

# The Growth Characteristics of Three Terrestrial Plants Cultivated with Biogas Slurry as a Hydroponic Medium

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## Abstract

Biogas slurry is a traditional high-quality organic liquid fertilizer. Three terrestrial plants with high economic value, fu gui cai, yang xin cai and strawberry, were selected for hydroponic cultivation using an optimal dilution of biogas slurry. The results of the experiments showed that strawberries could bloom and bear fruit and fu gui cai and yang xin cai grew well. However, in comparison with the control groups planted in soil, all three crops were subject to a certain degree of environmental stress, as shown by changes in growth, biomass indicators, physiological indicators and morphological indicators. The leaves and stems of yang cai and fu gui cai were tested for several types of heavy metals, and all met the requirements for human consumption. It is feasible to cultivate these three plants with biogas slurry. The results of this study can help guide hydroponic production practices and have practical significance and reference value.

## Keywords

Biogas Slurry, Fu Gui Cai, Yang Xin Cai, Strawberry, N/P/K, Vitamin C, Chlorophyll, Heavy Metals

## 1. Introduction

Biogas slurry is the residue from the anaerobic fermentation of livestock and poultry manure. It is rich in trace elements (e.g., iron, zinc, boron, etc.), many auxins (e.g., gibberellin, indole acetic acid, etc.), B vitamins and certain antibiotics [1] [2] [3]. It also contains abundant minerals [4] and is a good source of plant nutrition [5] [6] [7]. At present, the primary uses of biogas slurry in China include direct application to agricultural fields, as a soaking medium for seeds,

as a foliar fertilizer, as a feed additive, etc. Among these uses, direct application in the field is the primary use of biogas slurry [8]. With the rapid development of large-scale farms, the amount of biogas slurry produced has increased dramatically in many Asian countries, including China [7] [9]. In China alone, approximately 2 million tons of biogas slurry is produced annually [10]. Large-scale farms often do not have sufficient land to use all of the biogas slurry produced. Excessive application of biogas slurry as a fertilizer would not only change soil properties but also cause secondary pollution [4] [10] [11]. Traditional wastewater treatment methods are not suitable for the treatment of biogas slurry because of their high cost; in addition, this wastes the nutrients contained in biogas slurry [12]. Identifying a new green resource utilization method for the considerable amount of biogas slurry produced in China is important for the national economy and environmental protection.

Hydroponics is a new vegetable cultivation technology that is environmentally friendly. This technology can effectively address the constraints on the production of vegetables and other crops due to the shortage of soil resources and appropriate cultivation conditions. Therefore, studying the physiological response of economic crops to the biogas slurry hydroponic environment is important. Such studies can expand more ways to use biogas slurry, and solve the key problem that restricts the development of livestock and poultry breeding enterprises.

Strawberry (*Fragaria ananassa*) is a popular fruit that has been called the queen of fruits and is rich in vitamin C. However, due to the lack of advanced support technologies [13], the soilless cultivation of strawberries is still primarily based on substrate cultivation. The current research status on hydroponic strawberry production is as follows. Changjian Shi *et al.* [14] investigated a special fertilizer for hydroponic strawberries and identified three effective formulas. The authors found that hydroponic strawberries in a nutrient solution grew better than those cultivated in soil. Liang Danna, Wang Xinli *et al.* [15] studied the effects of different hydroponic methods on strawberry growth and development. Liang Danna [16] also studied the effects of hydroponics on the growth of different strawberry varieties and the effects of different hydroponic methods on the quantity and quality of strawberries [17]. Xueqing Jia *et al.* [18] studied the effects of potassium fertilizer on strawberry seedling growth and photosynthesis under Deep Flow Technique hydroponic conditions (DFT). Zhao Peng *et al.* [19] studied the hydroponic cultivation technology of strawberries in greenhouses in northern China. Dianyuan Huang and Kelin Yin [20] studied the effect of light intensity on fruit quality in hydroponic strawberries. The research literature on strawberries shows that this crop can be grown in a hydroponic environment. However, there are no reports on the use of liquid biogas as a hydroponic nutrient source for strawberry.

