Ammonium Hydroxide and Orange Juice Synergistic Effects on *Escherichia coli*

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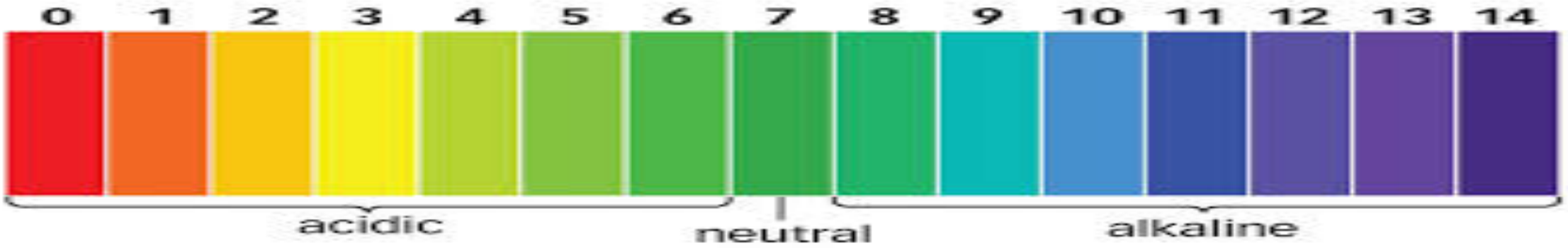
pH

pH Scale

- Measures how acidic or basic a substance is
- Scale from 0-14
- pH<7 acidic, pH>7 basic, pH=7 neutral

Neutralization

- The mixture of an acid and a base.
- Mixture of acid and base commonly yields water



Navel Orange Juice

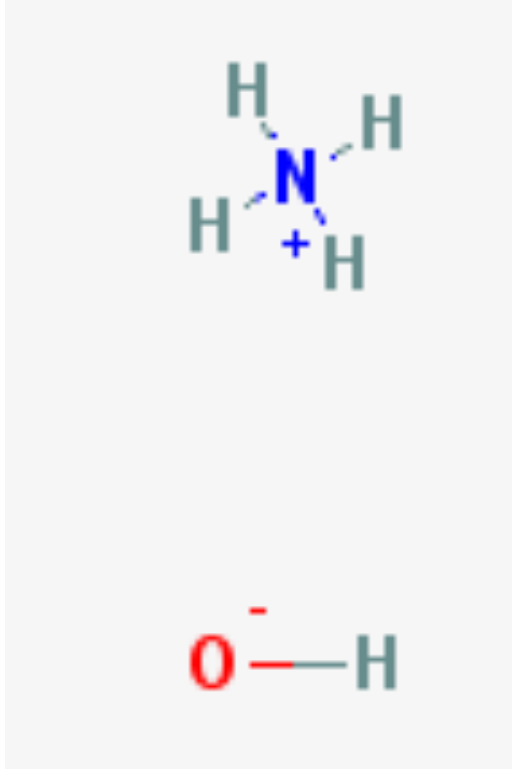
- Very commonly sold orange
- Measured pH of 3 (raw)
- Contains large amounts of Vitamin A and C
  - May help animals form connective tissue
  - Also contains many other Vitamins

Components of Orange Juice

- Sugars
- Proteins and amino acids
- Vitamins A,C,D,E,K, B12, B6
- Thiamin
- Pantotheic Acid
- Sodium
- Potassium
- Water
- Mono/Polysaturated fats
- Omega-3 fatty acids
- Calcium
- Iron
- Magnesium
- Phosphorus

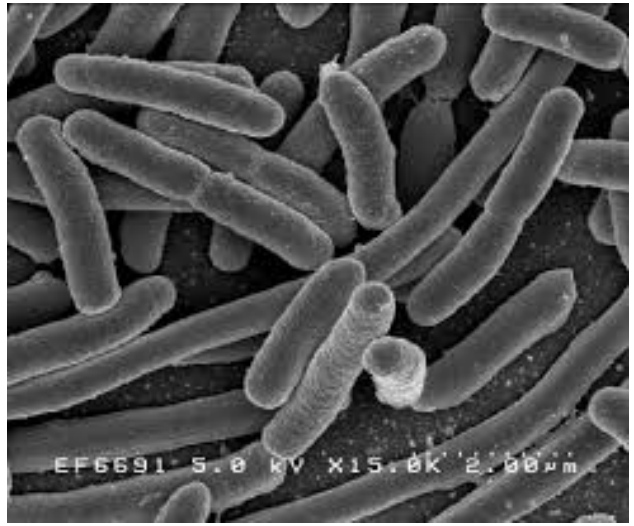
Ammonium Hydroxide

- Basic liquid
  - Measured pH of 10 (2 Molar)
- Very corrosive and antibacterial
  - Used at very small molarities in cleaners
- Releases Ammonia vapors when mixed with an acid or water.
  - Toxic to humans at high concentrations



