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Effects of cadmium perturbation on the microbial community structure and heavy metal resistome of a tropical agricultural soil

Lateef B. Salam^{1*} , Oluwafemi S. Obayori², Mathew O. Ilori³ and Olukayode O. Amund³

Abstract

The effects of cadmium (Cd) contamination on the microbial community structure, soil physicochemistry and heavy metal resistome of a tropical agricultural soil were evaluated in field-moist soil microcosms. A Cd-contaminated agricultural soil (SL5) and an untreated control (SL4) were compared over a period of 5 weeks. Analysis of the physicochemical properties and heavy metals content of the two microcosms revealed a statistically significant decrease in value of the soil physicochemical parameters ($P < 0.05$) and concentration of heavy metals (Cd, Pb, Cr, Zn, Fe, Cu, Se) content of the agricultural soil in SL5 microcosm. Illumina shotgun sequencing of the DNA extracted from the two microcosms showed the predominance of the phyla, classes, genera and species of *Proteobacteria* (37.38%), *Actinobacteria* (35.02%), *Prevotella* (6.93%), and *Conexibacter woesei* (8.93%) in SL4, and *Proteobacteria* (50.50%), *Alphaproteobacteria* (22.28%), *Methylobacterium* (9.14%), and *Methylobacterium radiotolerans* (12.80%) in SL5, respectively. Statistically significant ($P < 0.05$) difference between the metagenomes was observed at genus and species delineations. Functional annotation of the two metagenomes revealed diverse heavy metal resistome for the uptake, transport, efflux and detoxification of various heavy metals. It also revealed the exclusive detection in SL5 metagenome of members of RND (resistance nodulation division) protein *czcCBA* efflux system (*czcA*, *czrA*, *czrB*), CDF (cation diffusion facilitator) transporters (*czcD*), and genes for enzymes that protect the microbial cells against cadmium stress (*sodA*, *sodB*, *ahpC*). The results obtained in this study showed that Cd contamination significantly affects the soil microbial community structure and function, modifies the heavy metal resistome, alters the soil physicochemistry and results in massive loss of some autochthonous members of the community not adapted to the Cd stress.

Keywords: Cadmium, Agricultural soil, Heavy metals, Soil microcosm, Shotgun metagenomics, Microbial community structure and function, Heavy metal resistome

Introduction

Cadmium (Cd) is a highly toxic, carcinogenic heavy metal with an exceptionally high biological half-life (> 20 years) and propensity for accumulation in the food chain, drinking water and soil (Benavides et al. 2005; Khan et al. 2015; Fashola et al. 2016). Major sources of Cd in soil include

wet and dry atmospheric deposition (vehicular emission, incineration, burned fuel and tyre wear, residual ashes from wood, coal or other types of combustion) (Mielke et al. 1991; Steinnes and Friedland 2006); and geological weathering (Khan et al. 2010; Liu et al. 2013). Other primary anthropogenic sources of Cd in soil include mining, sewage sludge, composted municipal solid wastes, improper waste disposal practices, smelting, wastewater irrigation, manufacturing and agrochemicals (Alloway

*Correspondence: babssalaam@yahoo.com

¹ Department of Biological Sciences, Microbiology Unit, Summit University, Offa, Kwara, Nigeria

Full list of author information is available at the end of the article

and Steinnes 1999; Khan et al. 2016a, b; Nawab et al. 2016; Khan et al. 2017).

Elevated Cd concentration in soil poses significant threat to the quantity and diversity of soil microorganisms. Cd toxicity to microbial cells is believed to be due to depletion of glutathione and sulfhydryl groups in proteins, interaction with nucleic acids, oxidative damage by production of reactive oxygen species, and inactivation of metalloproteins due to displacement of Zn and Fe ions (Vallee and Ulmer 1972; Stohs and Bagchi 1995; Fortuniak et al. 1996; Stohs et al. 2001; Banjerdkij et al. 2005). This result in protein denaturation, cell membrane and nucleic acid disruption, and inhibition of transcription, cell division and enzyme activities (Fashola et al. 2016). Several workers have also highlighted the debilitating effects of Cd toxicity on the lung, kidney, bones, and the nervous and immune systems of humans (Adriano 2001; Waisberg et al. 2003; Edwards and Prozialeck 2009; Yazdankhah et al. 2010; Satarug et al. 2001; Moynihan et al. 2017). Furthermore, Cd cytotoxicity has been implicated in destruction of plant mitochondria as well as disruption of photosynthesis and transpiration (Imai and Siegel 1973; Toppi and Gabbrielli 1999; Lopez-Milla'n et al. 2009; Mohamed et al. 2012; Júnior et al. 2014; Khan et al. 2016a, b).

