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A mechanism for efficient cadmium phyto remediation and high bioethanol production by combined mild chemical pretreatments with desirable rapeseed stalks

Yue Wu^{a,b,d,1}, Meiling Wang^{a,b,d,1}, Li Yu^{c,*}, Shang-wen Tang^d, Tao Xia^{a,d,e}, Heng Kang^{a,b,d}, Chengbao Xu^{a,b,d}, Hairong Gao^{a,b,d}, Meysam Madadi^{a,b}, Aftab Alam^{a,b}, Liangliang Cheng^{a,b}, Liangcai Peng^{a,b,d,*}

^a Biomass & Bioenergy Research Centre, Huazhong Agricultural University, Wuhan 430070, China

^b College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

^c Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Key Laboratory of Biology and Genetic Improvement of Oil Crops and Key Laboratory of Detection for Mycotoxins, Ministry of Agriculture, Wuhan 430062, China

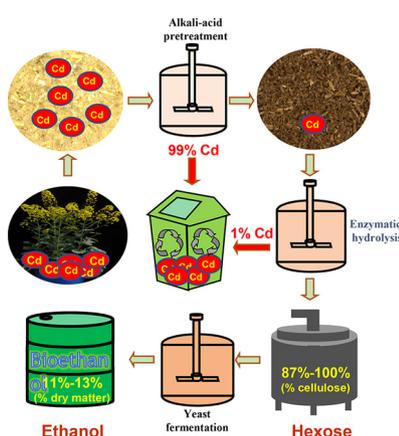
^d Laboratory of Biomass Engineering and Nanomaterial Application in Automobiles, College of Food Science and Chemical Engineering, Hubei University of Arts and Science, Xiangyang, China

^e College of Life Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

HIGHLIGHTS

- Highest Cd accumulation in rapeseed stalks among major food crops.
- Pectin deposition increased Cd accumulation in rapeseed stalks.
- A complete Cd release for recycling under optimal biomass process.
- Much enhanced biomass saccharification due to Cd accumulation.
- A mechanism for Cd accumulation and cellulosic ethanol production.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) is one of the most hazardous trace metals, and rapeseed is a major oil crop over the world with considerable lignocellulose residues applicable for trace metal phyto remediation and cellulosic ethanol co-production. In this study, we examined that two distinct rapeseed cultivars could accumulate Cd at 72.48 and 43.70 ug/g dry stalk, being the highest Cd accumulation among all major agricultural food crops as previously reported. The Cd accumulation significantly increased pectin deposition as a major factor for trace metal association with lignocellulose. Meanwhile, the Cd-accumulated rapeseed stalks contained much reduced wall polymers (hemicellulose, lignin) and cellulose degree of polymerization, leading to improved lignocellulose enzymatic hydrolysis. Notably, three optimal chemical pretreatments were performed for enhanced biomass enzymatic saccharification and bioethanol production by significantly increasing cellulose accessibility and lignocellulose porosity, along with a complete Cd release for

* Corresponding authors at: Biomass & Bioenergy Research Centre, Huazhong Agricultural University, Wuhan 430070, China (L. Peng).

E-mail addresses: yuli01@caas.cn (L. Yu), lpeng@mail.hzau.edu.cn (L. Peng).

¹ Equal contribution.

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collection and recycling. Hence, this study proposed a mechanism model interpreting why rapeseed stalks are able to accumulate much Cd and how the Cd-accumulated stalks are of enhanced biomass saccharification. It has also provided a powerful technology for both cost-effective Cd phytoremediation and value-added bioethanol co-production with minimum waste release.

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1. Introduction

Cadmium (Cd) is a major toxic trace metal with potential pollution to agricultural soil and water source over the world. Due to its strong mobility, Cd can pass through plant root and accumulate in various plant tissues (Lin & Aarts, 2012; Ronzan, 2018). Hence, phytoremediation has been considered as a cost-effective and environmental-friendly approach by growing crops in the Cd-polluted soil (Desai et al., 2019), and in particular, the mature crop straws could largely accumulate Cd (Mcgrath et al., 2002; Tangahu et al., 2011). Despite it has been documented that Cd accumulation affects plant cell wall biosynthesis (Douchiche and Azeddine, 2010; Yang et al., 2015) and biomass yield (Durenne et al., 2018; Ko et al., 2017), much remains unknown about its impact on lignocellulose features. In terms of the Cd accumulation with crop straws, it thus remains to explore an optimal biomass process technology for complete Cd release and recycling.

Agricultural crop straws provide huge lignocellulose residues convertible for bioethanol production with less net carbon release. In principle, lignocellulose conversion requires three major steps: initial physical and chemical pretreatment for wall polymers destruction, sequential enzymatic hydrolysis for soluble sugar release and final yeast fermentation for bioethanol production (Shuai et al., 2010). Over the past years, various physical and chemical pretreatments have been performed to enhance biomass enzymatic saccharification and bioethanol production by partially extracting lignin and hemicelluloses and characteristically altering wall polymer features. For instance, sulfuric acid (H_2SO_4) is used to partially digest hemicelluloses at high temperature, whereas sodium hydroxide (NaOH) can effectively extract lignin under high concentration (Huang et al., 2011; Jeong et al., 2010; Li et al., 2014). Furthermore, lignocellulose features have been examined as major factors affecting chemical pretreatment and sequential enzymatic hydrolysis. In particular, DP (degree of polymerization) of β -1,4-glucans is a negative factor accounting for lignocellulose hydrolysis, whereas both biomass porosity and cellulose surface accessibility positively affect biomass enzymatic saccharification in the lignocellulose residues examined (Alam et al., 2019; Li et al., 2017; Meng et al., 2015).

Rapeseed (*Brassica juncea* L.) is a major oil crop providing large amounts of lignocellulose residues (Kuglarz et al., 2018; Pei et al., 2016). In this study, we collected mature stalks of two rapeseed cultivars (Tapidor, Wanyou29) that grew in the soil pots co-supplied with $CdCl_2$, and examined the Cd accumulation. Meanwhile, this work performed various chemical pretreatments and determined much enhanced biomass enzymatic saccharification and bioethanol production, coupled with a complete Cd release from the combined alkali and acid pretreatments in the mature rapeseed stalks. Furthermore, this study proposed a mechanism model to sort out why Cd was much more accumulated in the rapeseed stalk compared to other major food crops, how Cd could be released from optimal biomass processing and why the Cd accumulation enhanced biomass enzymatic saccharification and bioethanol production. Hence, this study could provide a potential strategy for green-like Cd phytoremediation and valued-added cellulosic ethanol co-production with minimum waste release using rapeseed stalk and other crop straws.

2. Material and methods

2.1. Plant samples collection

The general experiment procedure is described in Fig. 1. Two local rapeseed (*Brassica napus* L.) cultivars (Tapidor, Wanyou29) were grown in the soil pots (25.0 kg soil) co-supplied with $CdCl_2$ (CAS No.10108-64-2) at different concentrations (0.0, 20.0, 40.0, 60.0, 120 mg Cd/kg dry soil). Before rapeseed seedlings were transferred, the soil pots were soaked with water for a few days until the $CdCl_2$ was well mixed with the soil. Each $CdCl_2$ concentration treatment used three soil pots and each pot contained four rapeseed seedlings. For the following experiments performed in this study, all mature stalks of 12 rapeseed plants from each $CdCl_2$ treatment were collected, dried at 50 °C and ground through a 40 mesh screen, and the well-mixed powders were stored in a sealed dry container until use.

2.2. Wall polymer determination

Plant cell wall fractionation procedure was used to extract cellulose and hemicelluloses as previously described by Peng et al. (2000) with minor modification by Wu et al. (2013), which was applicable for biomass residues at lab scale. The biomass powders were well ground with potassium phosphate buffer (pH 7.0), followed with chloroform-methanol (1:1, v/v), DMSO-water (9:1, v/v) and 0.50% (w/v) ammonium oxalate to respectively remove soluble compounds (sugars/proteins/Cd), lipid, starch and pectin. The remaining residues were extracted with 4 M KOH with 1.0 mg/mL sodium borohydride as KOH-extractable hemicelluloses, followed by H_2SO_4 (67%, v/v) to completely dissolve cellulose and non-KOH-extractable hemicelluloses. Cellulose content was measured by determining hexoses of the cellulose fraction, and total hemicelluloses were calculated by determining hexoses and pentoses of total hemicellulose fractions.

