Research About the Coupling Effect of Environmental Factors on the Quality of Storage Water

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Abstract. Although rainwater collection can alleviate water shortages in seasonally water-deficient area, the water’s sanitary condition is usually very poor. By 4 factors 3 levels orthogonal experiment, this paper studied the coupling effects of temperature, nutrient, suspended solids and total phosphorus on the bacteria content of storage water, and analyzed their variance and regression. The mechanism of how environmental factors affected storage water’s quality were explored, and a binary first-order linear correlation equation of bacterial content with temperature and nutrients was established. The research results can provide reference for the safe utilization of storage water in water-deficient area.

1. Introduction
Rainwater collection can do help to solve the problem of water shortage in seasonally water-deficient areas, affected by various environmental factors, the water’s sanitary condition is usually very poor [1-7]. Clear up the relationship between environmental factors and water quality can do help to take pre-control measures to control the main influencing factors in the process of water cellars’ planning, construction, and management, which will improve storage water’s sanitary condition.

This paper carried out a 4 factors 3 levels orthogonal experiment, of which the 4 factors included temperature, nutrient, suspended solids and phosphorus, to investigate and analyze the relationship between environmental factors and bacteria content in storage water.

2. Materials and Methods

2.1. Materials
The experimental water was rainwater collected from the roof. The rainwater’s CODCR, SS and TP were 13mg/l, 10mg/l and 0.06mg/l respectively. The rainwater’s bacteria content was 4350/ml. Glucose, diatomite and potassium dihydrogen phosphate were used to regulate the water samples’ nutrient, suspended solids and total phosphorus. Experimental equipment: conical flask, spx-150b-z biochemical incubator.

2.2. Methods
The water samples’ temperatures were controlled by biochemical incubators. The water samples’ bacteria content were determined by plate counting method.

The 4 factors 3 levels orthogonal experiment consisted of 9 different treatments, which were showed in Table 1 and Table 2. We cultured the water samples in biochemical incubators for 120 days, then obtained the water samples’ bacterial content by plate counting method. We used SPSS19.0 analysis software analyzed the variance and regression of the data.
### Table 1. Factors and levels of orthogonal experiment.

| Level | Factor | Level | Factor | Level | Factor | Level | Factor |
|-------|--------|-------|--------|-------|--------|-------|--------|
|       | A      |       | B      |       | C      |       | D      |
|       | (Temperature ℃) |       | (Nutrient mg/L) |       | (Suspended Solids mg/L) |       | (Total Phosphorus mg/L) |
| 1     | 5      |       | 0      |       | 0      |       | 0      |
| 2     | 20     | 10    | 0      |       | 0      |       | 0.5    |
| 3     | 35     | 20    | 20     |       | 1      |       | 1      |

### Table 2. Design and results of orthogonal experiment.

| No. | Temperate A | Nutrient B | SS C | TP D | Bacteria (number/ml) |
|-----|-------------|------------|------|------|----------------------|
| 1   | 3           | 1          | 3    | 2    | 26328                |
| 2   | 3           | 2          | 1    | 3    | 41627                |
| 3   | 1           | 2          | 2    | 2    | 19155                |
| 4   | 2           | 3          | 1    | 2    | 39599                |
| 5   | 2           | 1          | 2    | 3    | 21399                |
| 6   | 3           | 3          | 2    | 1    | 58596                |
| 7   | 1           | 3          | 3    | 3    | 21272                |
| 8   | 2           | 2          | 3    | 1    | 23852                |
| 9   | 1           | 1          | 1    | 1    | 1783                 |
|     | T1          | 42210      | 49510| 83009| 84231                |
|     | T2          | 84850      | 84634| 99150| 85082                |
|     | T3          | 126551     | 119467| 71452| 84298                |
|     | m1          | 14070      | 16503| 27669| 28077                |
|     | m2          | 28283      | 28211| 33050| 28360                |
|     | m3          | 42183      | 39822| 23817| 28099                |
|     | R           | 28113      | 23319| 9233 | 283                  |

Note: T is the sum of 3 same level treatments corresponding to one specific factor; m= T/3; R= Max {m1, m2, m3} - Min {m1, m2, m3}.

