Bioethanol Production from Sugarcane Grown in Heavy Metal-Contaminated Soils

Jun Xie,[a,b,c,*] Qiang Weng,[a,b,c] Guangying Ye,[a,b,c] Sishi Luo,[a,b,c] Rui Zhu,[a,b,c] Aiping Zhang,[a,b,c] Xiaoyang Chen,[a,b,c] and Chuxia Lin [d,*]

Field and laboratory experiments were conducted to evaluate the feasibility of bioethanol production using the juice of sugarcane grown in heavy metal-contaminated soils. The results suggest that the sugar concentration was not adversely affected when the sugarcane was grown in the heavy metal-contaminated soil. Although the juice of sugarcane grown in contaminated soil contained elevated levels of heavy metals, sugar fermentation and ethanol production were not adversely affected when five selected yeast species were used to mediate the processes. The preliminary research findings obtained from this study have implications for developing cost-effective technologies for simultaneous bioethanol production and soil clean-up using heavy metal-contaminated soils for energy sugarcane farming.

Keywords: Sugarcane; Ethanol; Fermentation; Yeast; Contamination; Heavy metals

Contact information: a: Institute of New Energy and New Materials, South China Agricultural University, Guangzhou 510642 P.R. China; b: Key Laboratory of Energy Plant Resources and Utilization under Ministry of Agriculture, South China Agricultural University, Guangzhou 510642 P.R. China; c: Key Laboratory of Guangdong Regular Higher Education Institutions, South China Agricultural University, Guangzhou 510642 P.R. China; d: School of Environment and Life Science, University of Salford, Salford, Greater Manchester, M5 4WT, UK;
** Corresponding authors: C.Lin@salford.ac.uk; xiejun@scau.edu.cn*

INTRODUCTION

The importance of biofuel has been increasingly recognized due to the ever-increasing price and negative environmental and human impacts of fossil fuels (Demirbas 2008; Amin 2009; Ibeto *et al*. 2011). Biofuel has the advantages of being renewable and arguably more eco-friendly than fossil fuels (Goldemberg 2007). Bioethanol is the principal biofuel that has been commercially used as a petrol substitute for road transport vehicles (Anderson 2012). Many countries have implemented or are implementing programs for the addition of ethanol to petrol/gasoline (Sánchez and Cardona 2008).

Bioethanol can be produced from biomass by hydrolysis and sugar fermentation processes (Jamai *et al*. 2007; Bai *et al*. 2008). Candidate crops for ethanol production include sugarcane, sugar beet, corn, cassava, wheat, barley, and rice. Currently, the US and Brazil are the two major bioethanol-producing countries in the world. Corn and sugarcane are used as major feedstocks for bioethanol production by the former and the latter, respectively (Motta and Ferreira 1988; Luchansky and Monks 2009). Sugarcane-based ethanol production is relatively simpler, as compared to the corn-based process, because it does not require an additional treatment to convert starch to sugar prior to fermentation. Sugarcane ethanol has an energy balance seven times greater than ethanol produced from corn (International Energy Agency (IEA) 2004).

Sugarcane is a C4 plant that has a high photosynthetic efficiency (Bull 1969). Warm and humid climate conditions favor the growth of sugarcane (Verheye 2010). Therefore, there is potential to use tropical and subtropical areas to produce energy sugarcane crops to meet the increasing demand for bioethanol. However, most of the humid tropical and subtropical areas are heavily populated and there is a strong demand for food from limited available arable lands. Development of energy crop production at the cost of reducing food production in such areas is unlikely to be politically and socially feasible, economically viable, and culturally acceptable. Therefore, energy crop farming has to be confined to marginal lands such as heavy metal-contaminated lands that are not suitable for food crop production due to elevated levels of heavy metals in the edible portion of the crops (Ustyak and Petrikova 1996; Wang *et al*. 2001; Lin *et al*. 2005). While there is no human health concern with sugarcane farming for bioethanol production, it remains unclear whether the elevated levels of heavy metals in the juice of sugarcane could affect fermentation processes. It has been noted that the presence of heavy metals might or might not affect the activities of some fermenting yeasts (Perego and Howell 1997; Pearce and Sherman 1999; Azenha *et al.* 2000). However, direct tests for fermentation of heavy metal-contaminated sugarcane juices are lacking. In this communication, we present experimental results to address this issue.

EXPERIMENTAL

Sugarcane Plants and Juice Preparation

A total of 16 sugarcane cultivars (Table 1) were grown in soils contaminated by heavy metal-laden acidic mine water derived from the Guangdong Dabaoshan Mine (Lin *et al*. 2005; Chen *et al.* 2010). The soil was amended by adding acid neutralizing agent (red mud) to raise soil pH, which aimed to simultaneously improve the soil conditions and reduce the pollution of groundwater. In addition, a selected cultivar (YT94-128) was also grown in a small plot of un-amended contaminated soil, as well as a small plot of the soil that was not affected by mine water (*i.e.* the non-contaminated soil, which was about 600 m away from the main experimental site). This was to allow a comparison of sugar concentration in the juice among the three different soil conditions: (a) non-contaminated soil (control), (b) contaminated soil (T1), and (c) amended contaminated soil (T2).