Fu gui cai (*Gynura divaricata*) is a perennial root herb belonging to the genus notoginseng in compositae [21]. This beneficial herb is a wild vegetable that can be used as both medicine and food [22]. Lanfen Huang *et al.* [23] studied the hydroponic cultivation of fu gui cai and obtained good results. Yi

Yang [24] added different concentrations of nitrogen fertilizer to the hydroponic solution and measured the yield, quality, and nitrate content of fu gui cai. In terms of yield and quality, the most suitable nitrogen fertilizer concentration was 12 mmol/L, and the ratio of ammonia nitrogen to nitrate nitrogen was 3 to 1. The research literature on fu gui cai shows that this herb can be grown in a hydroponic environment, but there are no reports on the use of biogas slurry as a hydroponic nutrient solution for this herb.

Yang xin cai (*Sedum aizoon*) is a perennial succulent herb [25]. Owing to its many functions as an ornamental, medicinal and edible plant, yang xin cai has high practical value. According to a survey by Lianfen Zhou [26] on the cultivation of yang xin cai, the current national planting area of yang xin cai is no more than 67,700 hm<sup>2</sup>, and the total output is less than 1 million tons.

It is estimated that the annual demand for yang xin cai in the national market is approximately 5 million tons. Because output is far below the market demand, the market prospect of growing yang xin cai is good. At present, the primary cultivation method is soil cultivation. There are no reports about using biogas liquid as a hydroponic nutrient solution to cultivate yang xin cai.

This study selected these three plants with high economic value for hydroponic experiments using diluted biogas as a nutrient solution. The aim of the experiment was to study the physiological and growth responses of the three plants to the dilute biogas slurry hydroponic environment. This paper fills a research gap regarding the use of biogas slurry in the hydroponic cultivation of these three plants. This study has reference significance for further research on new directions in biogas slurry utilization and the realization of facility-based agricultural production based on biogas slurry.

## 2. Experimental Materials and Methods

### 2.1. Experimental Materials

The biogas slurry was obtained from a biogas project at a swine farm in Jiangxia District, Wuhan. The strawberry seedlings were obtained from a strawberry farm in Hubei, whose variety was Chunxu. Fu gui cai and yang xin cai seedlings were obtained from a farm at the Hubei Agricultural Sciences Research Institute. The container used for hydroponics was a square glass jar with a maximum volume of approximately 10 liters. The floating bed material consisted of a foam floating plate with 4 equidistant holes.

### 2.2. Experimental Methods

The hydroponic experiment was carried out in a greenhouse at Hubei University in October, 2017. This greenhouse had plastic film shed. No other method for controlling the temperature was used during the experiment in winter.

#### 1) Determination of the optimal dilution of biogas slurry

Cress was grown in different dilutions of biogas slurry (5-, 10-, 15-, 20-, 25-, and 30-fold), and the growth of the cress was measured over the following 20

days. The optimal dilution of the biogas slurry was determined from the growth observations. The optimal dilution factor was used to perform the following hydroponic experiments.

#### 2) Experimental hydroponic production of strawberry using biogas slurry

Four strawberry plants were placed in each of two pots of organic soil as a control group ( $CK_{cm}$ ). Eight liters of 20-fold-diluted biogas slurry were placed into each of two square glass tanks. Perforated foam plates were used as floating beds. Each floating bed was planted with 4 strawberry seedlings ( $T_{cm}$ ). The biogas was changed every 30 days. The strawberry growth in each group was observed and recorded. The fruits were harvested on the 135th day.

#### 3) Experimental hydroponic production of fu gui cai and yang xin cai

Four fu gui cai seedlings were planted in each of two pots of organic soil as controls ( $CK_{FGC}$ ). Eight liters of 20-fold-diluted biogas slurry was placed into each of two square glass tanks. Perforated foam plates were used as floating beds. Each floating bed was planted with 4 fu gui cai seedlings ( $T_{FGC}$ ). The cultivation time was 176 days, and the diluted biogas slurry was replaced on the 14th, 40th, 80th, 120th, and 160th days of the experiment. After the 176<sup>th</sup> day, plant height and the nitrogen, phosphorus, potassium, chlorophyll, vitamin C, and moisture contents were measured. Plant height was measured with a ruler with accuracy to a millimeter. The methods of nitrogen, phosphorus, potassium, chlorophyll, vitamin C, and moisture contents come from “Soil testing and plant analysis” [27]. The levels of seven heavy metals in the roots, stems, and leaves were measured. Copper, zirconium, lead, cadmium, and chromium were determined by atomic absorption spectrophotometer (copper, zirconium by Flame method with model 4510; lead, cadmium, and chromium by graphite furnace method with model 4510F); Arsenic and mercury were determined by atomic fluorescence spectrometer (model: PF6-2).