Two-step acid hydrolysis method was applied for total lignin assay according to the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (USA) with minor modification by Wu et al. (2013) applicable for small amounts of biomass residues. Total lignin includes acid-insoluble and acid-soluble lignin. The acid-insoluble lignin was calculated gravimetrically after correction for ash, and the acid-soluble lignin was measured by UV spectroscopy. For acid-insoluble lignin assay, the biomass sample (0.500 g as W1) was extracted with benzene-ethanol (2:1, v/v) in a Soxhlet for 4 h, and air-dried in a hood overnight. The sample was then hydrolyzed with 10 mL 72% H_2SO_4 (v/v) in a shaker at 30 °C for 1.5 h. After hydrolysis, the acid was diluted to a concentration of 2.88%, and placed in the autoclave for 1 h at 121 °C (15 psi). The autoclaved hydrolysis solution was vacuum-filtered through the previously weighed filtering crucible, and the filtrate was captured in a filtering flask for acid-soluble lignin. The lignin was washed free of acid with hot distilled water and the crucible and acid-insoluble residue was dried in an oven at 80 °C until constant weight was achieved. Then, the samples were removed from the oven and cooled in a dry container. The weight of the crucible and dry residue was recorded to the nearest 0.1 mg (W2). Finally the dried residue was ashed in the muffle furnace at 200 °C for

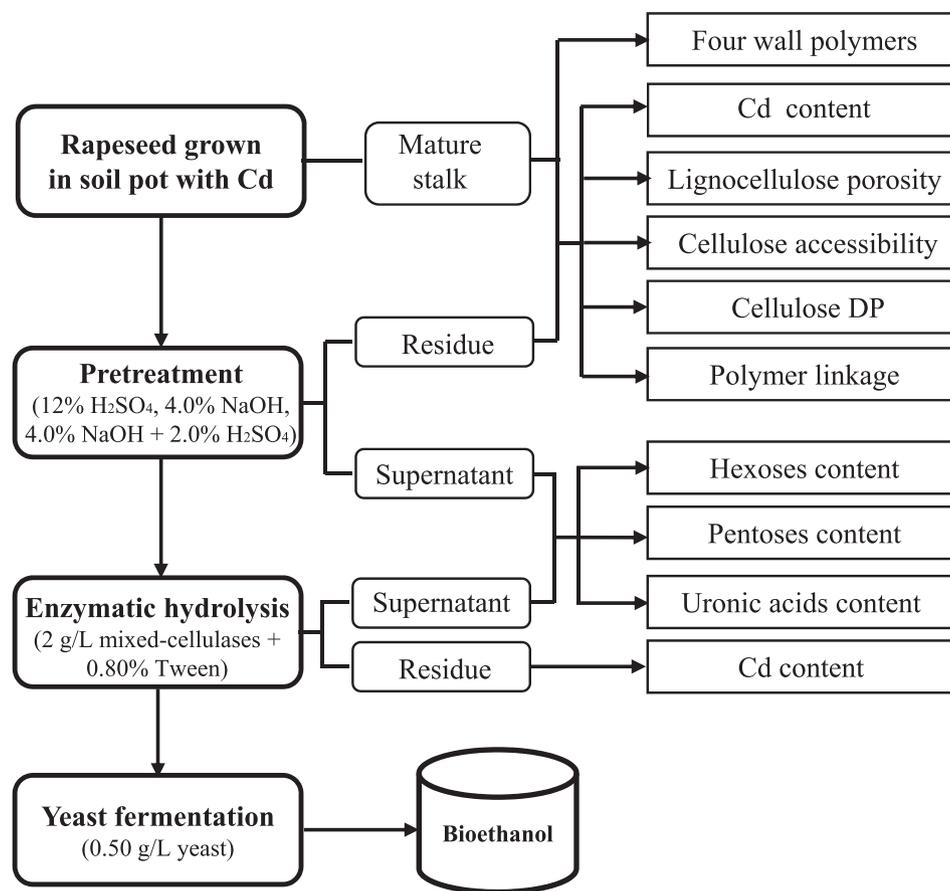


Fig. 1. General experimental procedure for Cd assay and bioethanol conversion.

30 min and at 575 °C for 4 h. The crucibles and ash were weighed to the nearest 0.1 mg recorded as W3. The acid-insoluble lignin (AIL) of the original sample was calculated as follows: $AIL (\%) = (W2 - W3) \times 100 / W1\%$. The acid-soluble lignin was solubilized during the hydrolysis process, and the hydrolysis liquor was transferred into a 250-mL volumetric flask and brought up to 250 mL with 2.88% sulfuric acid. The absorbance of the sample was read at 205 nm using UV-vis spectroscopy (Beckman Coulter Inc., Du800), and 2.88% sulfuric acid was used as blank. The calculation for acid-soluble lignin was performed as follows: $ASL(\%) = (A \times D \times V / 1000 \times K \times W1) \times 100\%$, Where A is the absorption value, D is the dilution ratio of the sample, and K (the absorptivity constant) = 110 L/g/cm.

For each Cd treatment described in Section 2.1, three experimental samples of the well-mixed biomass powders were equally weighed as independent triplicates for the wall polymer extraction and analysis.

2.3. Colorimetric assay

UV/VIS Spectrometer (V-1100D, MAPADA Instruments Co., Ltd., Shanghai, China) was applied for total hexoses and pentoses assay as described by Wu et al. (2014). For cellulose assay, sample was dissolved in 67% H₂SO₄ and hexoses were calculated by the anthrone/H₂SO₄ method. Hemicelluloses were calculated by determining total hexoses and pentoses of the hemicellulose fraction, and pectin was measured by calculating total hexoses, pentoses and uronic acids of the fraction. All experiments were performed in independent triplicates as described in Section 2.2.

2.4. Cadmium analysis

The dry biomass sample (0.100 g) was added into the porcelain crucible and transferred to the muffle furnace. The temperature of muffle furnace was gradually raised to 200 °C for 1 h, and set 600 °C for 6–8 h. The ash was dissolved with 1.0% HNO₃ (v/v), washed with 1.0% HNO₃ for 3 times, and all solutions were collected into a 25 mL volumetric flask. Atomic Absorption Spectrometer (Agilent 240Z GFAA) was used for Cd detection, and all experiments were conducted in independent triplicates as described in Section 2.2. In addition, the potassium phosphate (pH 7.0) fraction (Section 2.2) was used for soluble Cd assay of all rapeseed stalk samples.

2.5. Cellulose DP detection

The dry biomass powders (0.200 g) were extracted with 4 M KOH (containing sodium borohydride at 1.0 mg/mL) at 25 °C for 1 h. After centrifugation at 3000g for 5 min to remove supernatants, the remaining residues were washed once with 4 M KOH and five times with distilled water until pH at 7.0 to make sure of all alkali removal. The pellet was further extracted with 10 mL 8.0% NaClO₂ at 25 °C for 72 h (change NaClO₂ every 12 h). After centrifugation, the remaining residues were again washed five times with distilled water until pH at 7.0, and dried with vacuum suction filtration. The DP of crude cellulose sample was measured using the viscosity method (Puri, 2010) with minor modification by Li et al. (2017) and Wu et al. (2019). All experiments were performed in independent triplicates as described in Section 2.2.

2.6. Biomass porosity and cellulose accessibility measurements

Simons' stain (SS) was applied to determine the overall accessible surface area of biomass as previously described by [Chandra et al. \(2010\)](#) and [Sun et al. \(2017\)](#). Congo red (CR) stain was applied to estimate cellulosic surface area as previously described by [Alam et al. \(2019\)](#). All experiments were conducted in independent triplicates as described in [Section 2.2](#).

2.7. Fourier transform infrared (FTIR) spectroscopy scanning

FTIR spectroscopy was used to observe the chemical linkages in the raw and pretreated rapeseed samples using Perkin-Elmer Ppctro-photometer (NEXUS 470, Thermo Fisher Scientific, Waltham, MA, USA) as previously by [Alam et al. \(2019\)](#).

2.8. Chemical pretreatment and enzymatic hydrolysis analyses

One-step chemical (H_2SO_4 , NaOH) pretreatments were respectively performed as previously described by [Jin et al. \(2016\)](#) and [Li et al. \(2018\)](#). For acid pretreatment, the biomass powders were added with 6.0 mL H_2SO_4 at various concentrations (2.0%, 4.0%, 8.0%, 12%, 16%, v/v) and heated at 121 °C for 20 min in an autoclave. For alkali pretreatment, the biomass powders were added with 6 mL NaOH at various concentrations (1.0%, 2.0%, 4.0%, 8.0%, w/v) under shaking (150 rpm) at 50 °C for 2 h. The two-step alkali-acid chemical (NaOH + H_2SO_4) pretreatments were performed as previously described by [Si et al. \(2015\)](#). The biomass powders were treated with 6.0 mL 4.0% NaOH under shaking (150 rpm) at 50 °C for 2 h. After centrifugation at 3000 g for 5 min, the remaining residues were washed with 10 mL distilled water for 5–6 times until pH at 7.0, and then added with 3.0 mL H_2SO_4 (2.0%, 4.0%, 8.0%, w/v). Distilled water was added to make the final volume up to 6.0 mL and heated at 121 °C for 20 min in an autoclave. The samples added with 6.0 mL distilled water and shaken for 2 h at 50 °C were used as control.

For enzymatic hydrolysis, the sample was centrifuged at 3000 g for 5 min, and the remaining residues from pretreatments were washed with 10 mL distilled water for 5–6 times until pH at 7.0, and once more with 10 mL of mixed-cellulase reaction buffer until pH at 4.8. The washed residues were incubated with mixed-cellulases (containing β -glucanase $\geq 5.96 \times 10^4$ U and cellulase ≥ 596 U and xylanase $\geq 9.60 \times 10^4$ U, purchased from Imperial Jade Bio-technology Co., Ltd., China) with the final enzyme concentration at 2.0 g/L and Tween-80 concentration at 0.80% (v/v), and shaken under 150 rpm for 48 h at 50 °C. All experiments were conducted in independent triplicates as described in [Section 2.2](#).

