objective 3 - Expected Results (With Agaricus)

- Higher percentages of dark peat will have increased survival of *Listeria* and *Salmonella*

- Possible transfer of pathogens to mushrooms

Objective 4 - Food Safety Interventions

Supplementation of Irrigation Water with Sanitizers

- Hydrogen Peroxide as sanitizer
- Shown to reduce spoilage microorganisms
- No decrease in mushroom yield

Chikthimmah, et. al 2006

Objectives 4 and 5 – Expected Results

- Hydrogen peroxide will result in additional decreases in levels of *Listeria* and *Salmonella*

Summary

- Dark peats appear to enhance the survival of *Listeria* and *Salmonella*

- Studies are now underway to determine:
  1. The role of *Agaricus bisporus*
  2. How much dark peat is too much?
  3. The use of sanitizers in irrigation water

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Questions?

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