The experimental methods and time for yang xin cai cultivation were all as same as those for fu gui cai.

### 2.3. Data Processing Methods

GraphPad Prism 6.0 software was used for calculations and analysis of variance. A value of  $P < 0.05$  was considered to be statistically significant.

## 3. Experimental Results and Analysis

### 3.1. Strawberry Hydroponic Experiment

At the beginning of the experiment, the total weight of the four strawberry seedlings was approximately 350 g. The height change, flowering time, fruiting time, time to maturity, and number and weight of the fruit in each group were recorded; the data and calculation results are shown in **Table 1**.

As seen in **Table 1**, there were significant differences between  $T_{cm}$  and  $CK_{cm}$  in the average plant height, flowering time, fruit bearing and ripening time, fruit number and weight. The flowering time of  $T_{cm}$  was 4 days earlier than that of

**Table 1.** Comparison data from the strawberry experiment.

Average value	Height change rate (mm/day)	Flowering time (days)	Fruit bearing time (days)	Maturity time (days)	Number of fruits	Weight of the fruit (g)
CK <sub>cm</sub>	1.51 ± 0.04a	90.5 ± 0.71a	110.5 ± 0.71a	135.5 ± 0.71a	9 ± 0a	42.88 ± 3.39a
T <sub>cm</sub>	1.11 ± 0.01b	86.5 ± 0.71b	103.5 ± 0.52b	130.5 ± 0.71b	7 ± 1.41b	24.26 ± 1.85b

Note: Letter a and b mean that there is statistically significant difference between the data of CK<sub>cm</sub> and data of T<sub>cm</sub>.

CK<sub>cm</sub>, the fruiting time of T<sub>cm</sub> was 7 days earlier than that of CK<sub>cm</sub> and the ripening time of T<sub>cm</sub> was 4 days earlier than that of CK<sub>cm</sub>. There were decreases in the average growth rate (0.42 mm/d), the average fruit weight (22.33 g) and the average number of fruits (2) for T<sub>cm</sub> compared with CK<sub>cm</sub>.

The early flowering, fruiting and ripening may be related to the presence of auxins in the biogas slurry, which can promote strawberry flowering and fruiting. It is also possible that the plants blossomed and seeded early in response to environmental stress, accelerating plant growth and reproduction to produce offspring [28]. The decrease in fruit number and weight may be related to lower levels of iron, calcium, and other nutrients in the diluted biogas liquid compared with the soil. Lower levels of nutrients can decrease the chlorophyll content of the leaves and affect photosynthesis in the plant. If the nutrients lacking in the biogas liquid could be supplemented at an appropriate time, particularly the nitrogen, phosphorus and potassium needed for strawberry growth and fruit bearing, the production of strawberry would increase.

### 3.2. Hydroponic Experiments with Fu Gui Cai and Yang Xin Cai

#### 1) Plant height

For yang xin cai, both CK<sub>YXC</sub> and T<sub>YXC</sub> plants grew well. The average plant heights of CK<sub>YXC</sub> and T<sub>YXC</sub> were 13.5 cm, and 12.4 cm, respectively. The average height of CK<sub>YXC</sub> was significantly greater than that of T<sub>YXC</sub>. The leaf area of CK<sub>YXC</sub> looked larger than that of T<sub>YXC</sub>.

For fu gui cai, both CK<sub>FGC</sub> and T<sub>FGC</sub> plants grew well. The average plant heights of CK<sub>FGC</sub> and T<sub>FGC</sub> were 14.0 cm and 12.1 cm, respectively. The average height of CK<sub>FGC</sub> was significantly greater than that of T<sub>FGC</sub>. The leaf area of CK<sub>FGC</sub> looked larger than that of T<sub>FGC</sub>.

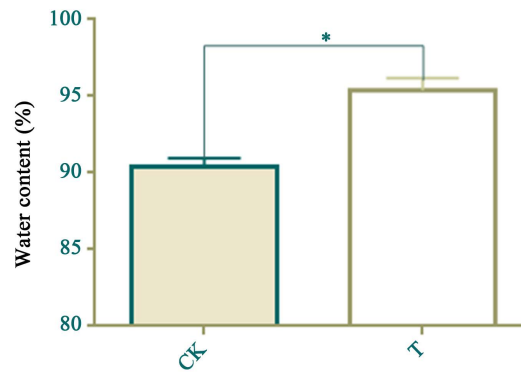
#### 2) Leaf moisture content

The moisture content of the leaves of each group of yang xin cai and fu gui cai was measured and analyzed by analysis of variance. The results are shown in **Figure 1** and **Figure 2**.