2.9. Yeast fermentation and ethanol detection

Yeast fermentation was conducted using total hexoses released from above enzymatic hydrolysis and ethanol was measured as previously described by [Li et al. \(2018\)](#). Yeast *Saccharomyces cerevisiae* strain (Angel yeast Co., Ltd., Yichang, China) was used in all fermentation reactions and all experiments were performed in independent triplicates as described in [Section 2.2](#).

2.10. Statistical analysis

Superior Performance Software Systems (SPSS version 16.0, Inc., Chicago, IL) was applied for any calculations. Means were separated by least significant difference (LSD) test at $p = 0.05$. Pair-wise comparisons were conducted between two measurements by Student's *t*-test. The line graphs were generated using Origin 8.5 software (Microcal Software, Northampton, MA). The average values were calculated from the original triplicate measurements for these analyses.

3. Results and discussion

3.1. High Cd accumulation in two rapeseed stalks

As illustrated in the general experimental procedure ([Fig. 1](#)), this study initially examined the Cd accumulation in mature stalks of two local rapeseed cultivars (Tapidor, Wanyou29) grown in the soil pots supplied with five concentrations of $CdCl_2$ (0.0, 20.0, 40.0, 60.0, 120 mg/kg dry soil). In general, as the $CdCl_2$ concentration was increasing in the soil pots, the Tapidor and Wanyou29 cultivars showed significantly increased Cd accumulation in the mature stalks at $p < 0.05$ level ([Fig. 2A](#)). In comparison, the Wanyou29 cultivar accumulated much more Cd than that of the Tapidor in their mature stalks. In particular, the Wanyou29 and Tapidor respectively contained Cd levels at 72.5 and 43.7 $\mu\text{g/g}$ dry stalk from the soil pots supplied with 120 mg/kg dry soil. Further compared

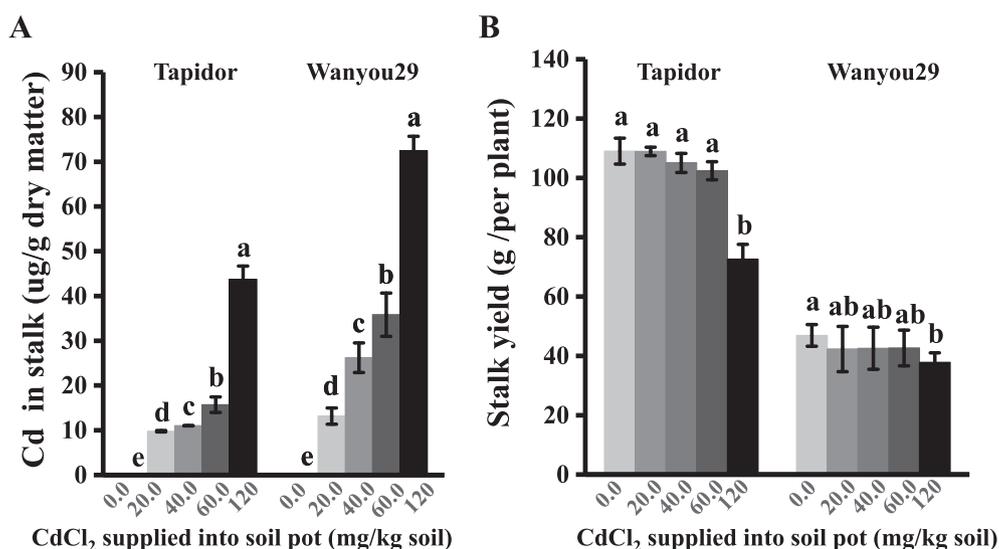


Fig. 2. Cd content (A) and biomass yield (B) in mature stalks of two rapeseed cultivars (Tapidor, Wanyou29) grown in the soil pots supplied with different concentrations of $CdCl_2$. Letter (a, b, c, d, e) indicated significant difference by LSD-test at $p < 0.05$. Bar as mean \pm SD ($n = 3$).

Table 1
Comparison of Cd content in rapeseed stalks with other plants as previously reported.

Plant species	CdCl ₂ supplied into soil (mg/kg soil)	Cd content in stalk (μg/g dry matter)	Reference
Rapeseed (Tapidor)	120	43.7	This study
(Wanyou29)	60.0	15.7	
	120	72.5	
	60.0	35.8	
Wheat	13.7	0.63	Shi et al., 2015
Rice	100	28.0	Lin et al., 2016
Maize	6.00	0.60	Liu et al., 2018
Cotton	20.3	3.30	Chen et al., 2015
Forage grasses	8.00	28.0	Zhang et al., 2010
Edible amaranth	2.28	0.55	Xu et al., 2017
Soybean	4.29	2.40	Murakami et al., 2007

to other major crops as previously reported (Table 1), both rapeseed cultivars could accumulate much more Cd in the stalks with the increased rates of Cd from 2.5 to 131 folds relative to the referred crops (Chen et al., 2015; Lin et al., 2016; Liu et al., 2018; Murakami et al., 2007; Shi et al., 2015; Xu et al., 2017; Zhang et al., 2010), indicating that the rapeseed should be distinctive for Cd phytoremediation.

Meanwhile, this study found that two rapeseed cultivars did not show significantly affected biomass yields while incubated with CdCl₂ at concentrations of 20.0, 40.0 and 60.0 mg/kg dry soil (Fig. 2B). But, two cultivars had significantly reduced biomass yields in the pots supplied with 120 mg CdCl₂/kg dry soil. On the other hand, despite that the Tapidor contained much lower Cd level than that of the Wanyou29, it had much higher biomass yield, leading to similar Cd amounts per plants in two rapeseed cultivars in almost all CdCl₂ supplement experiments (Table S1). Hence, the results revealed that two rapeseed cultivars could much accumulate Cd in their stalks.

3.2. Enhanced biomass saccharification and bioethanol production in Cd-accumulated stalks

Based on our previously-established approaches (Alam et al., 2019

Pei et al., 2016), this study performed various chemical (alkali, acid) pretreatments in the mature stalks of two rapeseed cultivars, and then determined biomass saccharification by calculating hexoses yields (% dry matter) released from enzymatic hydrolysis or total sugars (hexoses and pentoses) yields (% dry matter) released from both pretreatment and enzymatic hydrolysis. To find out the optimal chemical pretreatments, this study initially used a series of concentrations of NaOH (1.0%, 2.0%, 4.0%, 8.0%) and H₂SO₄ (2.0%, 4.0%, 8.0%, 12%, 16%) in two rapeseed cultivars. By comparison, both Wanyou29 and Tapidor cultivars showed the highest hexoses and total sugars yields under one-step pretreatment with 4.0% NaOH or 12% H₂SO₄ (Fig. S1A, B, D, E). Furthermore, this work performed two-step alkali and acid pretreatments and sorted out that two rapeseed cultivars could reach to the highest hexoses (or total sugars) yields under 4.0% NaOH pretreatment followed by 2.0% H₂SO₄ (Fig. S1C,F). Using three optimal pretreatments (4.0% NaOH 12% H₂SO₄ 4.0% NaOH + 2.0% H₂SO₄), we examined biomass saccharification in the Cd-accumulated rapeseed stalks (Fig. 3A-C). Compared to controls (CK/without Cd), two Cd-accumulated rapeseed cultivars showed significantly higher hexoses yields (% cellulose) at $p < 0.01$ level ($n = 3$) with the increased rates of 12%-17%, but the Wanyou29 had consistently higher hexoses yields than those of the Tapidor under three optimal pretreatments (Fig. 3A-

C). Notably, the Wanyou29 showed an almost complete biomass saccharification with hexoses yield of 100% (% cellulose) only under 4.0% NaOH + 2.0% H₂SO₄ pretreatment.

Using total hexoses released from enzymatic hydrolysis of pretreated biomass residues, this study further conducted a classic yeast fermentation for bioethanol production. As predicted, the Cd-accumulated stalks showed significantly higher bioethanol yields (% dry matter) than those of the controls in two cultivars at $p < 0.05$ or 0.01 ($n = 3$) under three optimal pretreatments with increased rates of 7.0%-12% (Fig. 3 D-F). Due to its relatively higher hexoses yields, the Wanyou29 remained consistently higher bioethanol yields than those of the Tapidor, and two rapeseed cultivars respectively had the highest bioethanol yields of 13% and 11% (% dry matter) under two-step (4.0% NaOH + 2.0% H₂SO₄) pretreatments. Therefore, this study demonstrated that the Cd accumulation in rapeseed stalks could significantly enhance biomass enzymatic saccharification and bioethanol production under two-step (4.0% NaOH + 2.0% H₂SO₄) chemical pretreatment.

