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Interactive effects between cadmium stabilized by palygorskite and mobilized by siderophores from *Pseudomonas fluorescens*

Jian-Jun Jiang, Jun-Feng Wang, Ping Yang, Zhi-Min Xu, Tao He, Qiong Gao, Li-Li Wang, Qu-Sheng Li*

School of Environment, Key Laboratory of Environmental Pollution and Health of Guangdong Province, Jinan University, Guangzhou, 510632, China

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ABSTRACT

The application of palygorskite (PAL) for potentially toxic trace elements (Cd^{2+}, Ni^{2+}, etc.) remediation in polluted soil can substantially reduce the bioavailability and toxicity of these hazard materials. However, the secretion of organic acids and siderophores by microorganisms might result in the re-mobilization of cadmium (Cd) in PAL-bound forms (PAL-Cd). In this study, the interactive effects between Cd stabilized by PAL and mobilized by siderophores from *Pseudomonas fluorescens* were performed with four flask-shaking experimental treatments, namely, strain with or without an ability of siderophores production respectively associated with or without PAL-Cd. The GC-MS and UHPLC-MS test methods were used to analyze the concentrations of metabolites. Results showed that the Cd mobilized by strain with siderophores production was 22.1% higher than that of strain without the ability of siderophores production ($p < 0.05$). The mobilization of Cd in PAL in turn significantly reduced the siderophores production of *Pseudomonas fluorescens* by 25.1% ($p < 0.05$). The numbers of metabolites significantly up-regulated and down-regulated were 9 and 22 in strain groups with PAL-Cd addition compared with the groups without PAL-Cd, respectively. Metabolomics analysis revealed that the mobilized Cd affects the signal transduction pathway and primary metabolic processes, reduces the metabolic capacity of pentose phosphate pathway, glycolysis and tricarboxylic acid cycle pathway. These changes inhibit the ability of strain to biosynthesize amino acids during the mobilization processes, further reducing the capacity of *Pseudomonas fluorescens* to produce siderophores. This study provides a useful information on how to select soil Cd-stabilizing materials in a targeted manner and how to avoid Cd re-mobilization by siderophores.

1. Introduction

Cadmium (Cd) is one of the potentially toxic trace elements (PTEs) with high toxicity and long biological half-life (Duffus, 2002; Hooda and Peter, 2010). Cd in soil can be transmitted through the food chain, thereby posing a serious risk to human health (Rizwan et al., 2016). During the remediation processes of Cd polluted soil, clay mineral can substantially reduce the exchangeable Cd concentration in soil matrix, effectively decrease the Cd uptake by plants with low cost (Li and Xu, 2018; Wu et al., 2016). Palygorskite (PAL), one of the clay minerals, is widely applies into the remediation of PTEs polluted soil because it is natural alkaline characteristic and high-adsorption properties (Sheikhhosseini et al., 2013), thereby effectively reducing the bioavailable content of Cd in soil (Liang et al., 2014). The percentage of eluting Cd from the acidic and calcareous polluted soil amended with PAL were 69.6% and 60.6%, respectively (Zhang and Pu, 2011). In addition, it has been reported that the Cd concentration of brown rice decreases from 0.26 mg/kg to 0.18 mg/kg when 15.0 g/kg PAL was mixed into soil (Li and Xu, 2017). Therefore, PAL has been considered as a widely used natural material for the remediation of Cd contaminated soil.

In Cd polluted soil amended with clay minerals, the wide distribution of microbes, such as *Rahnella* sp. *JN6*, *Burkholderia* sp. *J62*, and *Burkholderia* sp. *D54*, etc., can secrete organic acids to mobilize Cd (Guo et al., 2011; He et al., 2013). Generally, these acids re-mobilize Cd stabilized by PAL through the complexation effects of hydroxyl and carboxyl groups and their acid dissolution effects (Jeong et al., 2012; Yang et al., 2018), which has been considered as a serious risk for the remediation efficiency of clay minerals in Cd contaminated soils. Besides, the mobilization of Cd in soil can be also affected by soil microbes through their secondary metabolites, such as siderophores (Chen et al., 2014; He et al., 2013). Siderophores, low-molecular-weight substances, can chelate Fe^{3+} and then transfer the Fe into the intermembrane of microbial cells to ensure their normal growth (Schalk et al., 2011). In

* Corresponding author. School of Environment, Jinan University, Guangzhou, 510632, China.
E-mail address: liqusheng@21cn.com (Q.-S. Li).

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addition, these compounds also can mobilize and transfer the heavy metal ions, such as Ni^{2+} and Cd^{2+} (Ferret et al., 2015), possibly resulting in the secondary release and mobilization of PTEs in soil. Although the secretion of organic acids and siderophores by microorganism can re-mobilize the Cd amend with PAL, there are few reports about the metabolomic profiling of these compounds in microbes and their influence on the mobilization of Cd amended with PAL.

Pseudomonas fluorescens, one of the plant rhizosphere-promoting bacteria, owns the capacity of siderophores and organic acids secretion and is a dominant microbial species in the rhizosphere of crops (Príncipe et al., 2018). These biological functions have been considered as the main effects for the re-mobilization of Cd in remediation soil. Remarkably, the re-mobilized Cd might result in serious metabolic deficiencies, such as the biosynthesis and secretion of succinic acid, malic acid, fumaric acid and siderophores, of microorganisms (Ma et al., 2016; Welikala et al., 2018). The decreasing production of organic acids, amino acids and siderophores of bacteria might inhibit the re-mobilization effect of Cd in remediation soil. However, the ability of producing siderophores for some bacterial strains were stimulated by the toxic metals, such as Cu^{2+} and Ni^{2+} (Dimkpa et al., 2008). Therefore, the mechanism of toxic metal forms changed with the capability of strains of secreting siderophores is unclear completely.

Herein, the present study set out to investigate the interactive effects between Cd stabilized by PAL and mobilized by siderophore from *Pseudomonas fluorescens*. We aimed to reveal that: i) the re-mobilization effect of Cd amended with PAL by siderophores associated with *Pseudomonas fluorescens*; ii) the qualitatively and quantitatively analyses of metabolites produced by *Pseudomonas fluorescens*; and iii) the mechanism of siderophores production as affected by re-mobilized Cd with metabolomics research methods.

2. Materials and methods

2.1. Experimental materials preparation

Pseudomonas fluorescens is purchased from Guangdong Culture Collection (collection No. GIM 1. 209). A modified sugar-aspartic acid (MSA) medium (composition: 2.0 g/L sucrose, 2.0 g/L $(NH_4)_2SO_4$, 1.0 g/L K_2HPO_4, and 0.5 g/L $MgSO_4$) with pH 7.0 is used to acclimate this strain (Scher and Baker, 1982). Different concentrations of Cd^{2+} (5.0, 10.0, 15.0, 20.0, 40.0 mg/L) were used to perform a Cd tolerance test (48 h) of *Pseudomonas fluorescens* on this medium. The pH, OD_{600}, and siderophores parameters are monitored every 8 h (Fig.S1). As shown in Table 1, the strain does not produce siderophores in MSA medium with a Fe^{3+} concentration above 20 μM ($FeCl_3$, ≥99.99%, Aladdin).