At harvest, the average diameter and height of stalk for each sugarcane cultivar grown in the amended contaminated soil was obtained, and then the yield of each cultivar was estimated. For Cultivar YT94-128 grown in non-contaminated soil and un-amended contaminated soil, diameter and height of the stalks were not measured because the trial plots were too small to allow comparable estimate of the yield. In each of the trial plots, 15 randomly collected sugarcane stalks were used to obtain sugarcane juice samples. Sugarcane juice was squeezed out of sugarcane stalks by a mechanical crusher. Juice samples were then stored in a refrigerator at 4 ^{o}C before being used for the experiments.

Yeast Species and Inoculum Preparation

Five yeast species were purchased from the China Center of Industrial Culture Collection (CICC): Y1347, Y1384, Y1484, Ybeer, and Y2.82. The stocks of yeast species were maintained at 4 ^{o}C in a potato dextrose agar medium (PDA). The cells were sub-cultured from stock vials onto malt agar plates at 30 ^{o}C for 24 h. The inoculum was prepared by picking well-developed colonies from the plate and suspending the material

in a test tube containing 50 mL of malt agar medium. The content in the test tube was incubated at 30 ^{o}C for 48 h and stored in a refrigerator prior to experiments.

Characterization of Sugarcane Juice and Bagasse

The sugarcane juice and bagasse of the 16 selected sugarcane cultivars plus YT94-128 grown in the non-contaminated soil (control) were used to determine the concentrations of Cd, Cu, Pb, and Zn. The sugarcane juice samples were also used for the determination of sugar concentration.

Fermentation Experiment

The juice samples of YT94-128 grown in the non-contaminated soils (C) and amended soil (T2) were used to observe the changes in residual sugar and ethanol in the fermenting juices over a period of 63.5 h. For each juice sample, 50 mL of the juice was inoculated with 10 mL of Y2.82 inoculum at 31±1 ^{o}C. Samples of the fermenting juice were taken at the 25.75th, 39th, 48th, 54.5th, and 63.5th h of the experiment for determinations of residual sugar and ethanol.

A separate experiment was also conducted to compare the effects of the five different yeast species on the fermentation rate of the control and T2. Because the above experiment showed that no marked decrease in sugar and increase in ethanol were observed after the 25.75th h, the experiment was only run for 40 h. A sample of each treatment was taken for determination of residual sugar and ethanol at the end of the experiment.

All experiments were performed in triplicate.

Analytical Methods

Heavy metals in the bagasse were extracted by dissolving the non-combustible portion in a 2 M HCl solution following combustion of a crushed plant tissue sample. The concentration of heavy metals in the extracts was then determined by atomic absorption spectrometry (AAS). Heavy metals in the sugarcane juice were also determined by AAS following HNO_3-$HClO_4$ digestion.

The concentration of sugar in the sugarcane juices was determined by a dinitrosalicylic acid (DNS) assay (Chen 2008).

The concentration of ethanol was determined by gas chromatography. Nitrogen was used as a carrier gas at 30 mL/min. The column temperature was maintained at 140 ^{o}C. The temperatures of the injector and FID detector were 200 and 300 ^{o}C, respectively. n-Butyl alcohol at a concentration of 2.0% (v/v) was used as the internal standard.

RESULTS AND DISCUSSION

The yield of the sugarcane cultivars grown in the amended contaminated soils ranged from 14440 to 30156 kg/ha (Table 1). This suggests that selection of appropriate cultivars is important to maximize the capacity of the contaminated soils for sugarcane production.

Figure 1 gives a comparison of sugar concentration in the juice among the same sugarcane cultivar (YT94-128) grown in the three different soils. The juice from the sugarcane grown in the non-contaminated soil contained significantly lower sugar contents than that from either the contaminated soil or amended contaminated soil.

Table 1. Growth Performance and Yield of the Various Sugarcane Cultivars Grown in the Contaminated Soil

Sugarcane cultivar	Stalk diameter (cm)	Height (cm)	Yield (kg/ha)
YT94-128	2.35±0.21	276.3±14.29	15548
YT83-271	2.03±0.13	250.3±15.46	30156
YT91-600	2.69±0.20	277.0±12.96	22986
YT94-343	2.06±0.47	270.2±22.45	17199
YT95-128	2.19±0.32	270.4±16.43	19524
YT95-168	1.91±0.32	282.7±24.63	15486
YT95-354	2.12±0.25	269.9±36.81	18350
YT96-598	2.08±0.12	251.6±12.48	16375
YT00-236	2.24±0.33	246.6±34.80	18610
ROC-16	2.32±0.45	233.1±30.36	18820
ROC-22	2.57±0.12	265.3±19.73	26387
CP88-1762	2.27±0.25	239.0±26.08	18473
CP89-2143	2.58±0.16	263.6±19.53	26422
CP92-1666	2.11±0.20	312.0±12.06	20974
CP93-1382	1.92±0.25	260.6±18.78	14440
CP94-1340	2.20±0.44	278.2±21.78	20308