3.3. Complete Cd release from optimal chemical biomass processing

With respect to the Cd accumulation in mature stalks of two rapeseed cultivars, this work examined how much the accumulated Cd could be released during three optimal pretreatments and sequential enzymatic hydrolyses (Fig. 4). As a result, two optimal chemical pretreatments (12% H₂SO₄, 4.0% NaOH + 2.0% H₂SO₄) could release 99% (of total in raw stalk) Cd from the mature stalks of two rapeseed cultivars, and thus the sequential enzymatic hydrolysis led to an almost complete Cd release into the supernatants. By comparison, the 4.0% NaOH pretreatments only extracted 34% and 58% Cd (of total) in the Tapidor and Wanyou29 stalks, respectively (Fig. 4A), but the sequential enzymatic hydrolysis caused 91% and 97% Cd release in two cultivars (Fig. 4B). Hence, compared to other two optimal pretreatment, the optimal two-step chemical pretreatment also caused a complete Cd release for Cd collection in two rapeseed cultivars. Because acid and alkali pretreatments distinctively extract and destruct wall polymers (Huang et al., 2011; Jeong et al., 2010; Li et al., 2014), the results suggested that the acid pretreatments performed in this study could not only digest non-cellulosic polysaccharides (hemicellulose, pectin), but also efficiently release Cd probably due to Cd interaction with polysaccharides as described below. In addition, this study examined that the Tapidor and Wanyou29 respectively contained soluble Cd at 41% and 55% (of total) in their mature stalks (Fig. 4C), indicating that the Wanyou29 cultivar was of much more extractable Cd in the mature stalk relative to the Tapidor. It also suggested that the soluble Cd should be not interacted with wall polymers in the mature stalks.

3.4. Altered cell wall compositions and increased soluble sugars in the Cd-accumulated stalks

To understand why the Cd-accumulation could largely enhance biomass saccharification and bioethanol production in two rapeseed cultivars, this study determined cell wall compositions in the mature stalks (Fig. 5). Compared to the controls (CK, without Cd), two rapeseed cultivars showed significantly reduced wall polymers levels including cellulose, hemicellulose and lignin at $p < 0.05$ or 0.01 level. In particular, lignin was decreased by 13% and 18% in two rapeseed cultivars, whereas cellulose and hemicellulose had the reduced rates of 5.4%-8.3%. By contrast, both rapeseed cultivars showed largely increased pectin contents and soluble sugars in the mature stalks. Hence, despite that Cd-accumulated stalks contained relatively low cellulose levels, the increased soluble sugars by 14% and 25% could be directly applied for yeast fermentation. In addition, although it has been character-

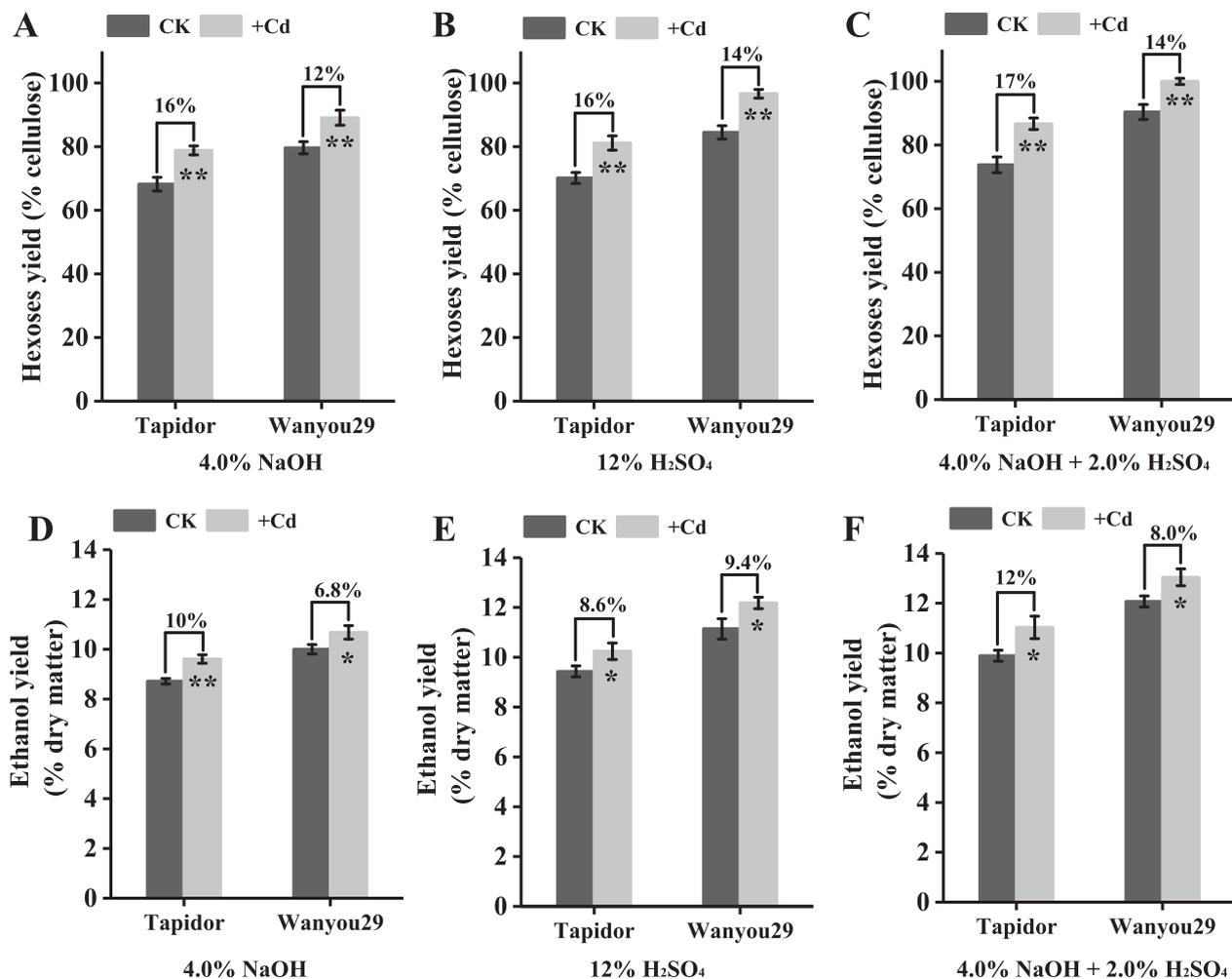


Fig. 3. Hexoses and bioethanol yields obtained in two rapeseed cultivars (Tapidor and Wanyou29). (A, B, C) Hexoses yields (% cellulose) released from enzymatic hydrolysis after one-step (4.0% NaOH, 12% H₂SO₄) and two-step (4.0% NaOH + 2.0% H₂SO₄) pretreatment with mature rapeseed stalks supplied with 120 mg/kg CdCl₂ (+Cd) or without CdCl₂ (CK). (D, E, F) Bioethanol yields (% dry matter) using hexoses from enzymatic hydrolysis as described (A, B, C). * and ** indicated significant difference between two samples by t-test at $p < 0.05$ and 0.01 ($n = 3$). Increased percentage calculated by subtraction between two samples divided by the CK. Bar as mean \pm SD ($n = 3$).

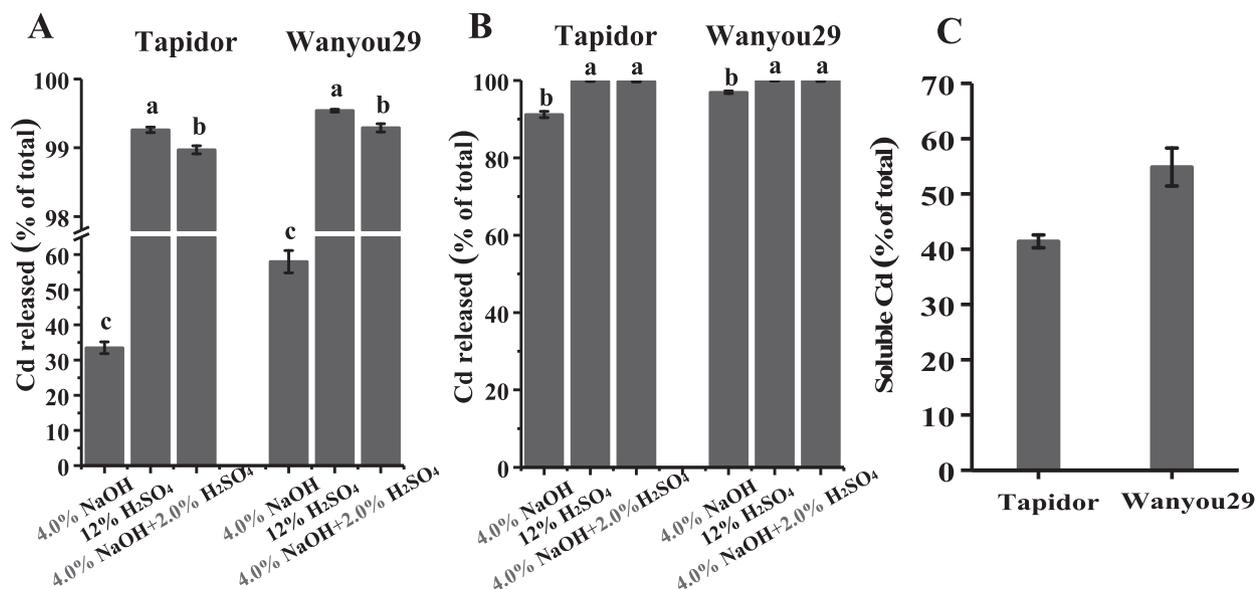


Fig. 4. Cd released from various chemical pretreatments (A), and total Cd released from chemical pretreatment and sequential enzymatic hydrolysis (B) in mature stalks of two rapeseed cultivars. Soluble Cd determined in the mature stalks (C). Letters (a, b, c, d, e) indicated significant difference by LSD-test at $p < 0.05$. Bar as mean \pm SD ($n = 3$).