PAL (Hebei, China) of fibrous crystal is washed with deionized water and absolute ethanol, dried, and sieved (100 mesh). 40 g of PAL and 1600 mL of 1 mM $Cd(NO_3)_2{\cdot}4H_2O$ solution are taken, shaken for 16 h to obtain PAL binding Cd (PAL-Cd), then dried at condition of vacuum freezer. After eluted with 0.1 M HCl, PAL-Cd is washed with deionized water until Cd is undetected in water, to acquire stable PAL-Cd, then dried again in a vacuum freezer. PAL-Cd is characterized through scanning electron microscopy (SEM, FEI Quanta 650, USA) and energy dispersive X-Ray spectroscopy analysis [Fig. S2 (A)]. The Cd concentration of PAL-Cd were determined with a flame atomic absorption spectrophotometer (900T, PerkinElmer) after the digestion with 10 mL mixed acids of nitric acid, hydrochloric acid and hydrofluoric acid (3:1:1, v/v/v) in microwave (Mars 6, CEM). Finally, the Cd content in PAL-Cd is 3.80 ± 0.28 mg/g.

Table 1
The characteristics of *Pseudomonas fluorescens* producing siderophores.

Fe^{3+} concentration (μM)	Siderophore yield (%)	Fluorescence
0	90.5	+
1	3.7	+
5	2.6	+
10	1.4	+
20	ND	-
50	ND	-
100	ND	-
200	ND	-

Note: ND is the abbreviated word of "not detected". "Fluorescence" and "non-fluorescence" are indicated by the symbol of "+" and "-" at a wavelength of 365 nm, respectively.

2.2. Cd re-mobilization experimental set up

PAL-Cd (0.51 g) was obtained and sterilized at 121 °C for 20 min in autoclave (LDZX-75KBS, Shanghai). Fifty milliliters of MSA medium is dispensed into a triangular flask and inoculated with *Pseudomonas fluorescens*. Four experimental treatments are prepared (eight duplicates in each treatment): (a) strain with PAL-Cd and 200 μM Fe^{3+} (Bcd + Fe + gim group, no siderophores production); (b) strain with PAL-Cd (Bcd + gim group, siderophores production); (c) strain with 200 μM Fe^{3+} (Fe + gim group, no siderophores production); (d) strain (gim group, siderophores production). These treatments are cultured in a biochemical shaker incubator for 48 h and monitored every 8 h. The bacteria liquid of MSA medium was centrifuged, the pH (Mettler, Switzerland), Cd concentration of supernatant was determined after filtered (0.22 μm polyether sulfone membrane), and a similar process was employed to analyze the Cd concentration of Bcd + gim and Bcd + Fe + gim groups. Meanwhile, the gas chromatography-mass spectrometry (GC-MS, Shimadzu, Japan) and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS, Agilent 1290 Infinity LC, Triple TOF 5600+ of AB SCIEX) respectively used to analyze the concentration of metabolites in extracellular solutions of each group. Finally, the siderophores content was tested according the standard methods with an ultraviolet spectrophotometer (UV-2600, Shimadzu, Japan) (Singh and Mishra, 2015). After freeze-dried, the surface characteristic of PAL-Cd was also determined by SEM and energy dispersive X-Ray spectroscopy with the above-mentioned analyzers.

2.3. Sampling pe-preparation and analysis

The powder sample of PAL-Cd is evenly sprinkled on the surface of conductive adhesive, fixed on the copper column, and vapor-deposited in gold plating instrument (EMS150R, UK). The surface is uniformly plated with gold powder with a thickness of 10 A°.

A universal method of Chrome azurol S (CAS) is selected as the quantitative detection method for siderophores (Schwyn and Neilands, 1987). Firstly, extracellular solutions of *Pseudomonas fluorescens* were centrifuged at 3000 × *g* at 4 °C for 10 min. Then the supernatant was passed through a 0.22 μm filter. After that, the filtrate and MSA medium liquid were mixed with an equal volume of 3 mL as CAS solution, and the absorbance was tested at a wavelength of 650 nm after light avoidance and reaction for 2 h and named as A_s and A_r, respectively. Siderophores concentration was finally calculated with the following formula: $SU = \frac{[A_r - A_s]}{A_r} \times 100\%$ (Singh and Mishra, 2015).

A 500 μL of extracellular solution was obtained, fully mixed with 100 μL salicylic acid with a concentration of 10 mg/L, and dried in a vacuum refrigerating machine (UATIL Coolsafe 55-4, China). After derivatization (orderly adding 40 μL 20 g/L methoxyamine acid salts pyridine and 70 μL of MSTFA, oscillatory reaction for 120 min and 30 min at 37 °C, respectively. *N*-hexane constant volume was set to 1000 μL), extracellular secretions were tested through GC-MS. The method and key parameter settings of instrument have been provided in previous study (Guo et al., 2018).

A 200 μL of each sample was collected and thoroughly mixed as the

quality control (QC) sample. Each sample (200 µL), including QC sample, was respectively mixed with 800 µL of cold methanol/acetonitrile solution (2:2, v/v), ultra-sounded 30 min at −20 °C, and placed for 10 min. Then, each solution was centrifuged at 14,000 × *g* for 20 min and dried in a vacuum freezing environment. A 100 µL of acetonitrile water solution (acetonitrile: water = 1:1, v/v) was used for reconstitution, vortex, and centrifugation. The separation process of sample components was conducted with an UHPLC (parameter: Table S1). Each sample was continuously analyzed, and the QC sample was randomly inserted into sample queue to evaluate the stability of system and the reliability of experimental data. MS was conducted in positive and negative ion modes after sample was separated through UHPLC. The setting conditions of MS are presented in Table S2.

2.4. Quality control and metabolic pathway analysis

A total of 1659 and 1363 metabolite ion peaks were extracted by XCMS software. Principal component analysis (PCA) of the samples of Groups QC, Bcd + gim, and gim were relatively aggregated [Figs. S3 (A) and (B)], indicating that the analysis is reproducible. Furthermore, the value of R2Y and Q2 are close to 1.0 after a seven cross-validations (Table S3), demonstrating that the orthogonal partial least squares discriminant analysis (OPLS-DA) mode is reliable [Figs. S3(C) and (D)]. Variable importance for the projection (VIP) was obtained in the OPLS-DA mode (Zhang et al., 2019), and metabolites that simultaneously meet VIP > 1 and $p < 0.05$ were considered as the significantly differential metabolites. After that, Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) was conducted to pathway enrichment analysis in this study, and metabolism information of *Pseudomonas fluorescens pfo-1* were used as a reference species.

2.5. Data analysis

The original MS data (wiff.scan files) were converted to an MZXML file by using ProteoWizard MS Convert and then analyzed with SIMCA-P (version 14.1, Umetrics, Umea, Sweden). Data analysis and graphing were respectively performed in SPSS V19 (IBM, USA) and Microsoft Office 2010. The variance analysis of metabolites was performed with Student's t-test, and $p < 0.05$ was considered as the significance level.

3. Results and discussion

3.1. Effect of siderophores on Cd mobilization

In Fig. 1(a), the concentration of mobilized Cd by *Pseudomonas fluorescens* that produces siderophores after 48 h is 22.1% higher than that in the group without siderophores production ($p < 0.05$). The pH of extracellular solutions (no PAL-Cd addition) of strain with or without siderophores production was respectively 3.81 ± 0.11 and 3.92 ± 0.14 ($p > 0.05$), whereas the pH of extracellular solutions (with PAL-Cd addition) of strain with or without siderophores production was 5.97 ± 0.10 and 6.05 ± 0.12 ($p > 0.05$), respectively.

No significant variance of pH value of extracellular solutions of strain indicate that the organic acids secreted by *Pseudomonas fluorescens* might present in a similar components or concentrations in MSA medium. In addition, GC-MS results show that no significant differences ($p > 0.05$) occur between the organic acid of extracellular solution with and without siderophores production in the MSA medium with PAL-Cd (Fig. S4). This observation reveal that the impact of organic acids secreted by strain is not the main reason for the difference of mobilized Cd content. However, the varied concentrations of Fe^{3+} can result in the significant difference of the secretion of siderophores by *Pseudomonas fluorescens* (Table 1). Additionally, pyoverdine, one of the siderophores, produced by *Pseudomonas fluorescens* can form a complex compound with Cd^{2+} (Ferret et al., 2015). Therefore, the difference concentration of mobilized Cd in the extracellular solution was resulted from the siderophores production of strain.