Bioremediation of Cd-inundated soil is predicated on the presence of highly efficient Cd uptake/transport/efflux/detoxification system within the soil microbial community well-adapted to Cd stress. Mechanisms such as intracellular or extracellular precipitation, active efflux, and transformation to less toxic species have been used by microorganisms to counteract heavy metal stress (Nies 1999, 2003; Hu et al. 2005). In Cd resistance, three families of efflux transporters are deployed by microorganisms. They are the P-type ATPases, which traverse the inner membrane and use ATP energy to pump metal ions from the cytoplasm (Nucifora et al. 1989; Rensing et al. 1997); the CBA (capsule biogenesis assembly) transporters, which act as cation–proton antiporters (Nies and Silver 1989; Nies 1995; Hassan et al. 1999); and the cation diffusion facilitator (CDF) transporters, which act as chemiosmotic ion–proton exchanger (Xiong and Jayaswal 1998; Anton et al. 1999; Grass et al. 2001; Nies 2003).

Previous works have deployed culture-based and culture-independent methods to monitor the effects of heavy metal contamination on autochthonous soil microbial community. In most cases, where culture-independent approach was used, specific resistance genes are amplified via PCR techniques (Rhee et al. 2004; Bhadra et al. 2005; Altimira et al. 2012). Information obtained from such studies cannot be adapted to design effective bioremediation strategies as it does not reflect the true picture of heavy metal resistome in such environments.

The use of shotgun metagenomics allows deep metagenomic sequencing providing unprecedented insight into the genetic potentials of microbial communities as well as underrepresented populations (Handelsman 2004; Oulas et al. 2015). It also reveals the communal nature of microbial existence and the interplay between diverse genes and processes produced and marshalled by members of the microbial community to counteract various environmental stressors. This exciting approach have been used to decipher the microbial community structure and function of diverse polluted and pristine soils (Salam et al. 2017, 2018; Feng et al. 2018; Salam et al. 2019).

In recent time, attempts have been made to use next-generation shotgun metagenomics to characterize the microbial community structure and function of heavy metal-inundated soils. However, to the best of our knowledge, none of the reports have used the approach to extensively decipher the specific resistance systems deployed by members of the microbial community to counteract the stress imposed by the studied heavy metal. Here, we report the use of shotgun metagenomics to decipher the effects of Cd contamination on the microbial community structure and heavy metal resistome of a tropical agricultural soil.

Materials and methods

Sampling site description

Soil samples were collected from an agricultural farm in Ilorin, Kwara State, Nigeria. The coordinates of the sampling site were latitude 8° 27' 45.36" N and longitude 4° 32' 7.08" E. Historically, farming at the sampling site dated back to 10–15 years and crops such as maize, cassava, cocoyam, beans and guinea corn were grown. In addition, livestock manures are routinely used to enhance soil nutrients while NIMBUS® Space Spray (5 g/kg soil pyrethrum + 40 g/kg soil piperonyl butoxide) is used on the farm to arrest grain weevil infestation.

Source of heavy metal

Cadmium chloride (CdCl₂), the source of cadmium used in this study was purchased from Sigma Aldrich Corp (St Louis MO, USA).

Sampling, microcosm setup, physicochemical and heavy metal content analysis

Soil samples were collected from upper 10–12 cm using a sterile hand trowel after removing the debris from the soil surface. The soil samples, collected via composite sampling were passed through a 2-mm mesh sieve. Sieved soils were made homogenous by thorough mixing in a large plastic bag. Sieved soil (1 kg) weighed and placed in an open pan was designated SL4. The second soil microcosm designated SL5 contained 1 kg of sieved

soil amended with 250 mg CdCl₂, respectively. The two setups (in triplicates) were incubated at room temperature for 5 weeks and flooded weekly with 50 ml distilled water to maintain a moisture content of 25%.

The pH of the soil samples was measured using a pH meter (model 3051, Jenway, UK) by dipping the glass electrode in a soil solution slurry that contains a fivefold volume of water containing 1 M KCl. Moisture and total organic matter contents were determined gravimetrically, while total nitrogen content was determined by macro-Kjeldahl digestion method. Potassium content was determined by flame photometry (Flame photometer model PFP-7, Buck Scientific Inc, USA) method while phosphorus content was determined spectrophotometrically. Heavy metals composition of the soils was determined using atomic absorption spectrophotometer (model Alpha 4, Chem Tech Analytical, UK) following mixed acid digestion and extraction of the soil samples.