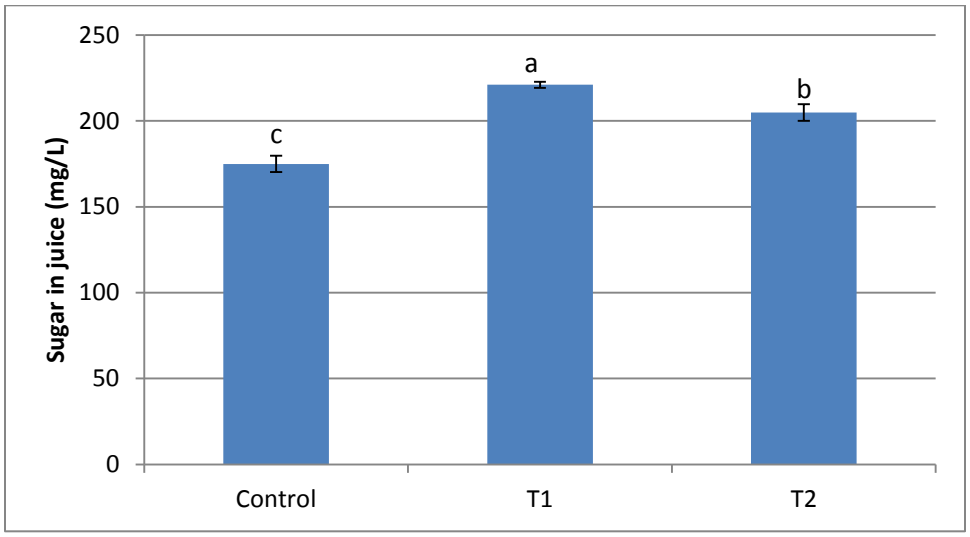

Fig. 1. Comparison of sugar concentration in the juice among the same sugarcane cultivar (YT94-128) grown in non-contaminated soil (control), contaminated soil (T1), and amended contaminated soil (T2). Different letters above bars indicate significant difference at $P < 0.05$.

There was also a significant difference in juice sugar concentration between the sugarcane grown in the un-amended soil and that grown in the amended soil; the former contained more sugar than the latter. However, it must be realized that the soil used for

the control was in another location, which had different fertility status. Therefore, this only allows a rough comparison being made. It is not intended to conclude that there was a higher juice sugar concentration in sugarcane grown in the contaminated soil than in the non-contaminated soil.

Table 2. Concentration of Heavy Metals in the Juice and Bagasse of Various Sugarcane Cultivars Grown in the Contaminated Soil

Sugarcane cultivar	Zn		Cu		Cd		Pb	
	Juice (mg/L)	Bagasse (mg/kg)	Juice (mg/L)	Bagasse (mg/kg)	Juice (mg/L)	Bagasse (mg/kg)	Juice (mg/L)	Bagasse (mg/kg)
YT94-128C	nd	nd	0.05	1.22	nd	0.18	nd	nd
YT94-128	22.86	28.64	0.08	6.55	0.1	nd	nd	0.83
YT83-271	32.96	32.76	0.13	nd	0.05	nd	nd	1.04
YT91-600	32.41	16.38	0.93	nd	0.4	nd	0.15	0.76
YT94-343	24.16	12.39	1.13	nd	0.25	nd	0.20	0.36
YT95-128	44.71	24.24	1.08	0.34	0.2	nd	0.50	1.12
YT95-168	34.41	22.1	1.28	nd	0.3	nd	0.15	0.07
YT95-354	28.46	12.91	1.18	0.24	0.3	nd	0.30	0.99
YT96-598	16.66	34.24	0.53	0.56	0.2	nd	0.15	1.96
YT00-236	24.56	34.37	0.25	nd	0.1	nd	0.05	1.26
ROC-16	18.11	9.63	0.73	nd	0.1	nd	0.10	1.28
ROC-22	28.51	31.08	1.48	1.26	0.4	nd	nd	1.73
CP88-1762	35.51	31.52	1.23	0.09	0.3	nd	0.05	1.73
CP89-2143	30.66	34.74	1.03	0.28	0.45	nd	nd	2.45
CP92-1666	31.91	15.84	0.88	nd	0.2	nd	nd	2.25
CP93-1382	18.96	15.14	0.13	0.17	0.1	nd	nd	2.31
CP94-1340	33.36	23.36	0.38	nd	0.1	nd	nd	0.94

YT94-128C, grown in non-contaminated soil, served as the control for YT94-128, grown in the amended contaminated soil

The heavy metal concentration in the juice and bagasse of the same sugarcane cultivar differed markedly between the non-contaminated soil (YT94-128C) and the contaminated soil (YT94-128). Sugarcane plants grown in contaminated soils tended to have higher heavy metal concentrations than did those grown in non-contaminated soil, particularly for Zn.