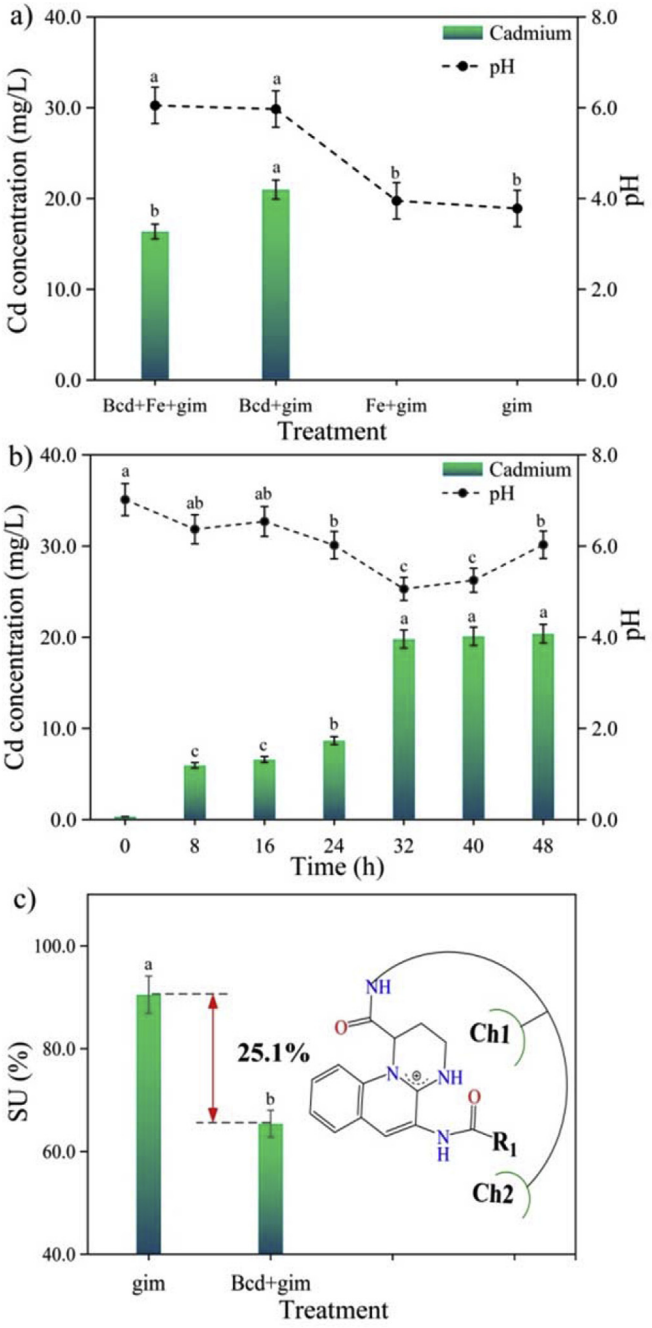

Fig. 1. The change of cadmium concentrations, siderophore yields and pH during a 48-h experimental period in different groups. The PAL-Cd were mobilized by *P. fluorescens* for 48 h with and without siderophores, named Group Bcd + gim and Group Bcd + Fe + gim, respectively. While Group gim and Group Fe + gim belongs to their CK with and without siderophore under lack of cadmium source (a). The PAL-Cd were mobilized by *P. fluorescens* secreting the siderophore, determining the level of cadmium release and pH at the time points of 8, 16, 24, 32, 40, 48 h (b). The siderophores yields secreting by *P. fluorescens* and the general structure of pyoverdine secreted by *Pseudomonas* (c).

As illustrated in Fig. 1(b), the pH of extracellular solution of Group Bcd + gim fluctuates from 7.02 ± 0.11 to 6.54 ± 0.08 within 0-16 h, and pH decreases to 6.02 ± 0.12 and 5.06 ± 0.07 at 24 h and 32 h, respectively. Conversely, pH increases to 6.03 ± 0.13 in 48 h. The concentration of mobilized Cd at 0, 8, 16, and 24 h is 0.00, 5.95 ± 0.48, 6.59 ± 0.53, and 8.66 ± 0.40 mg/L, respectively. However, the content

Table 2
Summary of the metabolites with significant differences.

Metabolites	Scan mode	Measured mass (Da)	Retention time (min)	Fold change (FC)	Elemental composition
3α-Mannobiose	+	343.1223	11.44	0.009	$C_{12}H_{22}O_{11}$
Sucrose	+	360.1493	11.46	0.009	$C_{12}H_{22}O_{11}$
α-D-(+)-Talose	+	163.0587	7.76	0.012	$C_6H_{12}O_6$
all cis-(6,9,12)-Linolenic acid	+	279.2315	0.91	3.804	$C_{18}H_{30}O_2$
D-Mannose	+	198.0962	7.76	0.007	$C_6H_{12}O_6$
Adenosine	+	268.1027	4.69	0.118	$C_{10}H_{13}N_5O_4$
Cytosine	+	112.0499	5.80	0.253	$C_4H_5N_3O$
4-Pyridoxic acid	+	184.0594	1.07	13.989	$C_8H_9NO_4$
5-Methylcytosine	+	126.0649	6.78	6.910	$C_5H_7N_3O$
Thymine	+	127.0491	2.90	15.198	$C_5H_6N_2O_2$
Thymidine	+	243.0966	1.41	5.456	$C_{10}H_{14}N_2O_5$
Adenosine 2',3'-cyclic monophosphate	+	330.0591	7.96	0.232	$C_{10}H_{12}N_5O_6P$
N-Acetyl-D-glucosamine	+	186.0748	4.05	5.183	$C_8H_{15}NO_6$
N-Acetyl-L-alanine	+	132.0640	7.40	0.030	$C_5H_9NO_3$
16-Hydroxypalmitic acid	+	314.2684	0.91	3.509	$C_{16}H_{32}O_3$
Betaine	+	118.0848	9.44	2.640	$C_5H_{11}NO_2$
1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	+	454.2913	1.24	5.613	$C_{21}H_{44}NO_7P$
Succinate	–	117.0191	12.26	0.032	$C_4H_8O_4$
m-Chlorohippuric acid	–	213.0172	3.53	0.066	$C_9H_8NO_3Cl$
Galactinol	–	401.1300	11.64	0.007	$C_{12}H_{12}O_{11}$
D-Tagatose	–	239.0774	9.60	0.004	$C_6H_{12}O_6$
DL-lactate	–	89.0243	7.26	0.040	$C_3H_6O_3$
DL-3-Phenyllactic acid	–	165.0554	1.24	0.068	$C_9H_{10}O_3$
D-Glucono-1,5-lactone	–	177.0406	3.35	0.016	$C_6H_{10}O_6$
D-gluconate	–	195.0513	11.74	0.005	$C_6H_{12}O_7$
D-Fructose	–	179.0565	9.57	0.007	$C_6H_{12}O_6$
Cyanuric acid	–	128.0101	2.16	0.343	$C_3H_3N_3O_3$
Adenosine 3',5'-cyclic phosphate (cAMP)	–	328.0455	7.88	0.107	$C_6H_{12}N_5O_6P$
Acetyl phosphate	–	176.9361	14.58	0.138	$C_2H_5O_5P$
2-Keto-D-gluconic acid	–	193.0358	11.13	0.002	$C_6H_{10}O_7$
(S)-2-Hydroxyglutarate	–	147.0298	12.49	0.029	$C_5H_8O_5$

Note: Fold change (FC) is the average intensity of Group Bcd + gim divided by the average intensity of Group gim in the UHPLC-MS. If the FC > 1, it means that the metabolites are up-regulated compared with those in Group gim, otherwise, it means the down-regulated metabolites. The significantly different metabolites meet "VIP > 1" and "$p < 0.05$" simultaneously. The symbols of "+" and "-" mean in the positive and negative modes, respectively.

of mobilized Cd stretched to 19.82 ± 0.62 mg/L at 32 h. No significant change occurs in Cd mobilization amounts between 32 and 48 h ($p > 0.05$).