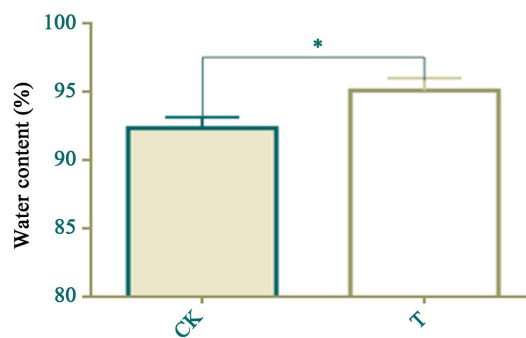
As shown in **Figure 1** and **Figure 2**, the leaf moisture content of CK<sub>FGC</sub> was significantly higher than that of T<sub>FGC</sub>. The leaf moisture content of CK<sub>YXC</sub> was significantly greater than that of T<sub>YXC</sub>.

#### 3) Nitrogen, phosphorus and potassium contents in leaves

The nitrogen, phosphorus and potassium contents in the leaves of each group



**Figure 1.** Water content in the leaves of fu gui cai. \* $P < 0.05$  for water content in the leaves of fu gui cai cultivated with slurry.



**Figure 2.** Water content in the leaves of yang xin cai. \* $P < 0.05$  for water content in the leaves of yang xin cai cultivated with slurry.

were determined and analyzed by analysis of variance, and the results are shown in **Figures 3-8**.

**Figures 3-8** show that the leaf nitrogen, phosphorus, and potassium contents of  $T_{FGC}$  were significantly lower than those of  $CK_{FGC}$ . The leaf phosphorus and potassium contents of  $T_{YXC}$  were significantly lower than those of  $CK_{YXC}$ ; the difference in leaf nitrogen content between  $T_{YXC}$  and  $CK_{YXC}$  was not significant.

#### 4) Chlorophyll content

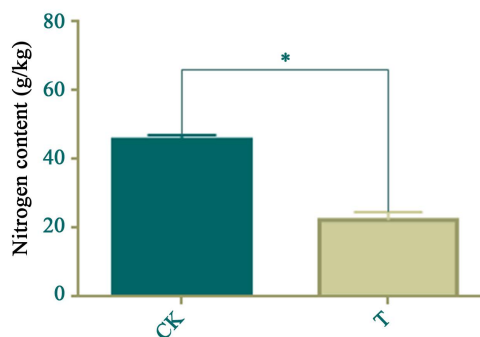
The chlorophyll content in the leaves and stems of each group was determined and analyzed by analysis of variance. The results are shown in **Figures 9-12**.

As seen in **Figures 9-12**, the chlorophyll content in the leaves of  $T_{FGC}$  was lower than that of  $CK_{FGC}$ , and there was no significant difference between the stem chlorophyll content of  $T_{FGC}$  and that of  $CK_{FGC}$ . The chlorophyll content in the leaves of  $T_{YXC}$  was lower than that of  $CK_{YXC}$ , and there was no significant difference between the stem chlorophyll content of  $T_{YXC}$  and that of  $CK_{YXC}$ .

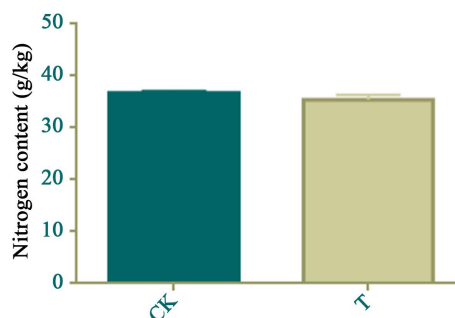
#### 5) Vitamin C content

The vitamin C contents in the leaves and stems of each group were determined and analyzed by analysis of variance. The results are shown in **Figures 13-16**.

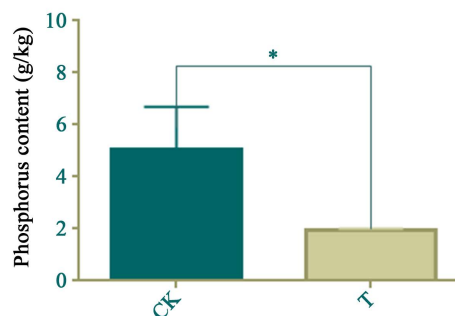
As shown in **Figures 13-16**, the vitamin C contents of the leaves and stems of  $T_{FGC}$  and  $T_{YXC}$  were significantly lower than those of  $CK_{FGC}$  and  $CK_{YXC}$ .