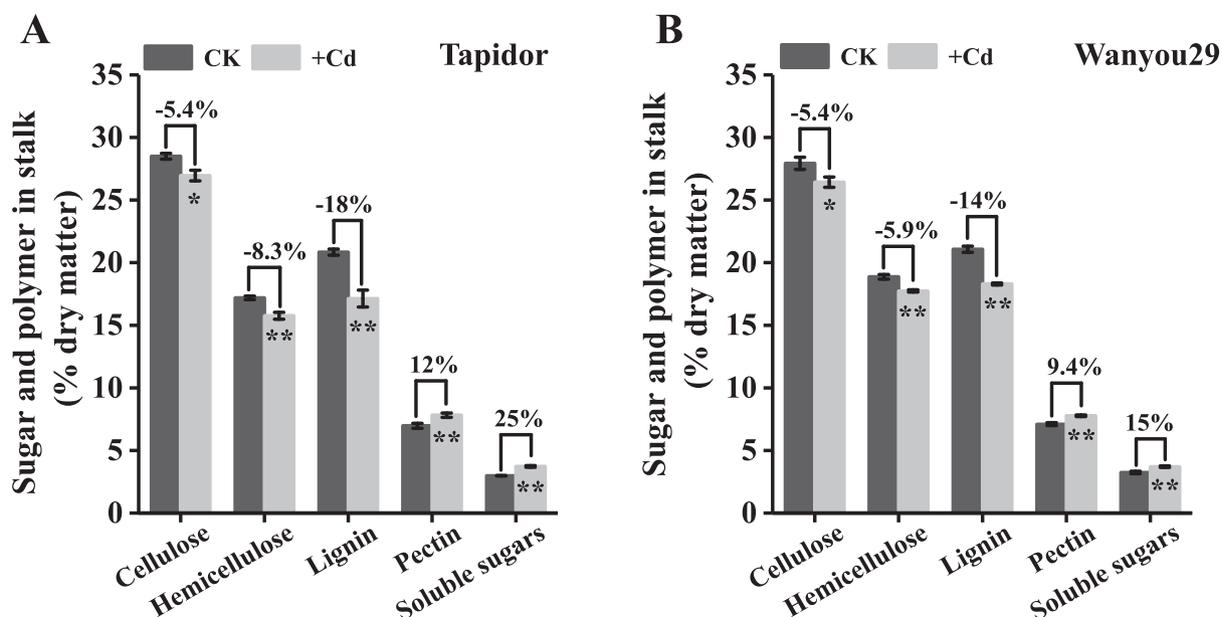


Fig. 5. Cell wall polymer and soluble sugars (% dry matter) in mature stalks of Tapidor (A) and Wanyou29 (B) supplied with 120 mg/kg CdCl₂ (+Cd) or without CdCl₂ (CK). * and ** indicated significant difference between two samples by *t*-test at $p < 0.05$ and 0.01 ($n = 3$). Increased or decreased percentage obtained by subtraction between two samples divided CK. Bar as mean \pm SD ($n = 3$).

ized that Cd accumulation could inhibit cell wall biosynthesis for reduced biomass yield (Durenne et al., 2018; Ko et al., 2017; Liu et al., 2010), the increased soluble sugars and pectin may be partially from the excess carbohydrates that were not used for cellulose and hemicellulose biosynthesis via dynamical carbon partitioning regulation in the Cd-accumulated stalks (Fan et al., 2017; Yao et al., 2018).

3.5. Reduced cellulose DP and raised lignocellulose porosity in the Cd-accumulated stalks

To further find out how the biomass enzymatic saccharification was enhanced in the Cd accumulated stalks, this work examined cellulose DP and lignocellulose porosity in two rapeseed cultivars. Compared to the controls (CK, without Cd), the Cd accumulated stalks showed significantly reduced cellulose DP by 7.6% and 9.5% in two rapeseed cultivars at $p < 0.01$ level (Fig. 6A; Fig. S2A), whereas the cellulose accessibility (cellulose surface area measured by Congo red dye staining) of two cultivars was respectively increased by 13% and 16%, probably due to the Cd accumulation

(Fig. 6B; Fig. S2B). Notably, the optimal two-step chemical (4.0% NaOH + 2.0% H₂SO₄) pretreatments could also reduce cellulose DP by 12%–21% with much increased accessibility by 120%–146% in both Cd accumulated stalks and controls (CK, without Cd) of two rapeseed cultivars, compared to their raw materials (Fig. 6A, B). Because the reduced cellulose DP is accountable for more ends of β -1,4-glucans allowed for cellobiohydrolase attack (Huang et al., 2011; Li et al., 2018), and the increased cellulose accessibility could provide more space for major cellulases enzymes loading (Alam et al., 2019), it should be understandable why largely enhanced biomass enzymatic saccharification occurred in the Cd-accumulated rapeseed stalks particularly after two-step chemical pretreatments as described above.

Using Simons' stain, this study evaluated that the Cd-accumulated stalks had significantly increased biomass porosity by 6.3%–14% including large pore (DY), small pore (DB) and total pore amounts in two rapeseed cultivars, compared with their controls (Fig. S2C–D). As Simons' stain is accounting for plant cell wall porosity (Alam et al., 2019; Macaskill et al., 2018; Meng et al., 2015), the increase of DY and DB should be mainly due to much

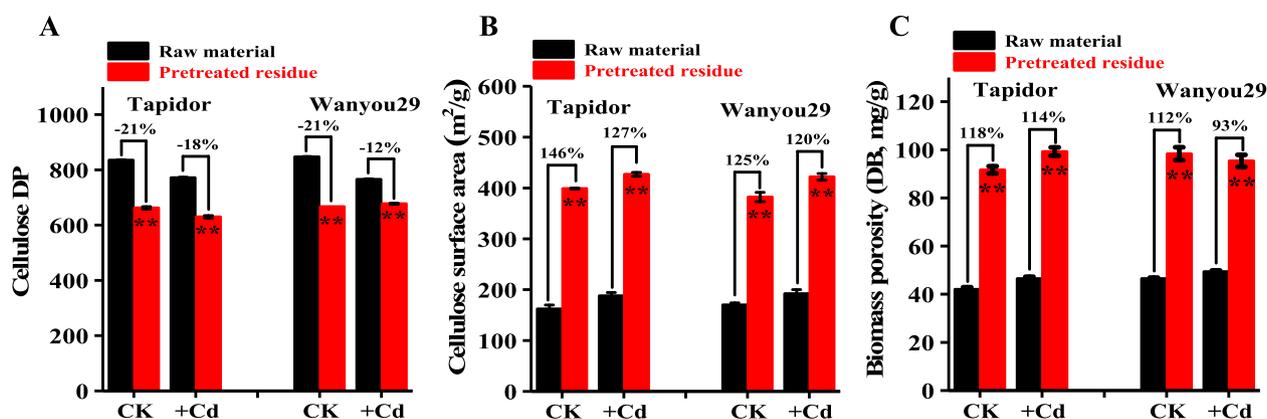


Fig. 6. Cellulose features and biomass porosity in raw materials and pretreated biomass residues of mature stalks in two rapeseed cultivars (Tapidor, Wanyou29) supplied with 120 mg/kg CdCl₂ (+Cd) or without CdCl₂ (CK). (A) Cellulose DP; (B) Cellulose surface area (accessibility) measured by Congo red (CR) stain; (C) Biomass porosity (total pore amounts) measured by Simons' stain. The pretreated residues were obtained using optimal two-step chemical (4.0% NaOH + 2.0% H₂SO₄) pretreatments. ** indicated significant difference between two samples by *t*-test at $p < 0.01$ ($n = 3$). Increased or decreased percentage obtained by subtraction between two samples divided by the raw material. Bar as mean \pm SD ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reduced wall polymers (cellulose, hemicellulose, lignin) in the Cd-accumulated stalks. Meanwhile, this work found that the optimal two-step chemical (4.0% NaOH + 2.0% H₂SO₄) pretreatment could only lead to significant increase of DB, rather than DY in two rapeseed cultivars (Fig. 6C), probably due to effective extraction of wall polymers from the two-step pretreatment. To confirm this, we observed fourier transform infrared (FTIR) spectroscopic profiling in the raw materials and pretreated residues of two rapeseed cultivars (Fig. 7; Table S2) (Goshadrou et al., 2013; Goshadrou & Lefsrud, 2017; Haque et al., 2013; Ravindran et al., 2017; Guan et al., 2018; Zhou et al., 2016). Compared to the raw materials, the pretreated residues exhibited distinct alterations of seven peaks corresponding for three major wall polymers inter-linkage styles (C-O-C, C = C, -C = O, C-H, C-H₂, C-H₃), in particular for the Cd-accumulated stalks (Fig. 7C,D). For instance, the peak for lignin linkage style (C = C located at 1590 cm⁻¹) was almost lost and the peak for hemicellulose and lignin linkage (C = O located at 1735 cm⁻¹) was large reduced in the pretreated residues compared to their raw materials, suggesting that the optimal two-step

chemical pretreatment could effectively extract major wall polymers (hemicellulose, lignin). The results were also consistent with almost complete Cd release from the optimal two-step chemical pretreatments in two rapeseed cultivars as described above.