Pseudomonas fluorescens can secrete a large amounts of low-molecular-weight organic acids, such as oxalic acid, citric acid, and gluconic acid, etc. (Yang et al., 2018). As the increasing accumulation of organic acids secreted by *Pseudomonas fluorescens*, pH of extracellular solutions gradually decreased. Organic acids lead to the release of Cd^{2+} on PAL-Cd into solution. This finding is similar to the previously reported results about Cd mobilized by organic acids secreted by bacteria, such as *Rahnella* sp. *JN6*, *Burkholderia* sp. *J62*, and *Burkholderia* sp. *D54* (Guo et al., 2011; He et al., 2013; Jiang et al., 2008). However, pH increases after 32 h possibly because the gradual release of Cd^{2+} and other cations (Mg^{2+}) from PAL leads to an increasing adsorption sites number on the surface of PAL (Fig. S2). Otherwise, alkaline oxides on PAL, such as MgO, are released into the solution during the Cd mobilization process, consuming H^+ in solution and resulting in an increase of pH (Li et al., 2018). This also could be concluded as the reasons for the pH value of Bcd + gim and Bcd + Fe + gim groups was significantly higher than that of gim and Fe + gim groups [Fig. 1(a)]. The concentration of mobilized Cd increased with the increasing secretion of organic acids and the decrease of pH value. However, the mobilization Cd maintained at a similar level at 32 and 48 h because a stable copolymer is formed on the surface of PAL by the influence of *Pseudomonas fluorescens* [Fig. S2 (B)], thereby resulting in a difficulty of Cd mobilization.

3.2. Effect of re-mobilization Cd on the siderophores generation of *Pseudomonas fluorescens*

In Fig. 1(c), PAL-Cd mobilization significantly reduces the amount of siderophores produced by *Pseudomonas fluorescens* with a decreasing value of 25.1% ($p < 0.05$), indicating that PAL-Cd mobilization also reversely influences the ability of *Pseudomonas fluorescens* to yield siderophores. Pyoverdine, secreted by *Pseudomonas fluorescens*, is a mixed type of siderophore containing two hydroxamic acids and a catechol group (EDBERG et al., 2010). Its general structure, bidentate chelation sites Ch1 and Ch2 are located on peptide racks with different lengths (6–12 amino acids) (Hider and Kong, 2010), is presented with Fig. 1(c). Therefore, the mechanisms of siderophore generation in *Pseudomonas fluorescens* may be associated with its individual amino acids biosynthesis process. As shown in Table S4, 112 metabolites are identified with UHPLC-MS. In siderophores production treatments, the up-regulation and down-regulation number of extracellular metabolites are 9 and 22 in the group with PAL-Cd compared with that of group without PAL-Cd, respectively (Table 2). The up-regulation of differential metabolites are γ-linolenic acid (GLA), *N*-acetyl-D-glucosamine (GlcNAc), betaine, 5-methylcytosine, thymidine, thymine, and 4-pyridinic acid. The down-regulation of differential metabolites are listed as follows: adenosine, adenosine 3′,5′-cyclic phosphate (cAMP), adenosine 2′,3′-cyclic phosphate, cytosine, D-mannose, galactitol, D-tagatose, D-fructose, D-gluconic acid, D-gluconic acid-1,5-lactone, succinic acid, and acetyl phosphate. The cluster analysis of those metabolites is presented in Fig. 2. KEGG pathway analysis show that the metabolic pathways, such as purine metabolism, pyrimidine metabolism, carbohydrate metabolism, tricarboxylic acid cycle (TCA) metabolism, ABC transporter, and signal transduction, are significantly affected in *Pseudomonas fluorescens* (Fig. 3).

It is generally accepted that a secondary metabolic pathway based on the production of primary metabolites is the main process to produce siderophores by bacteria to overcome the low-iron concentration environment (Kulakovskaya and Kulakovskaya, 2014). Therefore, the variances of primary metabolism pathways, including lipid metabolism, nucleotide metabolism, carbohydrate metabolism, and energy

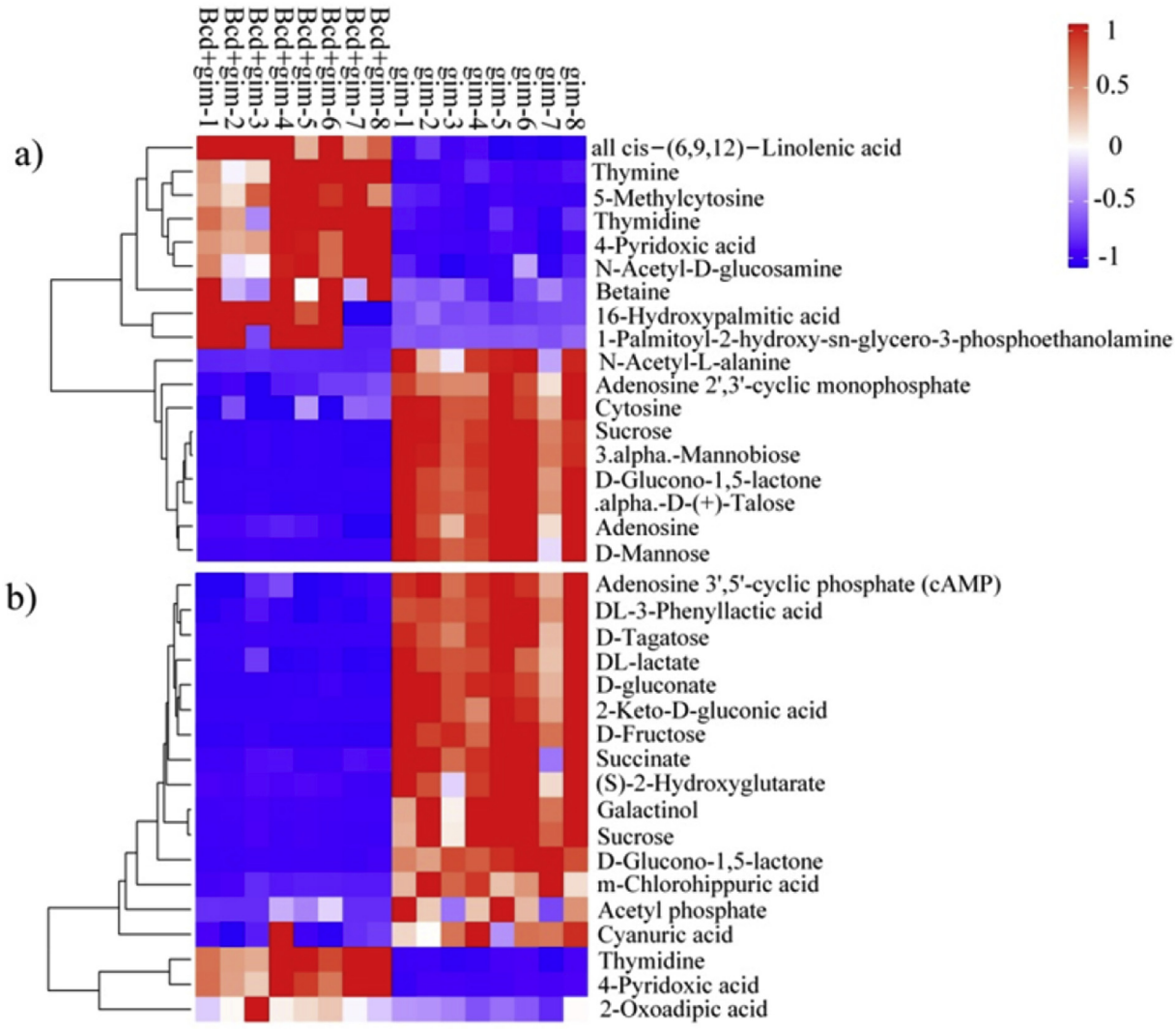

Fig. 2. The hierarchical clustering maps. The hierarchical clustering maps in the positive and negative modes, respectively (a and b). The red side means up-regulation and the blue side means down-regulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