Most of the other 15 sugarcane cultivars showed even higher concentrations of Zn, Cu, Cd, and Pb in the juice, as compared to YT94-128 (Table 2).

There was no clear relationship between the yield of sugarcane and any of the heavy metals tested in this study (Fig. 2). This suggests that the increased uptake of heavy metals by the sugarcane plants did not affect the growth of the plants.

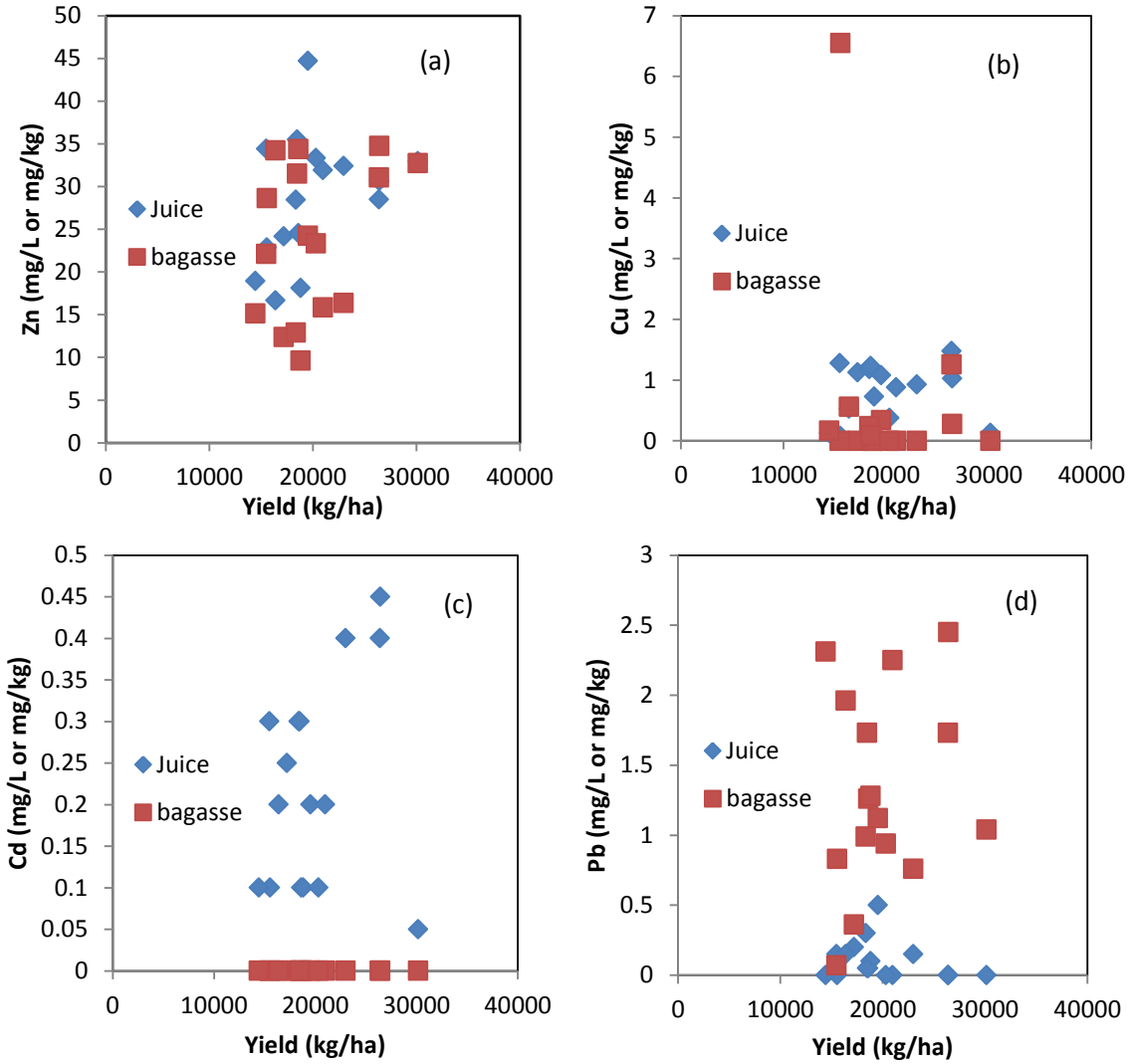

Fig. 2. Relationship between the yield of sugarcane and (a) Zn, (b) Cu, (c) Cd and (d) Pb contained in the juice and bagasse of the sugarcane cultivars

Both the control and T2 showed a similar pattern of temporal variation in sugar in the fermenting juice; the concentration of sugar dropped rapidly to a very low level within the initial 25 h (Fig. 3). This was accompanied by a sharp increase in ethanol content during the same period of time. After the 25.75th h, the sugar concentration underwent little change during the rest of the experiment. However, different variation trends of ethanol content were observed for the control and T2; the ethanol content in the control decreased from the 25.75th h to the 48th h and then increased to the 63.5th h while the ethanol content in T2 kept increasing until the 48th h and then decreased to the 54.5th h, followed by re-increase to the 63.5th h.