3.6. Mechanism of much Cd accumulation and high cellulosic ethanol production in rapeseed stalks

To sort out why the rapeseed stalks largely accumulated Cd and how the Cd-accumulated stalks showed high biomass enzymatic saccharification and bioethanol production, this study performed correlation analysis and proposed a hypothetic model (Fig. 8). Although it has been characterized that non-cellulosic polymers (hemicellulose, lignin, pectin) could be tightly associated with trace metals in the mature crop straws (Isaure et al., 2015; Ma et al., 2015), this study determined relatively higher pectin contents (ranged 7.0%-8.0% dry matter) in two rapeseed cultivars (Fig. 5), which may partially explain why rapeseed stalks accumulated much more Cd than those of other crop straws (Table 1).

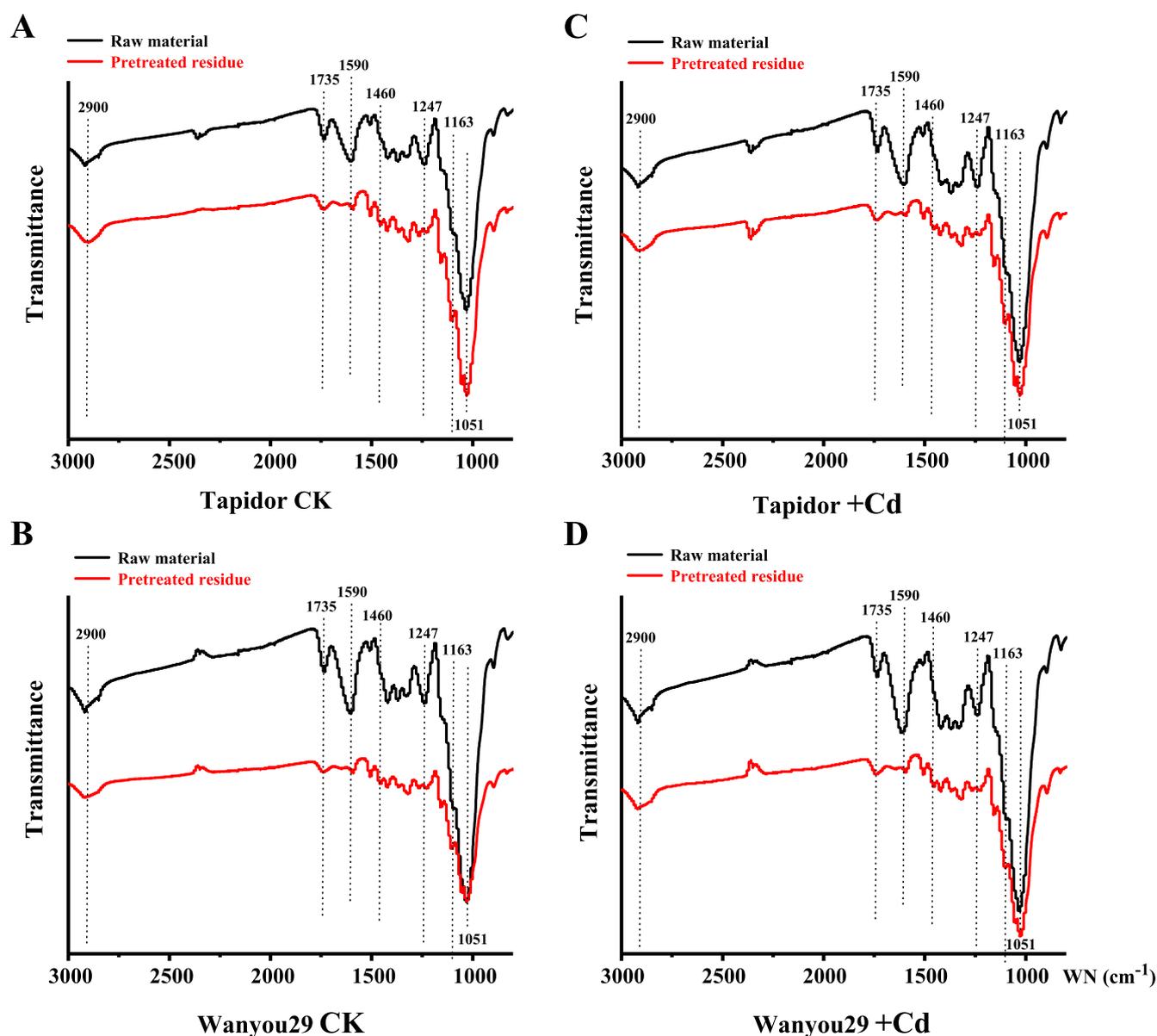


Fig. 7. Fourier transform infrared (FTIR) spectroscopic profiling in raw material and pretreated residues of mature stalks in two rapeseed cultivars (Tapidor, Wanyou29) supplied with 120 mg/kg CdCl₂ (+Cd) or without CdCl₂ (CK). The pretreated residues were obtained using optimal two-step chemical (4.0% NaOH + 2.0% H₂SO₄) pretreatments. Information about characteristic bands of the FTIR listed in Table S2.