metabolism, are substantially related to the biological mechanisms of produce siderophores in strain associated with Cd re-mobilization (Weselake et al., 2018).

In siderophores production group, compared with the treatment without PAL-Cd, the medium with PAL-Cd showed a significantly increasing level of GLA, GlcNAc and betaine in extracellular solution (Table 2). GLA is an important polyunsaturated fatty acid which plays an irreplaceable role in cell metabolism (Ronda et al., 2012). Furthermore, GLA can be converted to arachidonic acid by the effects of reduction and desaturation (Cinti et al., 1992). Arachidonic acid, an integral part of plasma membrane, can increase the fluidity and flexibility of membrane (Hanna and Hafez, 2018). Membrane-resident proteins and membrane-dependent processes depend on the performance of membrane fluidity (Ng et al., 2018). For example, ABC transporters, that are ATPase of transport on the plasma membrane, can carry different substrates across the bilayer lipid (Poolman and Van, 2003). GlcNAc and betaine are shuttled across cell membrane based on the transport function of ABC transporter family (Kempf and Bremer, 1995; Xiao et al., 2002). Therefore, in siderophores production group, an increasing to GLA concentration might promote the fluidity of plasma membrane, which finally promote the ABC transporter function of *Pseudomonas fluorescens* during the PAL-Cd mobilization.

In strain groups, by contrast to group without PAL-Cd addition, the concentration of three metabolites (adenosine, cAMP, adenosine 2′,3′-cyclic phosphate) in purine metabolic pathway is significantly reduced during the mobilization of Cd in PAL (Fig. 4). It has been reported that the purine metabolism of bacterial strain could be influenced by the enhancement of oxidative stress reaction (Kaddurahdaouk et al., 2013). Similar results were also observed during the Cd mobilization by *Pseudomonas fluorescens*. For pyrimidine metabolism, the contents of 5-methylcytosine, thymidine, and thymine significantly increased, whereas the concentration of cytosine significantly decreased with the Cd mobilization (Fig. 4). The strengthened methylation of cytosine in cells can be used to reflect the levels of 5-methylcytosine and cytosine in extracellular solution because 5-methylcytosine originates from the methylation metabolism process of cytosine at the 5' position (Wilson, 2008). In addition, the content of betaine significantly increases in extracellular solution of *Pseudomonas fluorescens* with PAL-Cd, $-CH_3$ contained in betaine can serve as a methyl donor for methylation (Stead et al., 2006). Therefore, i) the methylation process of strain can be promoted with the siderophores production; ii) the pathway of purine metabolic and pyrimidine was respectively inhibited and promoted in the metabolism of nucleotide by *Pseudomonas fluorescens*.

Compared with siderophores production without PAL-Cd group, the

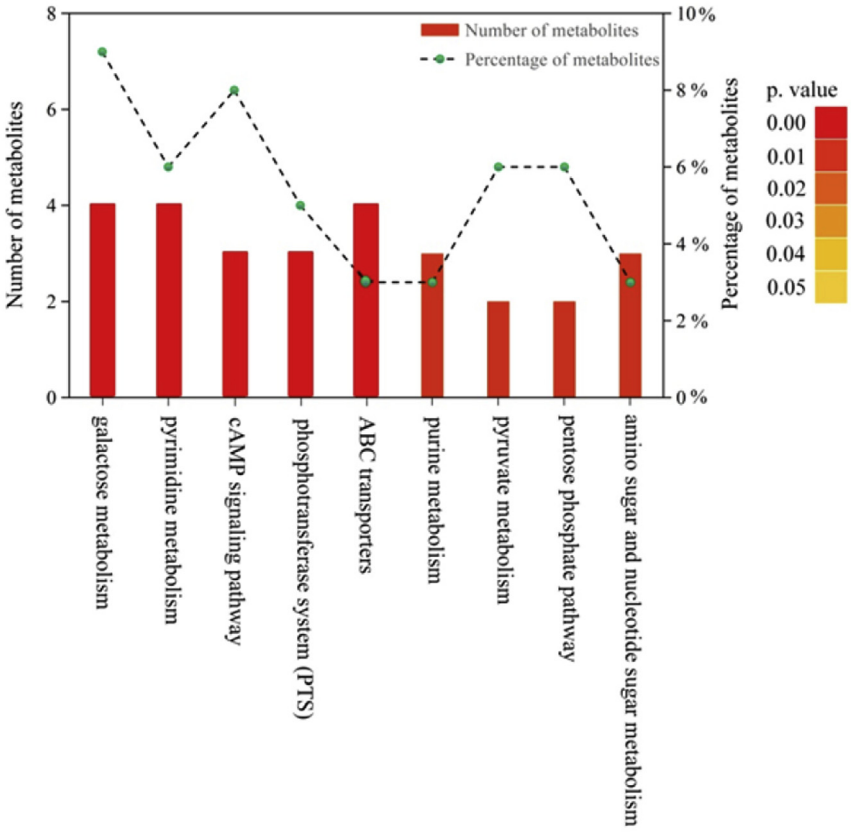

Fig. 3. Enriched pathways by Kyoto Encyclopedia of Genes and Genomes.