Escherichia coli (*E. coli*)

- Large and diverse group of gram (-) bacteria
- Free living, symbionts, or pathogens
- Most strains are not pathogenic
- Serve as a common prokaryotic cell model
- Common mammalian intestinal symbiont
- Environmental prokaryotic model



Rationale

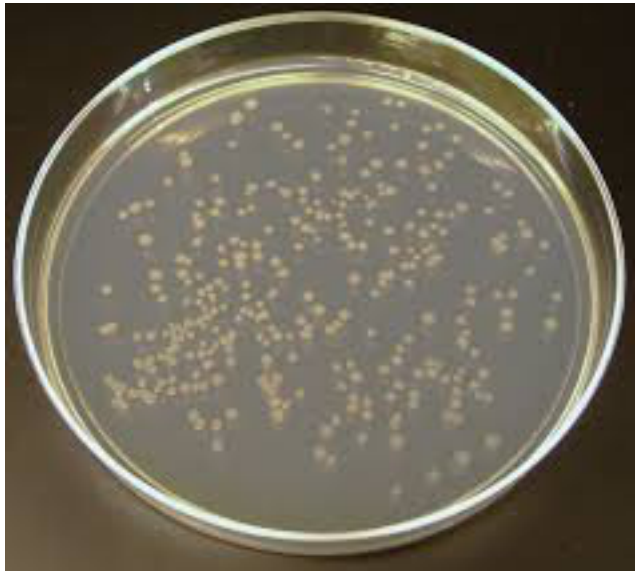
- Individual Orange Juice:
  - Orange falling from tree
  - Disposal of peels
- Individual Ammonium Hydroxide
  - Disposal of Ammonium-based cleaners
- Mixture of both:
  - Possible synergistic effects
  - Possible remedy for Ammonium-based cleaner spills

Purpose

- To determine the individual and synergistic effects of Navel orange juice and Ammonium Hydroxide on *E. Coli* survivorship.

Problem

- Will there be a significant effect when Ammonium Hydroxide, Navel orange juice, or a mixture of both is added to *Escherichia coli*?



Hypothesis

Alternative Hypothesis:

Ammonium Hydroxide and Orange juice will significantly reduce *E. coli* survivorship.

In combination, a synergistic effect will improve survivorship compared to individual exposures.

Materials

- Borosilicate sterile test tubes
- Latex Gloves
- Laboratory Goggles
- Micropipettes
- Sidearm Flask
- Sterile Filters
- Incubator (37^{o} C)
- Vortex Machine
- E. coli(DH5 Alpha)
- LB Agar Plates(LB Media) (1% Tryptone, 0.5% Yeast extract, 1% NaCl)
- Spread bars
- Ethanol
- pH strips
- Matches
- Sterile Dilution Fluid (SDF) (100mM KH_2PO_4, 100mM K_2HPO_4, 10mM $MgSO_4$, 1mM NaCl)
- Navel Orange Juice
- Juicer
- Knife
- 2 Molar Ammonium Hydroxide
- Sterile Dilution Fluid
- Bunsen Burner

Procedure

1. *E. coli* was grown until a density of 50 klett spectrophotometer density was reached. This was approximately 10^8 cells/mL
2. The culture was diluted in sterile dilution fluid to a concentration of approximately 10^5 cells/mL
3. Stocks of the variables were created;
    1. 10mL of orange juice was sterile filtered
    2. A 10^{-2} stock of Ammonium Hydroxide was made
4. The variables were added to the tubes as follows:

Experimental Exposures

Orange Juice \ Ammonium Hydroxide (10-2 stock)	Zero	Low	High
Zero	0 mL 0 mL 100 uL 9.9 mL	0 mL 10 uL 100 uL 9.89 mL	0 mL 1 mL 100 uL 8.8 mL
Low	100 uL 0 mL 100 uL 9.8 mL	100 uL 10 uL 100 uL 9.79 mL	100 uL 1 mL 100 uL 8.8 mL
High	1 mL 0 mL 100 uL 8.9 mL	1 mL 10 uL 100 uL 8.89 mL	1 mL 1 mL 100 uL 7.9 mL