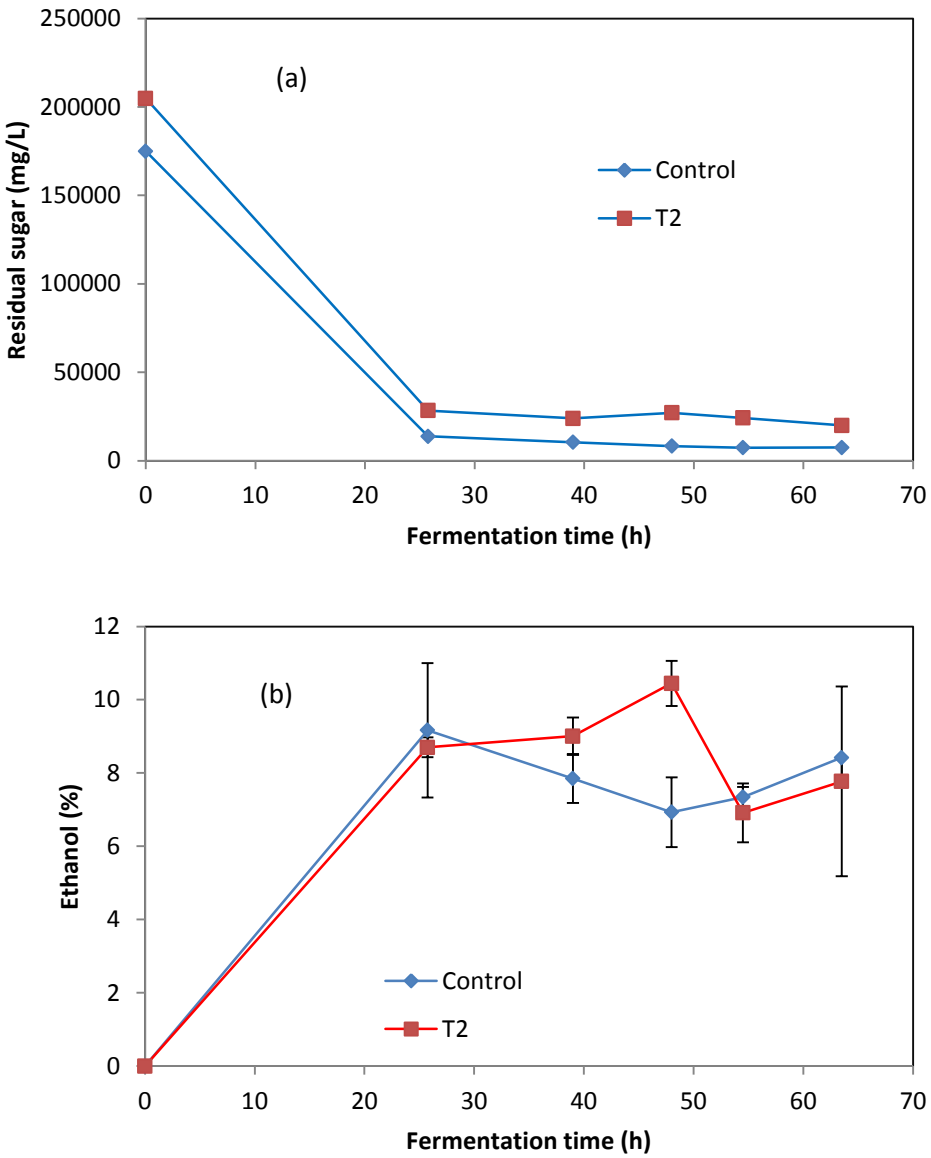

Fig. 3. Change in (**a**) residual sugar and (**b**) ethanol in the fermenting sugarcane juice (using Y2.82 as the fermenting yeast) during the experimental period

The content of ethanol in the fermented juices varied among the 9 selected cultivars grown in the contaminated soil (Table 3). However, there was no clear relationship between the ethanol content and the concentration of heavy metals present in the juices (Fig. 4). This further suggests that the heavy metals contained in the juices did not adversely affect the production of ethanol under the experimental conditions set for this study.

There was no statistically significant difference ($P < 0.05$) in sugar concentration of the fermented sugarcane juice among the fermentation experiments using the five different yeast species for either the control or T2. However, the concentration of residual sugar was consistently lower ($P < 0.05$) in the control than in T2.