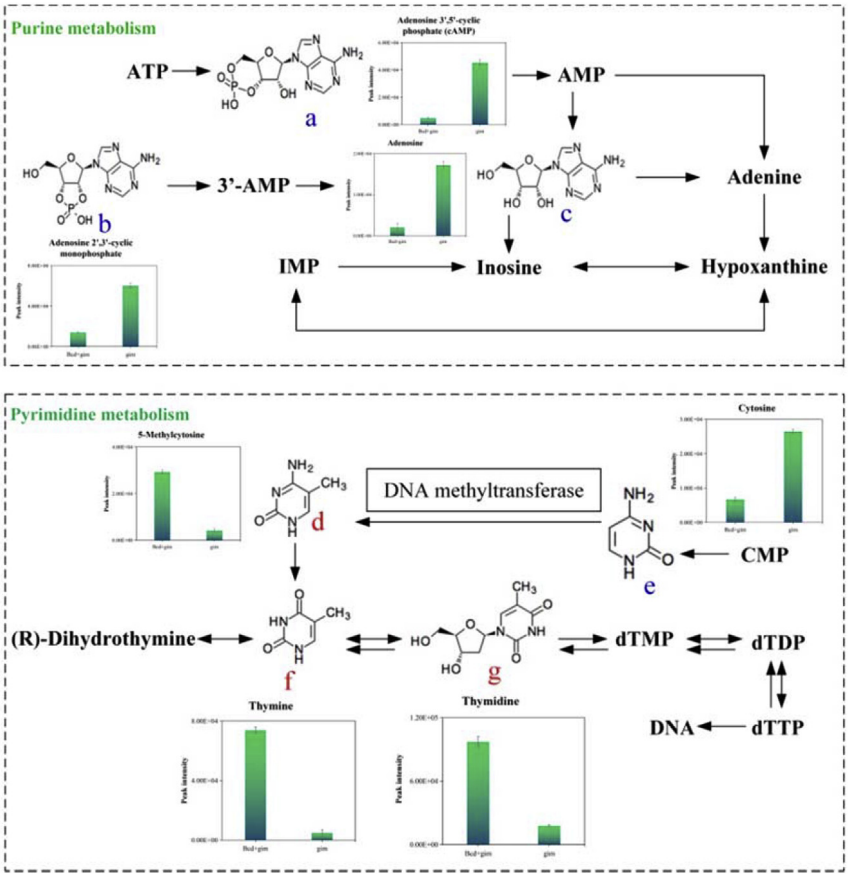

Fig. 4. The possible pathway of nucleotide metabolism of *P. fluorescens* affected by mobilized PAL- Cd. The blue and red alphabets are those down-regulated and up-regulated metabolites in the extracellular fluids of mobilization PAL-Cd by the strain compared with those without mobilizing PAL-Cd, respectively. The metabolites are as follows: 3',5'- cyclic AMP (a), 2',3'- cyclic AMP (b), Adenosine (c), 5-Methylcytosine (d), Cytosine (e), Thymine (f), and Thymidine (g). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

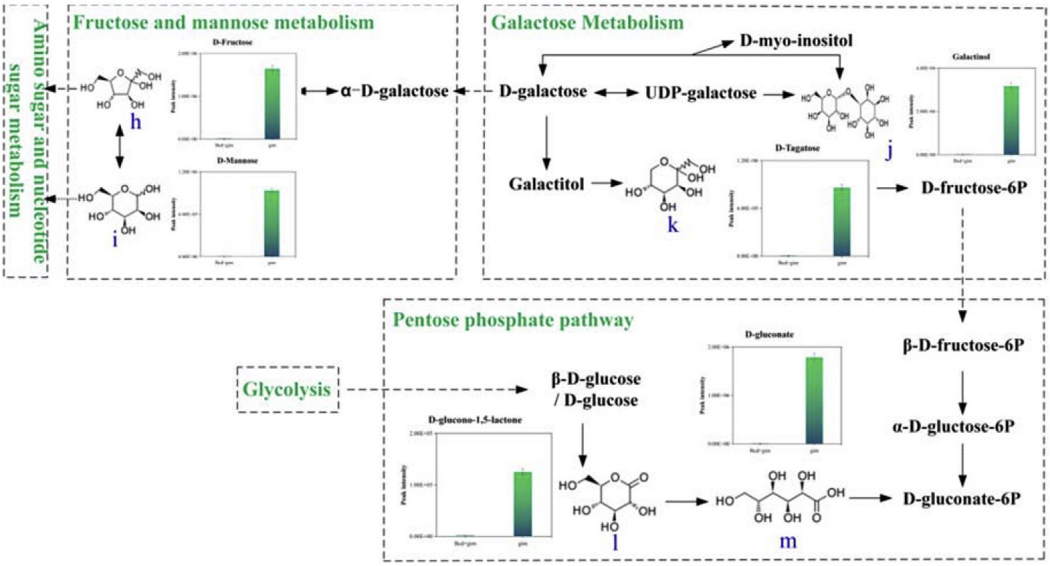

Fig. 5. The possible pathway of carbohydrate metabolism of *P. fluorescens* affected by mobilized PAL- Cd. The blue and red alphabets are those down-regulated and up-regulated metabolites in the extracellular fluids of mobilization PAL-Cd by the strain compared with those without mobilizing PAL-Cd, respectively. The metabolites are as follows: D-fructose (h), D-mannose (i), galactinol (j), D-tagatose (k), D-glucono-1,5-lactone (l), and D-gluconate (m). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contents of D-mannose, galactitol, D-tagatose, D-fructose, D-gluconic acid, D-gluconic-1,5-lactone significantly decrease in the extracellular solution during the mobilization process of Cd by strain (Table 2). The analysis of KEGG metabolic pathways reveals that those significantly decreasing differential metabolites are associated with carbohydrate metabolism pathways, including galactose metabolism, fructose and mannose metabolism, and pentose phosphate pathway (Fig. 5). Biosynthesis of histidine family are related to ribose-5-phosphate, which is the intermediate of pentose phosphate pathway (Cohen, 2011). Therefore, the reduce of pentose phosphate pathway might inhibit the biosynthesis of histidine family. The metabolism amino and nucleotide sugars may be affected by a decrease of the levels of upstream products D-mannose and D-fructose (Sarma et al., 2018). The glycolytic pathway may also be influenced by the decreasing content of upstream products, such as D-gluconate-1,5-lactone and D-gluconic acid (Fig. 5). The down-regulation of pyruvate metabolic pathway during mobilization of Cd in PAL can be used the significantly decreasing content of succinic acid and acetyl phosphate to reflect (Fig. 3 and Table 2). Pyruvate is the final product of glycolysis. Furthermore, mobilized Cd weakens the glycolysis metabolism of strain. Therefore, the biosynthesis of amino acids distributed in serine and pyruvate family are related to glycerate-3 phosphate and pyruvate, which are the intermediates of glycolytic pathway (Cohen, 2014). Thus, the down-regulation of glycolysis metabolism is not conducive to biosynthesis of these two types of amino acids. The inhibition of amino acids biosynthesis reduce siderophores production, because pyoverdine, the siderophore produced by *Pseudomonas fluorescens*, contains a peptide chain with 6-12 amino acids (Mossialos et al., 2002).

3.3. Change in oxidative stress, signal transduction and its inhibition on siderophores production

The concentration of 4-pyridic acid in extracellular solution in PAL-Cd group decreases significantly compared with that of group without PAL-Cd addition (Table 2). Under a higher concentration of Cd in medium solution mobilized by strain, the oxidative stress of *Pseudomonas fluorescens* could be activated with the replacement essential metal elements by Cd (Cuypers et al., 2010). 4-Pyridic acid is considered as one of the potential biomarkers with the presence of Cd (Chen et al., 2017), and its increasing concentration may suggest an the strengthened metabolism in vitamin B6 and the presence of oxidative stress (Chen et al., 2018; Taysi, 2005). Therefore, during the mobilization of Cd in PAL by *Pseudomonas fluorescens*, the oxidative stress of strain might be induced to promote, resulting in an imbalance of oxidation and antioxidant effects in cells (Storz and Imlay, 1999). GlcNAc has an capacity of excellent antioxidant (Azam et al., 2014). In the mobilization Cd in PAL by *Pseudomonas fluorescens*, an increasing GlcNAc content can help the oxidation and antioxidant balance in the intracellular (Table 2). Besides, oxidative stress is not the benefit for the biological of mitochondria, which are the sites for the accomplishment of TCA metabolism. The content of succinic acid may be reduced if TCA metabolic pathway is disturbed because the succinic acid is one of the most important intermediates in TCA cycle (Stryer et al., 2017). In our research, the concentration of succinic acid in the extracellular solutions with the addition of PAL-Cd decreases significantly compared with that of group without PAL-Cd (Table 2), indicating that the TCA metabolism of strain is disturbed during the process of Cd mobilization. Biosynthesis of amino acids distributed in glutamate and aspartate family are related to α-ketoglutarate and oxaloacetate, which are the intermediates of TCA metabolism (Ballinger et al., 2000; Cohen, 2011). Thus, in the mobilization process of Cd, the oxidative stress of strain may be induced to strengthen, which was considered the main reason to disrupt TCA metabolism and reduce ATP production, and then the biosynthesis of some amino acids and the production of siderophores in *Pseudomonas fluorescens* was inhibited.