### 3. Analysis of the Experiment Results

#### 3.1. Range analysis of the water samples’ bacterial content

The results of range analysis showed that: the range value caused by temperature factor A was $R_A=28113$, the range value caused by nutrient factor B was $R_B=23319$, the range value caused by suspended solids factor C was $R_C=9233$, the range value caused by total phosphorus factor D was $R_D=283$. It indicated that the influence of the 4 environmental factors on storage water’s bacteria content was temperature > nutrient > suspended solids > total phosphorus. Further analysis showed that: the water sample’s bacteria content increased with the increased of temperature, the bacteria content of the second and third level temperature treatments were 101.01% and 199.81% higher than that at the first level respectively; the water sample’s bacteria content increased with the increase of nutrient, the bacteria content of the second and third level nutrient treatments were 70.94% and 141.3% higher than that at the first level respectively; suspended solids had effect on the water sample’s bacteria content, the bacteria content of the second and third level suspended solids treatments were 19.4% and -13.9% higher than that at the first level respectively; total phosphorus had little effect on the water sample’s bacteria content, the bacteria content of the second and third level total phosphorus treatments were 1.0% and 0.08% higher than that at the first level respectively.
3.2. Variance analysis of the water samples’ bacterial content

Variance analysis was applied to investigate each environmental factor’s influence on the water samples’ bacterial content. Since there were no blank items or repetitive items in this orthogonal experiment, took the item, whose sum of squares of mean deviation was the smallest, as the error estimate. Ran the SPSS19.0 program, it showed that total phosphorus D’s sum of squares of mean deviation was the smallest. Took factor D as the error estimation and reanalyzed the data (Table 3), it showed that the significance of temperature A, nutrient B and suspended solids C were all less than 0.05, indicating that "A", "B" and "C" all had significant influence on bacteria content.

Table 3. Bacteria variance analysis.

| Source       | Type III Square Sum | Df  | Mean Square    | F     | Sig.  |
|--------------|---------------------|-----|----------------|-------|-------|
| Correction   | 2.130E9             | 6   | 3.551E8        | 4757.483 | 0.000 |
| Intercept    | 7.147E9             | 1   | 7.147E9        | 95758.709 | 0.000 |
| A            | 1.186E9             | 2   | 5.928E8        | 7943.261 | 0.000 |
| B            | 8.157E8             | 2   | 4.078E8        | 5464.724 | 0.000 |
| C            | 1.290E8             | 2   | 64515296.33    | 864.465 | 0.001 |
| Error        | 149260.667          | 2   | 74630.333      |       |       |
| Total        | 9.227E9             | 9   |                |       |       |
| Corrected Total | 2.130E9          | 8   |                |       |       |

Table 4. Bacteria content input/removal variables.

| Model | Input Variable | Removed Variable | Methods |
|-------|----------------|-------------------|---------|
| 1     | A              |                   | The probability of f-to-enter \( < = 0.050 \), and the probability of f-to-remover \( > = 0.100 \) |
| 2     | B              |                   | The probability of f-to-enter \( < = 0.050 \), and the probability of f-to-remover \( > = 0.100 \) |

Table 5. Summary of bacteria content model.

| Model | R       | \( R^2 \) | Adjusted \( R^2 \) | Standard Error | \( R^2 \) Change | F Change | Df1 | Df2 | Sig. | Durbin-Watson |
|-------|---------|-----------|-----------------|----------------|-----------------|-----------|-----|-----|------|--------------|
| 1     | 0.746*  | 0.556     | 0.493           | 11618.31795    | 0.556          | 8.883     | 1   | 7   | 0.021|              |
| 2     | 0.969b  | 0.939     | 0.919           | 4641.00460     | 0.383          | 37.869    | 1   | 6   | 0.001| 1.526        |

Note: *predictive variable: (constant), temperature A.
   bpredictive variable: (constant), temperature A, nutrient B.