Key:

Orange Juice

Ammonium Hydroxide

Escherichia coli

Sterile Dilution Fluid

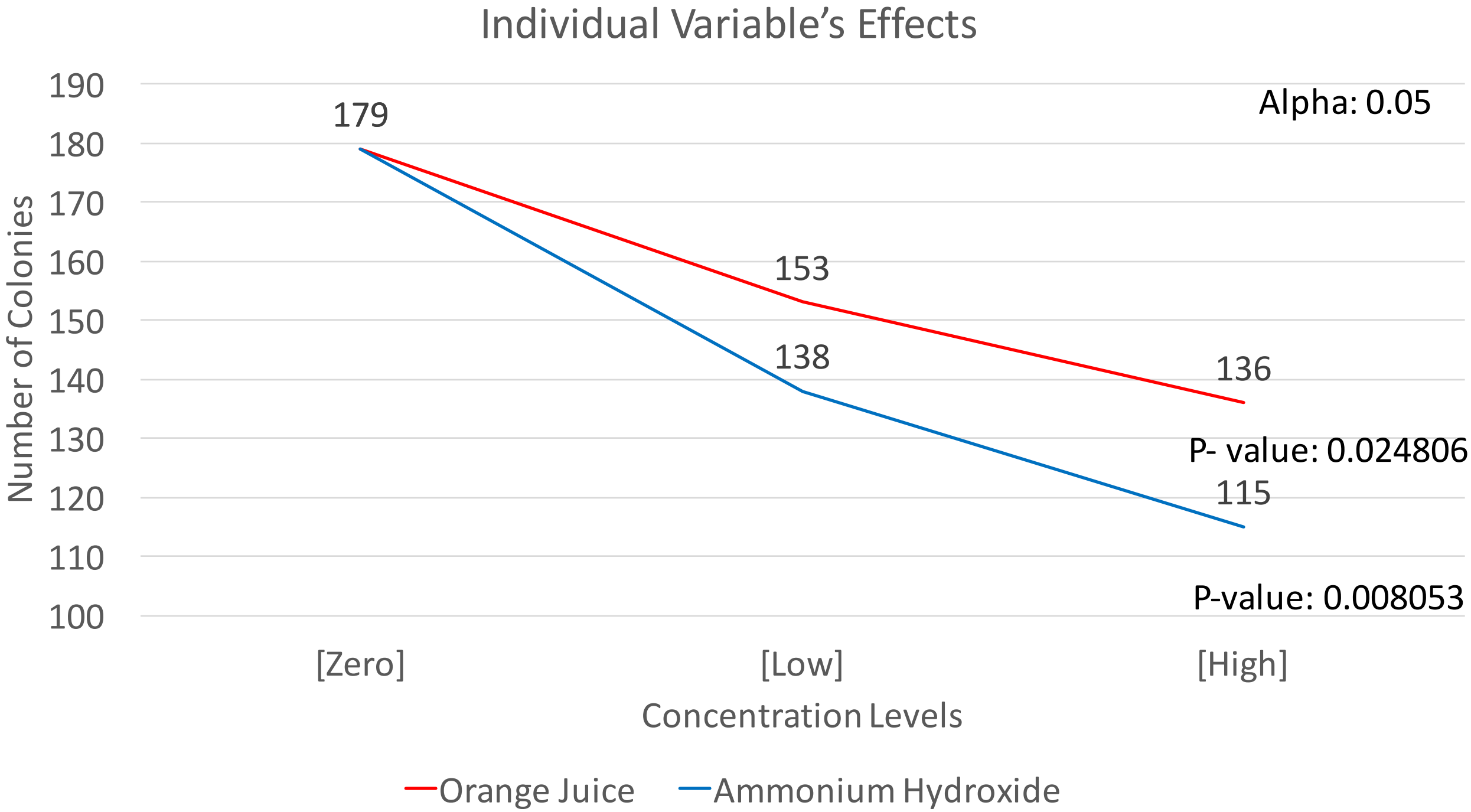
Concentration in %:

	Low	High
Ammonium Hydroxide	0.00005%	0.005%
Orange Juice	1%	10%

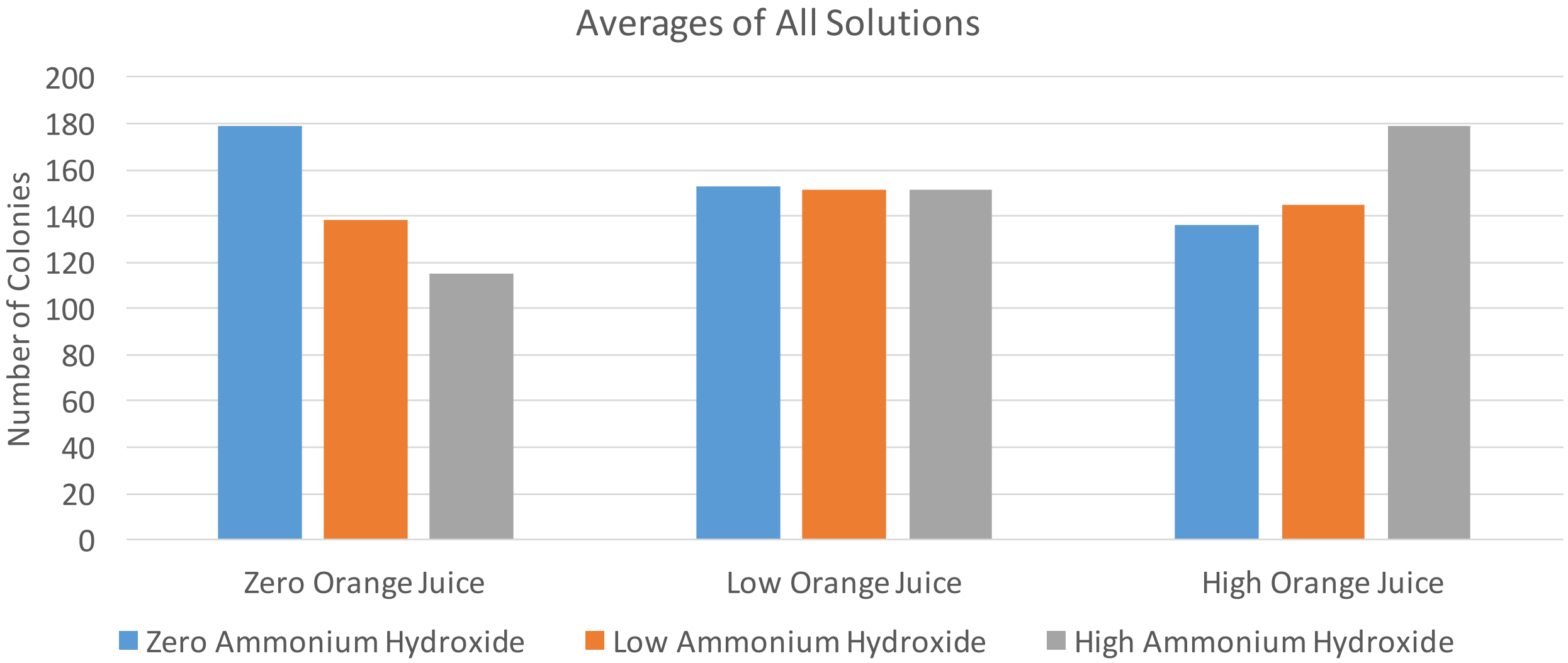
5. 0.1mL of *E. coli* stock was added to each tube, and allowed to incubate at room temperature for 15 minutes

6. 0.1mL of the aliquots were spread onto the agar plates

7. The plates were then incubated for 24 hours and the colonies were counted


Individual Variable's Effects
190
180
170
160
150
140
130
120
110
100
Number of Colonies
179
153
136
138
115
Alpha: 0.05
P- value: 0.024806
P-value: 0.008053
[Zero]
[Low]
[High]
Concentration Levels
Orange Juice
Ammonium Hydroxide

Synergistic Effects on E. Coli



Data Analysis: Two-factor ANOVA

Alpha: 0.05

- Two-Way ANOVA for both Variables:
  - Interaction P-value: Yes, the p value was at 0.000262
  - Interpretation: Reject null; there was a significant effect
  - Type of effect: The mixed variables appeared to reduce the negative effects