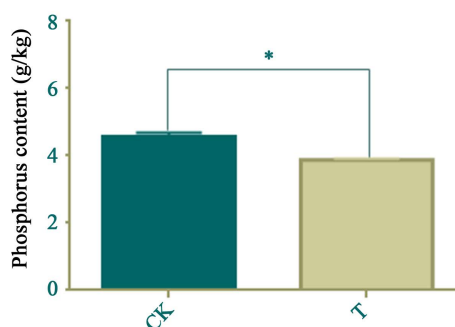
**Figure 3.** Nitrogen content in the leaves of fu gui cai. \* $P < 0.05$  for nitrogen content in the leaves of hydroponically grown fu gui cai.



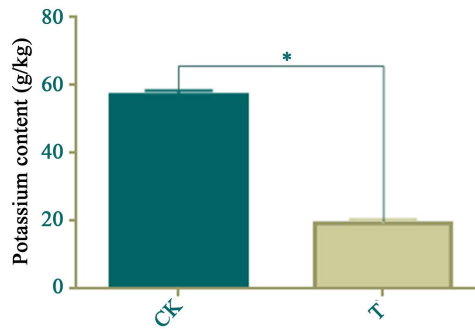
**Figure 4.** Nitrogen content in the leaves of yang xin cai. \* $P < 0.05$  for nitrogen content in the leaves of hydroponically grown yang xin cai.



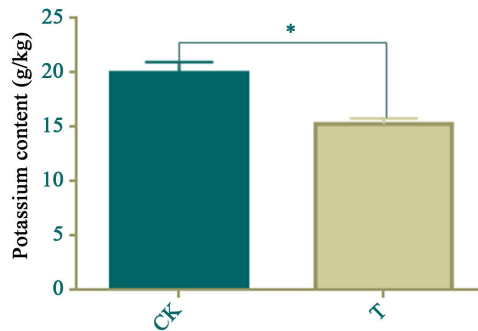
**Figure 5.** Phosphorus content in the leaves of fu gui cai. \* $P < 0.05$  for phosphorus content in the leaves of hydroponically grown fu gui cai.



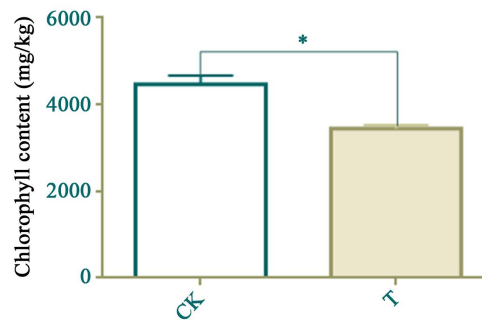
**Figure 6.** Phosphorus content in the leaves of yang xin cai. \* $P < 0.05$  for phosphorus content in the leaves of hydroponically grown yang xin cai.



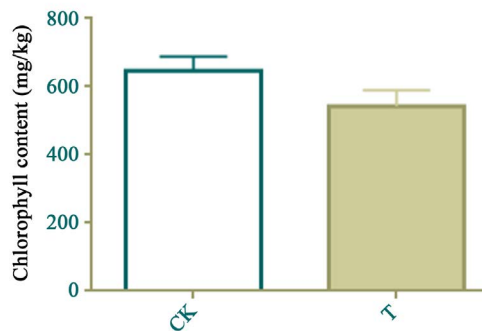
**Figure 7.** Potassium content in the leaves of fu gui cai. \* $P < 0.05$  for potassium content in the leaves of hydroponically grown fu gui cai.



**Figure 8.** Potassium content in the leaves of yang xin cai. \* $P < 0.05$  for potassium content in the leaves of hydroponically grown yang xin cai.

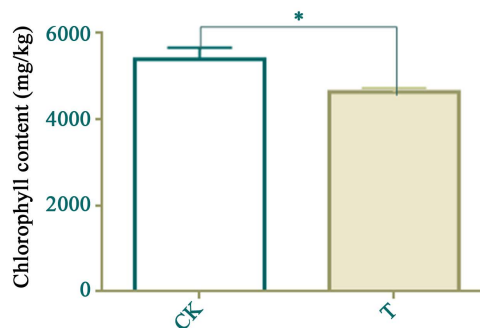


**Figure 9.** Chlorophyll content in the leaves of fu gui cai. \* $P < 0.05$  for chlorophyll content of the leaves of hydroponically grown fu gui cai.