Table 6. Analysis of variance of bacteria content regression model.

| Model | Sum of Squares | Df  | Mean Square | F     | Sig.  |
|-------|----------------|-----|-------------|-------|-------|
| 1     | Regression     | 1.186E9 | 1         | 1.186E9 | 8.783 | 0.021* |
|       | Residual       | 9.449E8 | 7         | 1.350E8 |       |       |
|       | Total          | 2.130E9 | 8         |       |       |       |
| 2     | Regression     | 2.001E9 | 2         | 1.001E9 | 46.456 | 0.000b |
|       | Residual       | 1.292E8 | 6         | 21538923.72 |       |       |
|       | Total          | 2.130E9 | 8         |       |       |       |

Note: *predictive variable: (constant), temperature A.
   bpredictive variable: (constant), temperature A, nutrient B.
Table 7. Bacteria content coefficient analysis table.

| Model | Nonnormalized Coefficient | Standard Coefficient | t | Sig. | B's 95.0% Confidence Interval |
|-------|---------------------------|----------------------|---|------|-----------------------------|
|       | Regression Coefficient    | Standard Error       | Trial Version |     | Lower Limit | Upper Limit |
| 1     | Constant                  | 9436.556             | 7415.795      | 1.272 | 0.244 | -8099.013 | 26972.124 |
|       | A                         | 937.122              | 316.211       | 2.964 | 0.021 | 1879.403 | 1684.841 |
| 2     | Constant                  | -2222.944            | 3516.381      | -0.632 | 0.552 | -10827.220 | 6381.331 |
|       | A                         | 937.122              | 126.312       | 7.419 | 0.000 | 628.048  | 1246.197 |
|       | B                         | 1165.950             | 189.468       | 6.154 | 0.001 | 702.338  | 1629.562 |

3.3. Regression analysis of the water samples’ bacterial content

Linear stepwise regression analysis was applied to investigate the relationship between environmental factors and bacterial content in water samples (Table 4, Table 5, Table 6 and Table 7).

As could be seen from Table 4 and Table 5, the first independent role to enter was temperature A, which was selected at the first stage (Model 1). Temperature A could independently explain 55.6% variation of bacteria content ($F(1,7)=8.883, p=0.021$). In terms of the adjusted determinant coefficient, Temperature A still had 49.3% explanatory power. The second independent variable being selected was nutrient B, which could explain 38.3% variation of dependent variable independently, the change of $F$ was 37.869 ($p=0.001$), which met the selected criteria. So Model 2 had two independent variables, temperature A and nutrient B, which could explain 93.9% of the variation of dependent variable and 91.9% after adjustment. According to the $F$ test result showed in Table 6, the explanatory power had statistical significance ($F(2,6)=46.456, p=0.000$). From Table 7, the following equation could be obtained:

$$S = 937.122A + 1165.95B - 2222.944$$

\(S\) is the number of bacteria per milliliter in the water sample; \(A\) is the Celsius temperature value of the water sample; \(B\) is the number of milligrams of nutrient per liter of the water sample.

Using equation (1) to predict the water sample’s bacteria content, the estimate standard error is 4641.00.

As could be seen from Table 7, temperature A had the best explanatory power and was first included in Model 1, which could independently predict dependent variables, it’s standard coefficient was 0.746 ($t=7.419, p=0.000$). The bacteria content in the water sample increased with the increase of temperature. Independent variable nutrient B was added to the coefficient estimation of Model 2, and nutrient B’s standard coefficient was 0.619 ($t=6.154, p=0.001$). The water sample’s bacteria content increased with the increase of nutrient.

The value of Durbin-Watson test was 1.526, ranging from 1.5 to 2.5, which indicated that there was no autocorrelation, so the regression model conformed to the hypothesis of multiple regression and had practical value.

4. Conclusion

The data analysis of the 4 factors and 3 levels orthogonal experiment showed that the order of influence of environmental factors on bacterial content in water sample was temperature > nutrient > suspended solids > total phosphorus. The effect of temperature, nutrient and suspended solids all reached significant level, and the effect of total phosphorus was weak.

Linear stepwise regression analysis showed that there was a bivariate first-order linear correlation between bacteria content, temperature and nutrient.

Acknowledgement

In this paper, the research is sponsored by "521" Project of Lianyungang (Stage 5).
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