The concentration of cAMP in extracellular solution associated with PAL-Cd decreases significantly compared with that of group without PAL-Cd (Table 2). As one of the most important messengers, it not only controls the key physiological processes of metabolism, gene transcription, secretion, cell activity, and calcium homeostasis, but also mediates the physiological response of extracellular signals (Ahuja et al., 2014; Oldenburger et al., 2012). Thus, in the process of Cd mobilization in PAL by *Pseudomonas fluorescens*, signal transduction of strain might be influenced by Cd. The capability of siderophore production by *Pseudomonas fluorescens* is also as affected by signal transduction, because the signal transduction participates in the regulation of cell synthesis and the secretion of siderophores under a low-iron condition (Martins et al., 2018).

4. Conclusion

Pseudomonas fluorescens that produces siderophores substantially affects the Cd mobilization bound with PAL, which might reduce the long-term immobility effect of PAL in the remediated soil. In turn, the Cd mobilization inhibits siderophores production of *Pseudomonas fluorescens*. Metabolome analysis shows that the mobilized Cd influences signal transduction and primary metabolism of *Pseudomonas fluorescens*, reduces the pathways of pentose phosphate, glycolysis, and TCA in the strain. This will result in the decreasing biosynthesis of amino acids. Finally, the ability of siderophores production in *Pseudomonas fluorescens* was also inhibited. This study provides a useful information about how to select soil Cd-stabilizing materials in a targeted manner and how to avoid Cd re-mobilization by siderophores.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.06.012.

References

Ahuja, M., Jha, A., Maléth, J., Park, S., Muallem, S., 2014. cAMP and Ca2+ signaling in secretory epithelia: Crosstalk and Synergism. Cell Calcium 55 (6), 385.
Azam, M.S., Kim, E.J., Yang, H.-S., Kim, J.K., 2014. High antioxidant and DNA protection activities of N-acetylglucosamine (GlcNAc) and chitobiose produced by exolytic chitinase from Bacillus cereus EW5. SpringerPlus 3 (1), 354.
Ballinger, S.W., Patterson, C., Yan, C.N., Doan, R., Burow, D.L., Young, C.G., Yakes, F.M., Van, H.B., Ballinger, C.A., Freeman, B.A., 2000. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. Circ. Res. 86 (9), 960.
Chen, L., Luo, S., Li, X., Wan, Y., Chen, J., Liu, C., 2014. Interaction of Cd-hyperaccumulator Solanum nigrum L. and functional endophyte Pseudomonas sp. Lk9 on soil heavy metals uptake. Soil Biol. Biochem. 68, 300–308.
Chen, M., Wang, P.N., Xie, Z.X., 2018. A complex mechanism involving LysR and TetR/AcrR that regulates iron scavenger biosynthesis in Pseudomonas donghuensis HYS. J. Bacteriol. 200 (13), 17.
Chen, S., Zhang, M., Bo, L., Li, S., Hu, L., Zhao, X., Sun, C., 2017. Metabolomic analysis of the toxic effect of chronic exposure of cadmium on rat urine. Environ. Sci. Pollut. Res. Int. 1, 1–10.
Cinti, D.L., Cook, L., Nagi, M.N., Suneja, S.K., 1992. The fatty acid chain elongation system of mammalian endoplasmic reticulum. Prog. Lipid Res. 31 (1), 1–51.
Cohen, G.N., 2014. Biosynthesis of amino acids derived from phosphoglyceric acid and pyruvic acid. In: Microbial Biochemistry. Springer Netherlands, Dordrecht, pp. 387–403.
Cohen, G.N., 2011. Microbial Biochemistry. University Park Press.
Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A.R., Munters, E., Artois, T.J., 2010. Cadmium stress: An oxidative challenge. Biometals 23 (5), 927–940.
Dimkpa, C.O., Svatoš, A., Dabrowska, P., Schmidt, A., Boland, W., Kothe, E., 2008. Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in Streptomyces spp. Chemosphere 74 (1), 19–25.
Duffus, J.H., 2002. Heavy metals—a meaningless term? Pure Appl. Chem. 74, 793–807. Availableon the IUPAC website at. http://www.iupac.org/publications/pac/2002/7405/7405x0793.htm.
EDBERG, F., KALINOWSKI, B.E., HOLMSTRÖM, S.J.M., HOLM, K., 2010. Mobilization of metals from uranium mine waste: The role of pyoverdines produced by Pseudomonas fluorescens. Geobiology 8 (4), 278–292.
Ferret, C., Cornu, J.Y., Elhabiri, M., Sterckeman, T., Braud, A., Jezequel, K., Lollier, M., Lebeau, T., Schalk, I.J., Geoffroy, V.A., 2015. Effect of pyoverdine supply on cadmium and nickel complexation and phytoavailability in hydroponics. Environ. Sci. Pollut. Res. Int. 22 (3), 2106–2116.
Guo, J.K., Tang, S.R., Ju, X.H., Ding, Y.Z., Liao, S.Q., Song, N.N., 2011. Effects of inoculation of a plant growth promoting rhizobacterium Burkholderia sp D54 on plant growth and metal uptake by a hyperaccumulator Sedum alfredii Hance grown on multiple metal contaminated soil. World J. Microbiol. Biotechnol. 27 (12), 2835–2844.
Guo, S.-H., Hu, N., Li, Q.-S., Yang, P., Wang, L.-L., Xu, Z.-M., Chen, H.-J., He, B.-Y., Zeng, E.Y., 2018. Response of edible amaranth cultivar to salt stress led to Cd mobilization in rhizosphere soil: A metabolomic analysis. Environ. Pollut. 241, 422–431.
Hanna, V.S., Hafez, E.A.A., 2018. Synopsis of arachidonic acid metabolism: A review. J. Adv. Res. 11, 23–32.
He, H.D., Ye, Z.H., Yang, D.J., Yan, J.L., Xiao, L., Zhong, T., Yuan, M., Cai, X.D., Fang, Z.Q., Jing, Y.X., 2013. Characterization of endophytic Rahnella sp JN6 from Polygonum pubescens and its potential in promoting growth and Cd, Pb, Zn uptake by Brassica napus. Chemosphere 90 (6), 1960–1965.
Hider, R.C., Kong, X., 2010. Chemistry and biology of siderophores. Nat. Prod. Rep. 27 (5), 637–657.
Hooda, Peter S. (Ed.), 2010. Trace Elements in Soils. John Wiley & Sons, Chichester, U.K.
Jeong, S., Moon, H.S., Nam, K., Kim, J.Y., Kim, T.S., 2012. Application of phosphate-solubilizing bacteria for enhancing bioavailability and phytoextraction of cadmium (Cd) from polluted soil. Chemosphere 88 (2), 204–210.
Jiang, C.Y., Sheng, X.F., Qian, M., Wang, Q.Y., 2008. Isolation and characterization of a heavy metal-resistant Burkholderia sp from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72 (2), 157–164.
Kaddurahdaouk, R., Bogdanov, M.B., Wikoff, W.R., Zhu, H., Boyle, S.H., Churchill, E., Wang, Z., Rush, A.J., Krishnan, R.R., Pickering, E., 2013. Pharmacometabolomic mapping of early biochemical changes induced by sertraline and placebo. Transl. Psychiatry 3 (1), e223.
Kempf, B., Bremer, E., 1995. OpuA, an osmotically regulated binding protein-dependent transport system for the osmoprotectant glycine betaine in Bacillus subtilis. J. Biol. Chem. 270 (28), 16701–16713.
Kulakovskaya, E., Kulakovskaya, T., 2014. Introduction. In: Kulakovskaya, E., Kulakovskaya, T. (Eds.), Extracellular Glycolipids of Yeasts. Academic Press, pp. ix–xi.
Li, J., Xu, Y., 2017. Immobilization remediation of Cd-polluted soil with different water condition. J. Environ. Manag. 193, 607–612.
Li, J.R., Xu, Y.M., 2018. Effects of clay combined with moisture management on Cd immobilization and fertility index of polluted rice field. Ecotoxicol. Environ. Saf. 158, 182–186.
Li, S., Wang, M., Zhao, Z., Ma, C., Chen, S., 2018. Adsorption and desorption of Cd by soil amendment: Mechanisms and environmental implications in field-soil remediation. Sustainability 10 (7), 2337.
Liang, X., Han, J., Xu, Y., Sun, Y., Wang, L., Tan, X., 2014. In situ field-scale remediation of Cd polluted paddy soil using sepiolite and palygorskite. Geoderma 235, 9–18.
Ma, Y., Rajkumar, M., Zhang, C., Freitas, H., 2016. Beneficial role of bacterial endophytes in heavy metal phytoremediation. J. Environ. Manag. 174, 14–25.
Martins, T.S., Costa, V., Pereira, C., 2018. Signaling pathways governing iron homeostasis in budding yeast. Mol. Microbiol. 109 (4), 422–432.
Mossialos, D., Ochsner, U., Baysse, C., Chablain, P., Pirnay, J.-P., Koedam, N., Budzikiewicz, H., Fernández, D.U., Schäfer, M., Ravel, J., Cornelis, P., 2002. Identification of new, conserved, non-ribosomal peptide synthetases from fluorescent pseudomonads involved in the biosynthesis of the siderophore pyoverdine. Mol. Microbiol. 45 (6), 1673–1685.
Ng, J., Kamm, R.D., Wohland, T., Kraut, R.S., 2018. Evidence from ITIR-FCS diffusion studies that the Amyloid-Beta (Aβ) peptide does not perturb plasma membrane fluidity in neuronal cells. J. Mol. Biol. 430 (18, Part B), 3439–3453.
Oldenburger, A., Maarsingh, H., Schmidt, M., 2012. Multiple facets of cAMP signalling and physiological impact: cAMP compartmentalization in the lung. Pharmaceuticals 5 (12), 1291–1331.
Poolman, B., Van, d.H., T., 2003. ABC proteins: From bacteria to man. ABC Proteins 263–275.
Príncipe, A., Fernandez, M., Torasso, M., Godino, A., Fischer, S., 2018. Effectiveness of tailocins produced by Pseudomonas fluorescens SF4c in controlling the bacterial-spot disease in tomatoes caused by Xanthomonas vesicatoria. Microbiol. Res. 212–213, 94–102.
Rizwan, M., Ali, S., Abbas, T., Zia-ur-Rehman, M., Hannan, F., Keller, C., Al-Wabel, M.I., Ok, Y.S., 2016. Cadmium minimization in wheat: A critical review. Ecotoxicol. Environ. Saf. 130, 43–53.
Ronda, S.R., Bokka, C.S., Ketineni, C., Rijal, B., Allu, P.R., 2012. AERATION EFFECT ON SPIRULINA PLATENSIS GROWTH AND gamma-LINOLENIC ACID PRODUCTION. Braz. J. Microbiol. 43 (1), 12–20.
Sarma, S.N., Saleem, A., Lee, J.Y., Tokumoto, M., Hwang, G.W., Chan, H.M., Satoh, M., 2018. Effects of long-term cadmium exposure on urinary metabolite profiles in mice. J. Toxicol. Sci. 43 (1–3), 89–100.
Schalk, I.J., Hannauer, M., Braud, A., 2011. New roles for bacterial siderophores in metal transport and tolerance. Environ. Microbiol. 13 (11), 2844–2854.
Scher, F.M., Baker, R., 1982. Effect of Pseudomonas putida and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology 72 (12), 1567–1573.
Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160 (1), 47–56.
Sheikhhosseini, A., Shirvani, M., Shariatmadari, H., 2013. Competitive sorption of nickel, cadmium, zinc and copper on palygorskite and sepiolite silicate clay minerals. Geoderma 192, 249–253.
Singh, A., Mishra, A.K., 2015. Influence of various levels of iron and other abiotic factors on siderophorogenesis in paddy field cyanobacterium anabaena oryzae. Appl. Biochem. Biotechnol. 176 (2), 1–15.
Stead, L.M., Brosnan, J.T., Brosnan, M.E., Vance, D.E., Jacobs, R.L., 2006. Is it time to reevaluate methyl balance in humans? Am. J. Clin. Nutr. 83 (1), 5–10.
Storz, G., Imlay, J.A., 1999. Oxidative stress. Curr. Opin. Microbiol. 2 (2), 188–194.
Stryer, L., Berg, J.M., Tymoczko, J.L., 2017. Biochemistry, fifth ed. .
Taysi, S., 2005. Oxidant/antioxidant status in liver tissue of vitamin B deficient rats. Clin. Nutr. 24 (3), 385–389.
Welikala, D., Hucker, C., Hartland, A., Robinson, B.H., Lehto, N.J., 2018. Trace metal