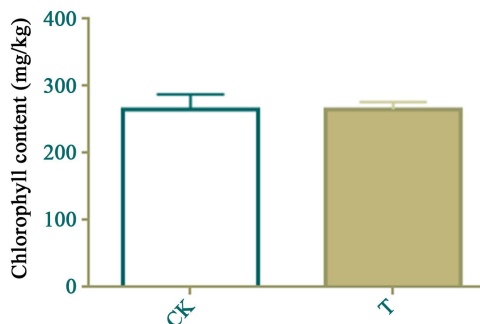


**Figure 10.** Chlorophyll content in the stems of fu gui cai.

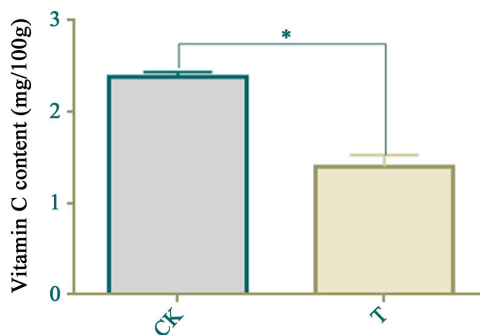




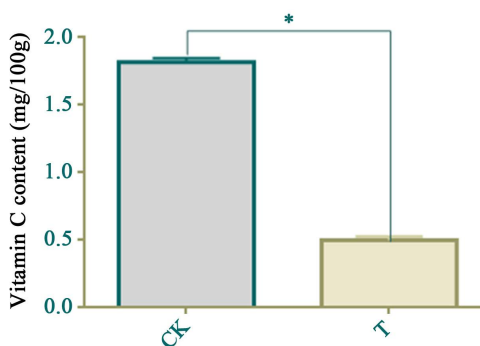
**Figure 11.** Chlorophyll content in the leaves of yang xin cai. \* $P < 0.05$  for chlorophyll content of the leaves of hydroponically grown yang xin cai.



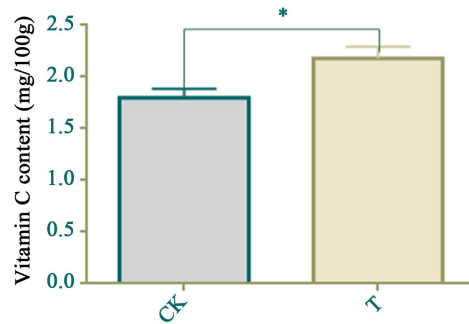
**Figure 12.** Chlorophyll content the in stems of yang xin cai.



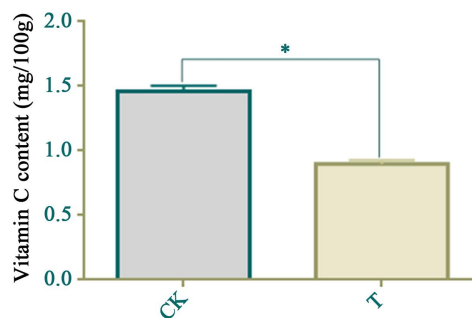
**Figure 13.** Vitamin C content in the leaves of fu gui cai. \* $P < 0.05$  for vitamin C content in the leaves of hydroponically grown fu gui cai.



**Figure 14.** Vitamin C content in the stems of fu gui cai. \* $P < 0.05$  for vitamin C content in the stems of hydroponically grown fu gui cai.



**Figure 15.** Vitamin C content in the leaves of yang xin cai. \* $P < 0.05$  for vitamin C content in the leaves of hydroponically grown yang xin cai.



**Figure 16.** Vitamin C content in the stems of yang xin cai. \* $P < 0.05$  for vitamin C content in the stems of hydroponically grown yang xin cai.

#### 6) Analysis of seven heavy metals

The levels of seven heavy metals in the roots, stems and leaves of  $T_{FGC}$ ,  $CK_{FGC}$ ,  $T_{YXC}$  and  $CK_{YXC}$  are shown in **Table 2**.