Table 3. Content of Ethanol in the Fermented Juice of Various Sugarcane Cultivars Grown in the Contaminated Soil

Sugarcane cultivar	Ethanol (%)	Fermentation rate (%)
YT83-271	9.54±3.11	71
YT95-168	11.30±2.72	89
YT00-236	10.89±0.72	90
ROC22	11.22±1.76	86
CP88-1762	8.60±0.11	66
CP89-2143	8.87±0.13	73
CP92-1666	7.13±0.27	66
CP93-1382	7.80±1.60	68
CP94-1340	6.94±1.06	62

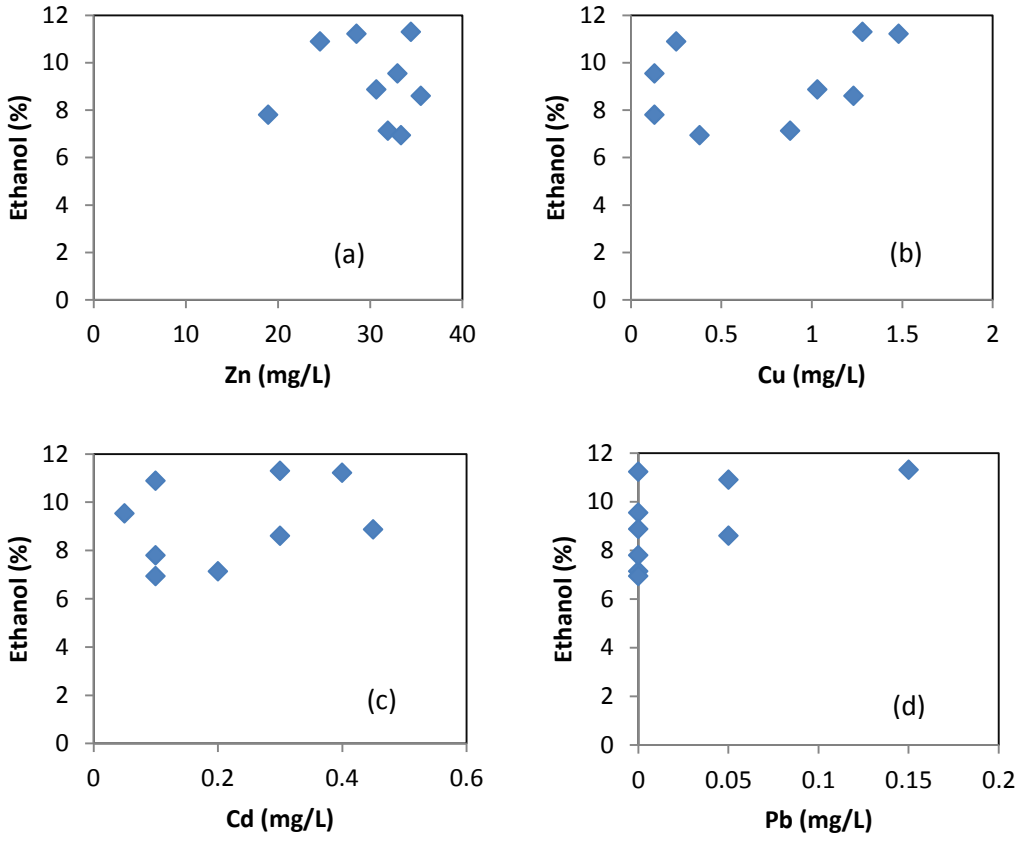

Fig. 4. Scatter plots showing the relationship between the ethanol produced during fermentation experiment and (**a**) Zn, (**b**) Cu, (**c**) Cd and (**d**) Pb in the sugarcane juice samples extracted from the 9 selected sugarcane cultivars

Unlike residual sugar, the ethanol content in the fermented juice varied significantly ($P < 0.05$) among the experiments using different yeast species. For the control, the use of Y1484 and Y1347 resulted in the highest and lowest ethanol contents, respectively. The other three experiments produced similar amounts of ethanol. For T2, the effect of yeast species on the production of ethanol showed the following decreasing order: Y2.82 > Y1347 > Ybeer > Y1484 > Y1384 (Table 4).

Table 4. Residual Sugar and Ethanol in the Fermented Sugarcane Juice of Cultivar Yuetan 94-128 using the Five Selected Yeast Species

	Yeast species	Residual sugar (mg/mL)	Ethanol content (%)	Fermentation rate (%)
Control	Y1347	6.10±0.23a	8.14±0.71d	74
	Y1484	5.89±0.14a	11.34±2.34bc	115
	Y1384	5.89±0.18a	9.30±1.12abc	85
	Ybeer	5.69±0.16a	9.94±1.43cd	90
	Y2.82	5.98±0.39a	9.27±0.78d	87
T2	Y1347	7.32±0.13b	12.27±1.24a	96
	Y1484	6.83±0.61b	10.48±0.39d	81
	Y1384	7.29±0.45b	9.76±0.11ab	76
	Ybeer	7.07±0.48b	11.08±0.88bc	86
	Y2.82	6.89±0.50b	12.75±1.29abc	99

Means with different letters in the same columns indicate statistically significant differences at $P < 0.05$.

The higher level of heavy metals in the juice and bagasse of the sugarcane grown in the contaminated soil, relative to that grown in the non-contaminated soil, was consistent with what has been found for many other crops (Liu *et al*. 2005; Jia *et al*. 2010). However, the sugar concentration in the sugarcane juice was not adversely affected by the elevated level of heavy metals in the soil. As a matter of fact, the contaminated soil enhanced the accumulation of sugar in the tested sugarcane cultivar (YT94-128), and amendment of the contaminated soil reduced the sugar content in the juice. These results suggest that soil amendment is not necessary when the contaminated soil is used for the production of energy sugarcane.