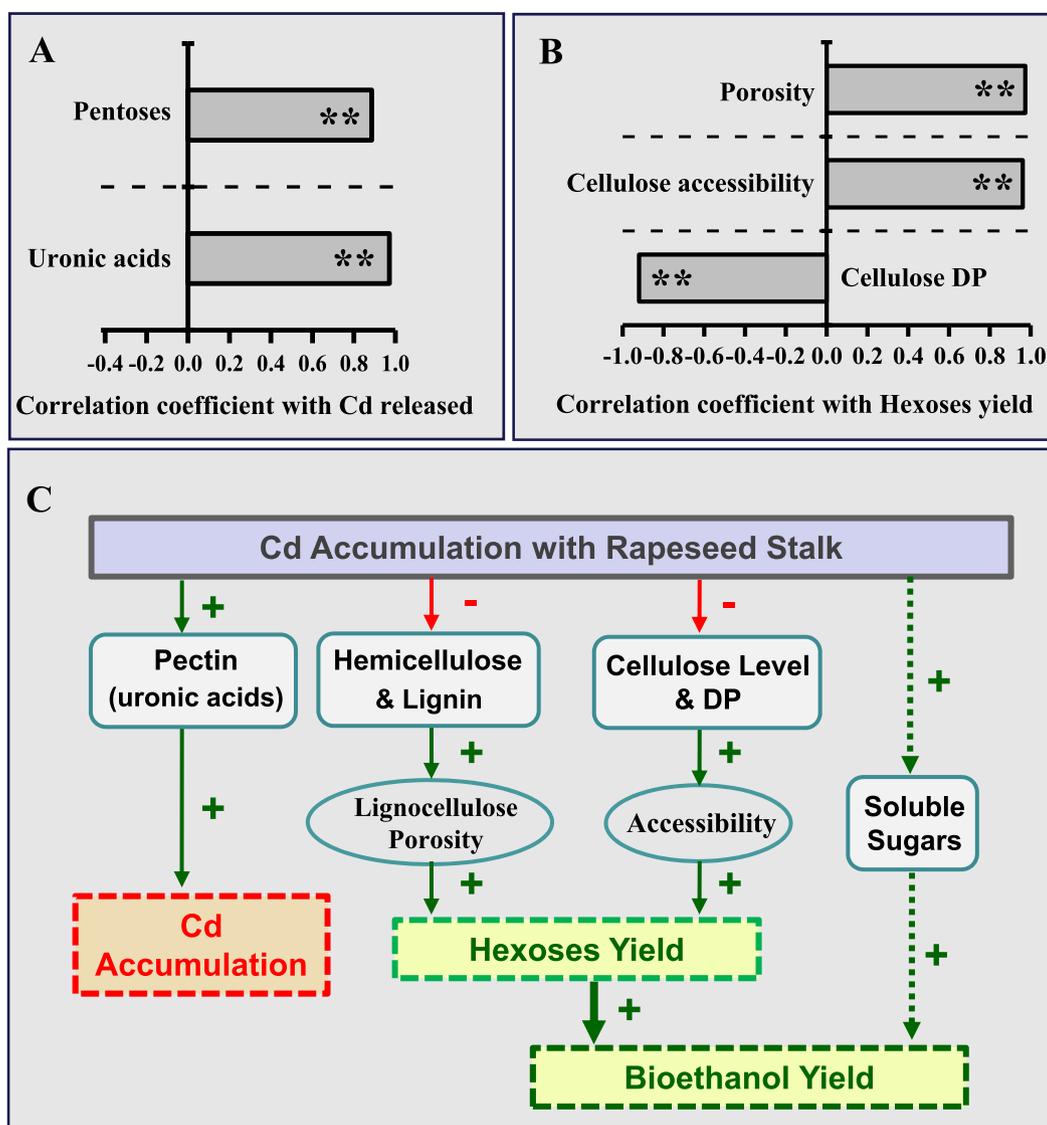


Fig. 8. Correlation analysis and hypothetical model on Cd accumulation and bioethanol co-production in rapeseed stalks. (A) Correlation coefficients between uronic acids of pectin or pentoses of hemicellulose and Cd released from various chemical pretreatments and enzymatic hydrolysis ($n = 12$); (B) Correlation coefficients between cellulose DP or cellulose accessibility (surface area) or biomass porosity (total pore amounts) and hexose yields released from enzymatic hydrolysis ($n = 6$). ** indicated significant correlation at $p < 0.01$ level. (C) A hypothetical model highlighting major factors or impacts on the Cd accumulation and hexoses and ethanol yields in mature rapeseed stalks; "+" and "-" indicated as positive and negative impacts, respectively.

Notably, despite of significantly reduced hemicellulose and lignin in the Cd-accumulated stalks of two rapeseed cultivars (Romero-Güiza et al., 2017), their pectin levels were remarkably increased, suggesting that pectin may be a major factor for Cd adsorption in the rapeseed stalk probably due to chemical binding between Cd^+ and uronic acids of pectin (Higuchi et al., 2016; Krzesłowska, 2011). To confirm this finding, correlation analyses were performed between one major component (uronic acids) of pectin and the Cd amounts released from various chemical pretreatments and enzymatic hydrolysis as described above (Fig. 4). Significantly, the uronic acids of pectin showed a positive correlation with the Cd at $p < 0.01$ level, with extremely high relative coefficient value at 0.972 (Fig. 8A). Meanwhile, this study also examined a strongly positive correlation between the pentoses of hemicellulose and Cd released from various chemical pretreatments and enzymatic hydrolysis with relative coefficient value of 0.886 (Fig. 8A), which confirmed that there was a strong Cd interaction with hemicellulose and pectin in the mature rapeseed stalks.

With regard to much enhanced biomass enzymatic saccharification, this work performed correlation analyses between major lignocellulose features and hexoses yields released from enzymatic hydrolysis in two rapeseed cultivars (Fig. 8B). By contrast, cellulose DP was negatively correlated with hexoses yield at $p < 0.01$ level, whereas both cellulose accessibility and biomass porosity showed significantly positive correlations with hexoses yields, consistent with the previous reports about their distinct impacts on biomass enzymatic saccharification in various lignocellulose residues examined (Alam et al., 2019; Hu et al., 2018; Karimi & Taherzadeh, 2016; Li et al., 2018; Meng et al., 2015). Based on the major findings achieved in this study, a hypothetical model was proposed to highlight major factors accounting for much Cd accumulation and high biomass enzymatic saccharification and bioethanol production in rapeseed cultivars (Fig. 8C). Therefore, this study could not only provide a green-like strategy for Cd phytoremediation by increasing pectin level in rapeseed and other crops, but it also indicated an applicable approach for value-

added cellulosic ethanol production with minimum waste release into the environment.

4. Conclusion

Two rapeseed cultivars were examined with remarkably more Cd accumulation in their mature stalks compared to other major food crops, mainly due to much pectin deposition and significantly altered cell wall compositions and polymer features. Under three optimal chemical pretreatments, the Cd-accumulated rapeseed stalks showed largely enhanced biomass enzymatic saccharification and bioethanol production by significantly increasing cellulose accessibility and lignocellulose porosity, along with a complete Cd release. Therefore, this study has provided a powerful strategy for both Cd phytoremediation and value-added cellulosic ethanol co-production in rapeseed and beyond.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.135096>.