Further Interpretation

AVERAGES OF COLONIES

Ammonium Hydroxide

Orange Juice	Zero	Low	High
Zero	179	138	115
Low	153	151	151
High	136	145	179

pH Chart and Interpretation

pH Levels			**Ammonium Hydroxide**	
		Zero	Low	High
Orange	Zero	7	7	7
Juice	Low	7	7	7
	High	6	6	6

- pH solely doesn't seem to be the affecting factor

Key Questions

- Did the Navel Orange Juice alone have a significant effect on *E. coli*?
  - Yes, p-value was less than 0.05
- Did the Ammonium Hydroxide alone have a significant effect on *E. coli*?
  - Yes, p-value was less than 0.05
- Did the mixture of both have a significant effect on the *E. coli*?
  - Yes, p-value was less than 0.05

Overall Conclusions

- There were negative individual effects of the variables, but a positive synergistic effect.

Limitations and Extensions

Limitations:

- Only one exposure time
- Chance of contamination
- Uneven distribution of E. coli in solution
- Small exposure to light
- Narrow range of variable concentrations

Extensions:

- Vary exposures and concentrations
- More replicates
- Other cultures of bacteria
- Different types of orange juice
- Other synergistic effects

Sources

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Data Chart

		Ammonium Hydroxide		
		Zero	**Low**	**High**
Orange Juice	**Zero**	193	179	88
		127	107	118
		195	130	115
		174	217	111
		148	134	116
	Low	140	145	237
		240	163	178
		139	158	132
		169	136	144
		165	105	149
	High	119	55	177
		130	157	171
		147	167	196
		165	151	170
		147	105	128

One-Way ANOVA: Ammonium Hydroxide

SUMMARY

Group	Count	Sum	Average	Variance
C1	4	710	177.5	476.333
C2	4	550	137.5	907
C3	4	460	115	8.66667

ANOVA

Source	SS	df	MS	Ftest	P-value	F-Critical
Between	8016.67	2	4008.33	8.63865	0.008053	4.2565
Within	4176	9	464			
Total	12192.7	11				

Conclusions

Ho:	μ1=μ2=μ3=...
Ha	At least 2 μ's differ
F-Test	8.63865
F-Critical	4.2565
Alpha	0.05
P-Value	0.008053
Decision:	Reject Ho

One-Way ANOVA: Orange Juice

SUMMARY

Group	Count	Sum	Average	Variance
C6	4	710	177.5	476.333
C7	4	613	153.25	254.917
C8	4	543	135.75	188.917

ANOVA

Source	SS	df	MS	Ftest	P-value	F-Critical
Between	3516.5	2	1758.25	5.73238	0.024806	4.2565
Within	2760.5	9	306.722			
Total	6277	11				

Conclusions

Ho:	μ1=μ2=μ3=...
Ha	At least 2 μ's differ
F-Test	5.73238
F-Critical	4.2565
Alpha	0.05
P-Value	0.024806
Decision:	Reject Ho

Two Factor ANOVA With Replicationa

Summary	O	L	H	Total
O				
Count	4	4	4	12
Sum	710	550	460	1720
Average	177.5	137.5	115	143.333
Variance	476.333	907	8.66667	1108.42
L				
Count	4	4	4	12
Sum	613	602	603	1818
Average	153.25	150.5	150.75	151.5
Variance	254.917	151	380.917	216.273

H

Count	4	4	4	12
Sum	543	580	714	1837
Average	135.75	145	178.5	153.083
Variance	188.917	754.667	145.667	664.992

ANOVA

Source	SS	df	MS	Ftest	P-value	F-Critical
Samples(rows)	657.056	2	328.528	0.904735	a	3.35413
Columns	775.056	2	387.528	1.06722	0.358032	3.35413
Interaction	11307.3	4	2826.82	7.7848	0.000262	2.72777
Within	9804.25	27	363.12			
Total	22543.6	35				

Conclusions

Interaction		
Ho:	NO Interaction	
Ha	Interaction Exists	
F-Test		7.7848
F-Critical		2.72777
Alpha		0.05
P-Value		0.000262
Decision:	Reject Ho	
Samples(rows)		
Ho:	μ1=μ2=μ3=...	
Ha	At least 2 μ's differ	
F-Test		0.904735
F-Critical		3.35413
Alpha		0.05
P-Value		0.416567
Decision:	Fail to reject Ho	
Columns		
Ho:	μ1=μ2=μ3=...	
Ha	At least 2 μ's differ	
F-Test		1.06722
F-Critical		3.35413
Alpha		0.05
P-Value		0.358032