An additional beneficial effect of growing energy sugarcane in heavy metal-contaminated soil is the cleanup of soil through phytoextraction of soil-borne heavy metals. Sugarcane has the ability to produce a large amount of biomass per unit area of land within a given period of time. This may make it more efficient and effective than the use of heavy metal hyperaccumulating plants in terms of soil cleanup purposes.

Hyperaccumulating plants are usually of low biomass output per unit of land area per unit of time (Robinson *et al.* 2009; Conesa *et al.* 2012).

In spite of elevated levels of heavy metals in the juice of sugarcane grown in the contaminated soil, the fermentation rate of sugarcane juice was not adversely affected. The higher ethanol content in T2 than in the control can be partly attributed to the higher initial sugar concentration in the former than in the latter. However, the residual sugar at and after the 25.75th h was also higher in T2 than in the control. If we assume that the residual sugar represents the non-fermentable sugar fraction in the sugarcane juice, then the proportion of non-fermentable sugar in the total sugar can be calculated using the following formula,

$$\text{Non-fermentable sugar (\%)} = (S_t - S_r)/S_t \times 100 \quad (1)$$

where S_t denotes the initial sugar concentration (mg/L) in the sugarcane juice and S_r stands for sugar concentration (mg/L) in the fermented juice.

Figure 5 gives a comparison of non-fermentable sugar (%) in the fermented sugarcane juice between the control (the non-contaminated soil) and T2 (the amended contaminated soil) for the same sugarcane cultivar (YT94-128). It is clear that T2 had a higher percentage of non-fermentable sugar than the control did. Therefore, the higher ethanol content in T2 is also attributable to the higher fermentation rate in the former than in the latter. Calculation showed that the fermentation rate of T2 was 82% at the 25.75th h, as compared to 70% for the control at the same time. The decrease in ethanol content in the fermented juice after the 25.75th h indicated volatilization of ethanol from the fermented juice.

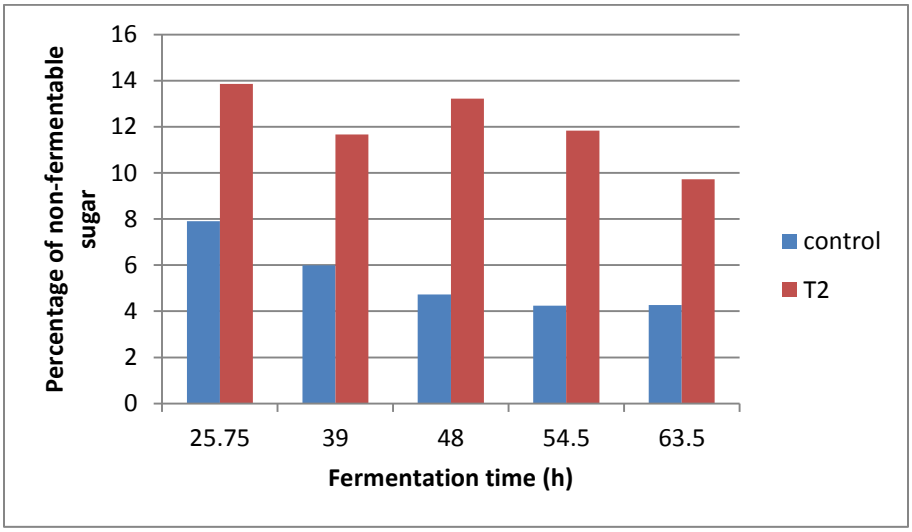

Fig. 5. Comparison of non-fermentable sugar (%) in the fermenting sugarcane juice between the control (the non-contaminated soil) and T2 (the amended contaminated soil) for the same sugarcane cultivar (YT94-128). Y2.82 was used as the fermenting yeast.

The similar fermentation effects of the five yeasts used in this study (Table 4) suggests that fermenting yeasts suitable for producing bioethanol using heavy metal-containing sugarcane juice are readily available, although the fermentation rate may vary slightly from species to species.

The findings obtained from this preliminary study have implications for developing cost-effective technologies for simultaneous bioethanol production and soil clean-up using heavy metal-contaminated soils for energy sugarcane farming. Further work is currently underway to obtain further insights into the mechanisms and kinetics related to the uptake of heavy metals by sugarcane plants, tolerance of sugar fermenting yeasts, *etc*., which can be used to optimize the technologies for using heavy metal-contaminated soils to produce sugar ethanol.

CONCLUSIONS

1. The sugar content was not adversely affected when the sugarcane was grown in heavy metal-contaminated soil.
2. The elevated levels of heavy metals in the sugarcane juice did not adversely affect the fermentation and the resulting ethanol production when appropriate yeast species were used to mediate the processes.
3. The uptake of heavy metals by energy sugarcane may allow the cleanup of contaminated soils in a more economical way, which makes it superior to the use of low-biomass heavy metal hyperaccumulating plants.