References

- Alam, A., Zhang, R., Liu, P., Huang, J.F., Wang, Y.T., Hu, Z., et al., 2019. A finalized determinant for complete lignocellulose enzymatic saccharification potential to maximize bioethanol production in bioenergy *Miscanthus*. *Biotechnol. Biofuels* 12 (1), 99–120.
- Chandra, R., Ewanick, S., Hsieh, C., Sessler, J.N., 2010. The characterization of pretreated lignocellulosic substrates prior to enzymatic hydrolysis, part 1: a modified Simons' staining technique. *Biotechnol. Prog.* 24 (5), 1178–1185.
- Chen, Z.F., Zhao, Y., Fan, L.D., Xing, L.T., Yang, Y.J., 2015. Cadmium (Cd) localization in tissues of cotton (*Gossypium hirsutum* L.), and its phytoremediation potential for Cd-contaminated soils. *Bulletin of Environmental Contamination and Toxicology* 95 (6), 784–789.
- Desai, M., Haigh, M., Walkington, H., 2019. Phytoremediation: Metal decontamination of soils after the sequential forestation of former opencast coal land. *Sci. Total Environ.* 656, 670–680.
- Douchiche, O. D., Azeddine, & Morvan, C. (2010). Spatial regulation of cell-wall structure in response to heavy metal stress: cadmium-induced alteration of the methyl-esterification pattern of homogalacturonans. *Annals of Botany*, 105(3), 481–491.
- Durenne, B., Druart, P., Blondel, A., & Fauconnier, M. L. (2018). How cadmium affects the fitness and the glucosinolate content of oilseed rape plantlets. *Environmental and Experimental Botany*, 155, 185–194.
- Fan, C.F., Feng, S.Q., Huang, J.F., Wang, Y.T., Wu, L.M., Li, X.K., et al., 2017. *AtCesA8*-driven *OsSUS3* expression leads to largely enhanced biomass saccharification and lodging resistance by distinctively altering lignocellulose features in rice. *Biotechnol. Biofuels* 10 (1), 221–232.
- Goshadrou, A., Karimi, K., Lefsrud, M., 2013. Characterization of ionic liquid pretreated aspen wood using semi-quantitative methods for ethanol production. *Carbohydr. Polym.* 96 (2), 440–449.
- Goshadrou, A., Lefsrud, M., 2017. Synergistic surfactant-assisted [EMIM]OAc pretreatment of lignocellulosic waste for enhanced cellulose accessibility to cellulase. *Carbohydr. Polym.* 166, 104–113.
- Guan, R., Li, X., Wachemo, A.C., Yuan, H., Liu, Y., Zou, D., et al., 2018. Enhancing anaerobic digestion performance and degradation of lignocellulosic components of rice straw by combined biological and chemical pretreatment. *Sci. Total Environ.* 637–638, 9–17.
- Haque, M.A., Barman, D.N., Kang, T.H., Kim, M.K., Kim, J.H., Kim, H., et al., 2013. Effect of dilute alkali pretreatment on structural features and enhanced enzymatic hydrolysis of *Miscanthus sinensis* at boiling temperature with low residence time. *Biosyst. Eng.* 114 (3), 294–305.
- Higuchi, K., Ito, N., Nukada, T., 2016. Prediction of the structure of a novel amylopectin-based Cd-associated molecule in the stem of common reed grown in the presence of Cd. *Biosci. Biotechnol. Biochem.* 80 (10), 1960–1965.
- Hu, M., Yu, H., Li, Y., Li, A., Cai, Q., Liu, P., et al., 2018. Distinct polymer extraction and cellulose DP reduction for complete cellulose hydrolysis under mild chemical pretreatments in sugarcane. *Carbohydr. Polym.* 202, 434–443.
- Huang, T., Cheng, K.H., Wang, C.H., 2011. Effects of Tween-80 and alkali pretreatment on the content of lignin of straw and enzymatic hydrolysis. *Journal of Cellulose Science and Technology* 19 (2), 52–58.
- Isaure, M.P., Hugué, S., Meyer, C.L., Castillomichel, H., Testemale, D., Vantelon, D., et al., 2015. Evidence of various mechanisms of Cd sequestration in the hyperaccumulator *Arabidopsis halleri*, the non-accumulator *Arabidopsis lyrata*, and their progenies by combined synchrotron-based techniques. *J. Exp. Bot.* 66 (11), 3201–3214.
- Jeong, T.S., Um, B.H., Kim, J.S., Oh, K.K., 2010. Optimizing dilute-acid pretreatment of rapeseed straw for extraction of hemicellulose. *Appl. Biochem. Biotechnol.* 161 (1–8), 22–33.
- Jin, W.X., Chen, L., Hu, M., Sun, D., Li, A., Li, Y., et al., 2016. Tween-80 is effective for enhancing steam-exploded biomass enzymatic saccharification and ethanol production by specifically lessening cellulase absorption with lignin in common reed. *Appl. Energy* 175, 82–90.
- Karimi, K., Taherzadeh, M.J., 2016. A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. *Bioresour. Technol.* 203, 348–356.
- Ko, C.H., Yu, F.C., Chang, F.C., Yang, B.Y., Chen, W.H., Hwang, W.S., et al., 2017. Bioethanol production from recovered napier grass with heavy metals. *J. Environ. Manage.* 203, 1005–1010.
- Krzyszowska, M., 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. *Acta Physiologiae Plantarum* 33 (1), 35–51.
- Kuglarz, M., Alvarado-Morales, M., Dąbkowska, K., Angelidaki, I., 2018. Integrated production of cellulosic bioethanol and succinic acid from rapeseed straw after dilute-acid pretreatment. *Bioresour. Technol.* 265, 191–199.
- Li, F. C., Xie, G. S., Huang, J. F., Zhang, R., Li, Y., Zhang, M. M., et al. (2017). OsCESA9 conserved-site mutation leads to largely enhanced plant lodging resistance and biomass enzymatic saccharification by reducing cellulose DP and crystallinity in rice. *Plant Biotechnology Journal*, 15(9), 1093–1104.
- Li, M., Si, S.L., Hao, B., Zha, Y., Wan, C., Hong, S.F., et al., 2014. Mild alkali-pretreatment effectively extracts guaiacyl-rich lignin for high lignocellulose digestibility coupled with largely diminishing yeast fermentation inhibitors in *Miscanthus*. *Bioresour. Technol.* 169 (5), 447–454.
- Li, Y., Liu, P., Huang, J.F., Zhang, R., Hu, Z., Feng, S.Q., et al., 2018. Mild chemical pretreatments are sufficient for bioethanol production in transgenic rice straws overproducing glucosidase. *Green Chem.* 20 (9), 2047–2056.
- Lin, H.M., Fang, C.X., Li, Y.Z., Lin, W.W., He, J.Y., Lin, R.Y., et al., 2016. Effect of silicon on grain yield of rice under cadmium-stress. *Acta Physiologiae Plantarum* 38 (7), 186–198.
- Lin, Y.F., Aarts, M.G.M., 2012. The molecular mechanism of zinc and cadmium stress response in plants. *Cell. Mol. Life Sci.* 69 (19), 3187–3206.
- Liu, L., Li, J.W., Yue, F.X., Yan, X.W., Wang, F.Y., Bloszies, S., et al., 2018. Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. *Cosmosphere* 194, 495–503.
- Liu, J.G., Cai, G.L., Qian, M., Wang, D.K., Xu, J.K., Yang, J.C., et al., 2010. Effect of Cd on the growth, dry matter accumulation and grain yield of different rice cultivars. *J. Sci. Food Agric.* 87 (6), 1088–1095.
- Ma, J., Cai, H. M., He, C. W., Zhang, W. J., & Wang, L. J. (2015). A hemicellulose-bound form of silicon inhibits cadmium ion uptake in rice (*Oryza sativa*) cells. *New Phytologist*, 206(3), 1063–1074.
- Macaskill, J.J., Suckling, I.D., Lloyd, J.A., Manley-Harris, M., 2018. Unravelling the effect of pretreatment severity on the balance of cellulose accessibility and substrate composition on enzymatic digestibility of steam-pretreated softwood. *Biomass Bioenergy* 109, 284–290.
- Mcgrath, S.P., Zhao, J., Lombi, E., 2002. Phytoremediation of metals, metalloids, and radionuclides. *Adv. Agron.* 75 (02), 1–56.
- Meng, X.Z., Wells Jr., T., Sun, Q.N., Huang, F., Ragauskas, A., 2015. Insights into the effect of dilute acid, hot water or alkaline pretreatment on the cellulose accessible surface area and the overall porosity of populus. *Green Chem.* 17 (8), 4239–4246.
- Murakami, M., Ae, N., Ishikawa, S., 2007. Phytoextraction of cadmium by rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.). *Environ. Pollut.* 145 (1), 96–103.
- Pei, Y.J., Li, Y.Y., Zhang, Y.B., Yu, C.B., Fu, T.D., Zou, J., et al., 2016. G-lignin and hemicellulosic monosaccharides distinctively affect biomass digestibility in rapeseed. *Bioresour. Technol.* 203, 325–333.
- Peng, L., Hocart, C.H., Redmond, J.W., Williamson, R.E., 2000. Fractionation of carbohydrates in *Arabidopsis* root cell walls shows that three radial swelling loci are specifically involved in cellulose production. *Planta* 211 (3), 406–414.
- Puri, V.P., 2010. Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. *Biotechnol. Bioeng.* 26 (10), 1219–1222.
- Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K., 2017. A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresour. Technol.* 248, 69–75.

- Romero-Güiza, M.S., Wahid, R., Hernández, V., Møller, H., Fernández, B., 2017. Improvement of wheat straw anaerobic digestion through alkali pre-treatment: Carbohydrates bioavailability evaluation and economic feasibility. *Sci. Total Environ.* 595, 651–659.
- Ronzan, M., Piacentini, D., Fattorini, L., Rovere, F. D., Eiche, E., Riemann, M., et al. Cadmium and arsenic affect root development in *Oryza sativa* L. negatively interacting with auxin *Environmental and Experimental Botany* 151 2018 64 75
- Shi, G.L., Zhu, S., Bai, S.N., Xia, Y., Lou, L.Q., Cai, Q.S., 2015. The transportation and accumulation of arsenic, cadmium, and phosphorus in 12 wheat cultivars and their relationships with each other. *J. Hazard. Mater.* 299, 94–102.
- Shuai, L., Yang, Q., Zhu, J.Y., Lu, F.C., Weimer, P.J., Ralph, J., et al., 2010. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. *Bioresour. Technol.* 101 (9), 3106–3114.
- Si, S.L., Chen, Y., Fan, C.F., Hu, H.Z., Li, Y., Huang, J.F., et al., 2015. Lignin extraction distinctively enhances biomass enzymatic saccharification in hemicelluloses-rich *Miscanthus* species under various alkali and acid pretreatments. *Bioresour. Technol.* 183, 248–254.
- Sun, D., Alam, A., Tu, Y.Y., Zhou, S.G., Wang, Y.T., Xia, T., et al., 2017. Steam-exploded biomass saccharification is predominately affected by lignocellulose porosity and largely enhanced by Tween-80 in *Miscanthus*. *Bioresour. Technol.* 239, 74–81.
- Tangahu, B.V., Abdullah, S.R.S., Basri, H., Idris, M., Anuar, N., Mukhlisin, M., 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng.* 2011, 1–31.
- Wu, L.M., Feng, S.Q., Deng, J., Yu, B., Wang, Y.M., He, B.Y., et al., 2019. Altered carbon assimilation and cellulose accessibility to maximize bioethanol yield under low-cost and green-like biomass processing in corn brittle stalk. *Green Chem.* 16 (21), 4388–4399.
- Wu, Z.L., Zhang, M.L., Wang, L.Q., Tu, Y.Y., Zhang, J., Xie, G.S., et al., 2013. Biomass digestibility is predominantly affected by three factors of wall polymer features distinctive in wheat accessions and rice mutants. *Biotechnol. Biofuels* 6 (1), 183–183.
- Z.L. Wu H.H. Hao Tu. Zahoor Y. Y., Zheng, H., Feng, W., et al. Diverse cell wall composition and varied biomass digestibility in wheat straw for bioenergy feedstock *Biomass and Bioenergy* 70 2014 347 355
- Xu, Z.M., Li, Q.S., Yang, P., Ye, H.J., Chen, Z.S., Guo, S.H., et al., 2017. Impact of osmoregulation on the differences in Cd accumulation between two contrasting edible amaranth cultivars grown on Cd-polluted saline soils. *Environ. Pollut.* 224, 89–97.
- Yang, J., Li, K., Zheng, W., Zhang, H., Cao, X., Lan, Y., et al., 2015. Characterization of early transcriptional responses to cadmium in the root and leaf of Cd-resistant *Salix matsudana* Koidz. *BMC Genomics* 16 (1), 705.
- Yao, W.Q., Lei, Y.K., Yang, P., Li, Q.S., Wang, L.L., He, B.Y., et al., 2018. Exogenous glycinebetaine promotes soil cadmium uptake by edible amaranth grown during subtropical hot season. *Int. J. Environ. Res. Public Health* 15 (9), 1794–1804.
- Zhang, X.F., Xia, H.P., Li, Z., Zhuang, P., Gao, B., 2010. Potential of four forage grasses in remediation of Cd and Zn contaminated soils. *Bioresour. Technol.* 101 (6), 2063–2066.
- Zhou, X.F., Li, Q., Zhang, Y.L., Gu, Y., 2016. Effect of hydrothermal pretreatment on *Miscanthus* anaerobic digestion. *Bioresour. Technol.* 224, 721–726.