**Table 2** shows that the copper and zinc contents in the leaves, stems and roots of TFGC were higher than those of CKFGC and the copper and zinc contents in the leaves, stems and roots of TYXC were higher than those of CKYXC. Lead was not detected in any group. Arsenic was detected only in the roots of TFGC. Mercury and chromium were detected in the roots, stems and leaves of CKFGC and TFGC. Chromium, cadmium and arsenic were detected in the roots, stems and leaves of TYXC and CKYXC.

The chromium, cadmium, arsenic and mercury detected in both yang xin cai and fu gui cai were below the food pollutant limit (GB2762-2012). The hydroponic production of these two vegetables using biogas slurry is acceptable based on the levels of toxic heavy metals.

## 4. Conclusions and Prospects

### 4.1. Conclusions

This is the first use of diluted biogas slurry as a hydroponic nutrient solution for strawberries, fu gui cai and yang xin cai. Compared with the controls, some changes were found in the biomass, physiology and morphology of the three plants.

**Table 2.** Heavy metal contents in the leaves, stems, and roots of fu gui cai and yang xin cai.

Plant Name	Group	Tissue	Heavy metal content (mg/kg)					
			Cu	Zn	Cr	Cd	As	Hg
Fu gui cai	CK <sub>FGC</sub>	Leaves	1.22	0.70	0.29	0.035	/	0.0074
		Stems	1.11	0.82	0.20	0.034	/	0.0066
		Roots	1.4	1.2	0.32	0.033	/	0.0081
	T <sub>FGC</sub>	Leaves	1.51	2.7	0.2	0.009	/	0.0046
		Stems	1.42	4.1	0.24	0.012	/	0.0038
		Roots	1.99	1.309	0.49	0.011	0.029	0.0049
Yang xin cai	CK <sub>YXC</sub>	Leaves	1.19	3.21	0.24	0.022	0.044	0.0029
		Stems	1.18	1.01	0.30	0.013	0.029	0.0025
		Roots	1.20	0.54	0.49	0.010	0.126	0.0039
	T <sub>YXC</sub>	Leaves	1.53	4.80	0.23	0.032	0.069	0.0028
		Stems	1.28	1.38	0.33	0.008	0.072	0.0029
		Roots	1.38	0.68	0.40	0.012	0.078	0.0030

Note: “/” means that content of heavy metal cannot be detected.

1) Strawberries cultivated with diluted biogas liquid in hydroponic production were able to grow, blossom and bear fruit, and their flowering, fruiting and fruit ripening times were significantly earlier than those of CK. However, the average plant height, fruit number and total fruit weight were significantly lower than those of CK.

2) The plant height and nitrogen, phosphorus, potassium, chlorophyll, and vitamin C contents in the leaves of T<sub>FGC</sub> and T<sub>YXC</sub> were significantly lower than those of CK<sub>FGC</sub> and CK<sub>YXC</sub>. The moisture content of the leaves of T<sub>FGC</sub> was significantly higher than that of CK<sub>FGC</sub>, but the moisture content of the leaves of T<sub>YXC</sub> was not significantly different from that of CK<sub>FGC</sub>.

3) In the stems, the vitamin C contents of T<sub>FGC</sub> and T<sub>YXC</sub> were significantly lower than those of CK<sub>FGC</sub> and CK<sub>YXC</sub>. The chlorophyll contents of the stems of T<sub>FGC</sub> and T<sub>YXC</sub> were not significantly different from those of CK<sub>FGC</sub> and CK<sub>YXC</sub>.

4) The copper and zinc contents in the leaves, stems and roots of T<sub>FGC</sub> and T<sub>YXC</sub> were higher than those of CK<sub>FGC</sub> and CK<sub>YXC</sub>. Lead was not detected in any groups. The chromium, cadmium, arsenic and mercury contents detected in both yang xin cai and fu gui cai were all below the food pollutant limits (GB2762-2012). The two hydroponic vegetables cultivated with biogas slurry qualified for human consumption based on their toxic heavy metal contents.

## 4.2. Outlook

It is feasible to use diluted biogas slurry for hydroponic cultivation of strawberry, Fugui cai and Yang xin cai.

The next step would be to evaluate whether the environmental stress on the three hydroponic plants can be reduced by adding the necessary deficient trace elements to the biogas slurry at different growth stages and by increasing dissolved oxygen content of biogas slurry to improve the yield and quality of the three plants. Using diluted biogas slurry as a hydroponic nutrient solution has good prospects.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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