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REFERENCES CITED

Amin, S. (2009). "Review on biofuel oil and gas production processes from microalgae," *Energ. Convers. Manage*. 50(7), 1834-1840.

Anderson, S. T. (2012). "The demand for ethanol as a gasoline substitute," *J. Environ. Econ. Manage*. 63(2), 151-168.

Azenha, M., Vasconcelos, M. T., and Moradas-Ferreira, P. (2000). "The influence of Cu concentration on ethanolic fermentation by *Saccharomyces cerevisiae*," *J. Biosci. Bioeng*. 90(2), 163-167.

Bai, F. W., Anderson, W. A., and Moo-Young, M. (2008). "Ethanol fermentation technologies from sugar and starch feedstocks," *Biotechnol. Adv*. 26(1), 89-105.

Bull, T. A. (1969). "Photosynthetic efficiencies and photorespiration in Calvin cycle and C4-dicarboxylic acid plants," *Crop Sci*. 9(6), 726-729.

Chen, A., Lin, C., Lu, W., Ma, Y., Bai, Y., Chen, H., and Li, J. (2010). "Chemical dynamics of acidity and heavy metals in a mine water-polluted soil during decontamination using clean water," *J. Hazard. Mater*. 175(1-3), 638-645.

Chen, Y. (2008). *Experimental Methods and Techniques for Biochemical Research*, Science Press, Beijing.

Conesa, H. M., Evangelou, M. W. H., Robinson, B. H., and Schulin, R. (2012). "A critical view of current state of phytotechnologies to remediate soils: Still a promising tool?," *Sci. World J*. 2012, 173829.

Demirbas, A. (2008). "Biofuels sources, biofuel policy, biofuel economy and global biofuel projections," *Energ. Convers. Manage*. 49(8), 2106-2116.

Goldemberg, J. (2007). "Ethanol for a sustainable energy future," *Science* 315(5813), 808-810.

Ibeto, C. N., Ofoefule, A. U., and Agbo, K. E. A. (2011). "Global overview of biomass potentials for bioethanol production: A renewable alternative fuel," *Trends Applied Sci. Res*. 6(5), 410-425.

International Energy Agency (IEA) (2004). *Biofuels for Transport: An International Perspective*.

Jamai, L., Ettayebi, K., El Yamani, J., and Ettayebi, M. (2007). "Production of ethanol from starch by free and immobilized *Candida tropicalis* in the presence of α-amylase," *Bioresource Technol*. 98(14), 2765-2770.

Jia, L., Wang, W., Li, Y., and Yang, L. (2010). "Heavy metals in soil and crops of an intensively farmed area: A case study in Yucheng City, Shandong Province, China," *Int. J. Environ. Res. Public Health* 7(2), 395-412.

Lin, C., Lu, W., and Wu, Y. (2005). "Agricultural soils irrigated with acidic mine water: Acidity, heavy metals, and crop contamination," *Aust. J. Soil Res.* 43(7), 819-826.

Liu, H., Probst, A., and Liao, B. (2005). "Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China)," *Sci. Total Environ*. 339(1-3), 153-166.

Luchansky, M. S., and Monks, J. (2009). "Supply and demand elasticities in the U.S. ethanol fuel market," *Energ. Econ*. 31(3), 403-410.

Motta, R. S. D., and Ferreira, L. D. R. (1988). "The Brazilian national alcohol programme: An economic reappraisal and adjustments," *Energ. Econ*. 10(3), 229-234.

Pearce, D., and Sherman, F. (1999) "Toxicity of copper, cobalt, and nickel salts is dependent on histidine metabolism in the yeast *Saccharomyces cerevisiae*," *J. Bacteriol*. 181, 4774-4779.

Perego, P., and Howell, S.B. (1997). "Molecular mechanisms controlling sensitivity to toxic metal ions in yeast," *Toxicol. Applied Pharmacol*. 147(2), 312-318.

Robinson, B. H., Bañuelos, G., Conesa, H. M., Evangelou, M. W. H., and Schulin, R. (2009). "The phytomanagement of trace elements in soil," *Critical Reviews in Plant Sciences* 28(4), 240-266.

Sánchez, O. J., and Cardona, C. A. (2008). "Trends in biotechnological production of fuel ethanol from different feedstocks," *Bioresource Technol*. 99(13), 5270-5295.

Ustyak, S., and Petrikova, V. (1996). "Heavy metal pollution of soils and crops in northern Bohemia," *Applied Geochem*. 11(1-2), 77-80.

Verheye, W. (2010). "Growth and production of sugarcane," in: *Soils, Plant Growth and Crop Production*, Vol. II, W. H. Verheye and M. B. Bayles (eds.), UNESCO-EOLSS.

Wang, Q., Dong, Y., Cui, Y., and Liu, X. (2001). "Instances of soil and crop heavy metal contamination in China," *Soil Sediment Contam.* 10(5), 497-510.

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