mobilization by organic soil amendments: Insights gained from analyses of solid and solution phase complexation of cadmium, nickel and zinc. Chemosphere 199, 684–693.
Weselake, R.J., Singer, S.D., Chen, G., 2018. Introduction to plant biomolecules and cellular metabolism. In: Chen, G., Weselake, R.J., Singer, S.D. (Eds.), Plant Bioproducts. Springer New York, New York, NY, pp. 9–25.
Wilson, A.G., 2008. Epigenetic regulation of gene expression in the inflammatory response and relevance to common diseases. J. Periodontol. 79 (8 Suppl. l), 1514.
Wu, Y.-J., Zhou, H., Zou, Z.-J., Zhu, W., Yang, W.-T., Peng, P.-Q., Zeng, M., Liao, B.-H., 2016. A three-year in-situ study on the persistence of a combined amendment (limestone + sepiolite) for remedying paddy soil polluted with heavy metals. Ecotoxicol. Environ. Saf. 130, 163–170.
Xiao, X., Wang, F., Saito, A., Majka, J., Schlosser, A., Schrempf, H., 2002. The novel Streptomyces olivaceoviridis ABC transporter Ngc mediates uptake of N-acetylglucosamine and N,N '-diacetylchitobiose. Mol. Genet. Genom. 267 (4), 429–439.
Yang, P., Zhou, X.-F., Wang, L.-L., Li, Q.-S., Zhou, T., Chen, Y.-K., Zhao, Z.-Y., He, B.-Y., 2018. Effect of phosphate-solubilizing bacteria on the mobility of insoluble cadmium and metabolic analysis. Int. J. Environ. Res. Public Health 15 (7).
Zhang, M., Pu, J., 2011. Mineral materials as feasible amendments to stabilize heavy metals in polluted urban soils. J. Environ. Sci. 23 (4), 607–615.
Zhang, W., Jiang, H., Yang, J., Song, G., Wen, D., Liu, W., Jin, M., Wang, Q., Du, Y., Sun, Q., Cao, L., Xu, H., 2019. A high-throughput metabolomics approach for the comprehensive differentiation of four Pulsatilla Adans herbs combined with a non-targeted bidirectional screen for rapid identification of triterpenoid saponins. Anal. Bioanal. Chem. 411 (10), 2071–2088.