Total DNA extraction and shotgun metagenomics

Total DNA used for metagenomic analysis was extracted directly from the two soil microcosms, SL4 and SL5. To unravel the microbial community structure of the agricultural soil prior to Cd amendment, total DNA was extracted from the agricultural soil (SL4) immediately after sampling. For metagenomic evaluation of the effects of cadmium contamination (250 mg kg⁻¹) on the microbial community of the agricultural soil, the total DNA was extracted from SL5 microcosm 5 weeks post-Cd amendment. Total DNA were extracted from the sieved soil samples (0.25 g) using ZYMO soil DNA extraction Kit (Model D 6001, Zymo Research, USA) following manufacturer's instructions. The quality and concentration of the extracted total DNA was ascertained using NanoDrop spectrophotometer and electrophoresed on a 0.9% (w/v) agarose gel, respectively. Shotgun metagenomics of SL4 and SL5 microcosms was prepared using the Illumina Nextera XT sample processing kit and sequenced on a MiSeq. The protocols for total DNA preparation for Illumina shotgun sequencing were as described previously (Salam 2018; Salam and Ishaq 2019).

Processing of fastq raw reads, quality control, assembly and taxonomic classification

Processing and quality control of fastq raw reads, assembly and taxonomic classification were carried out using the analysis tools in EDGE Bioinformatics web server (Li et al. 2017). The pre-processing of the raw Illumina fastq file of the two metagenomes (SL4 and SL5) for quality control check, de novo assembly of the trimmed reads and assembly validation were carried out using FastQ Quality Control Software (FaQCs) (Lo and Chain 2014),

IDBA-UD (Peng et al. 2012), and Bowtie2 (Langmead and Salzberg 2012), respectively.

Read-based and contig-based classifications in the EDGE Bioinformatics web-server were deployed for taxonomic classification of the SL4 and SL5 metagenomes. Although there are several read-based classification tools (GOTTCHA, Kraken, MetaPhlan, BWA) in the EDGE, Kraken (Wood and Salzberg 2014) was selected for read-based taxonomic classification of the metagenomes due to the depth and accurateness of its database. Contig-based taxonomic classification is premised on alignment of the SL4 and SL5 contigs to NCBI's RefSeq database using the BWA-mem aligner. Metagenomic data of SL4 and SL5 have been deposited and made public in EDGE Bioinformatics web server.

Functional annotation of metagenomics reads

Sequence reads generated from each of the metagenome were assembled individually using the `make.contig` command in the MOTHUR metagenomic analysis suite (Schloss et al. 2009). Gene calling was performed on the SL4 and SL5 sequence reads using MetaGene (Noguchi et al. 2006) to predict open reading frames (ORFs). The predicted genes were functionally annotated using the KEGG KofamOALA (Aramaki et al. 2019), which assigns K numbers to the predicted genes by HMMER/HMMSEARCH against Kofam (a customized HMM database of KEGG Orthologs). Other functional annotation tools used include the NCBI's conserved domain database CDSEARCH/cdd v 3.15 (CDD; Marchler-Bauer et al. 2015), PANNZER2 (Protein Annotation with Z-score) designed to predict the functional description (DE) and GO (Gene Ontology) classes (Törönen et al. 2018), and BacMet (Pal et al. 2014), a function-specific bioinformatics resource for detection of antibacterial biocide and metal-resistance genes.

In BacMet, the predicted genes (protein sequences of SL3 and SL4) were presented as query to the BacMet database (version 2.0) of predicted resistance genes (using default parameters) for identification of metal-resistance genes in the query sequences. A modified stand-alone version of the BLAST program (NCBI, version 2.2.2) implemented in the BacMet web server was used for similarity searches against the BacMet sequence databases.

Statistical analysis

The effects of Cd contamination on the soil physico-chemistry and the microbial community structure was statistically analysed using the *t* test tool in the Analysis ToolPak of Microsoft Excel 2013 software.

Results

Physicochemical properties and heavy metals content

The physicochemical properties and heavy metal content of the agricultural soil (SL4) and cadmium-contaminated agricultural soil (SL5) are shown in Table 1. The pH of the soil, which is close to neutral (6.87 ± 0.28) in SL4 became weakly acidic in SL5 (6.60 ± 0.06). The moisture content, which is less than 7% (6.75 ± 0.01) in SL4 dropped further to 4% in SL5 (4.32 ± 0.01). All the other physicochemical parameters also showed a declining trend in SL4 (Table 1). Statistical analysis of the physicochemical parameters of the two metagenomes revealed that the difference is statistically significant ($P < 0.05$; $P = 0.036$). In addition, significant traces of heavy metals were detected in the soil. While the concentrations of lead (0.02 ± 0.002 mg/kg), selenium (0.006 ± 0.001 mg/kg), and Cd (0.15 ± 0.001 mg/kg) detected in the agricultural soil are considerably low, high concentrations of zinc, iron, copper, and chromium were detected in the agricultural soil SL4. However, apart from Cd, the concentrations of the heavy metals substantially decrease in SL5 (Table 1).

General characteristics of the metagenomes

Illumina shotgun next-generation sequencing of the total DNA from the two soil microcosms revealed 73,402 and 46,294 sequence reads for SL4 and SL5, respectively. The SL4 and SL5 metagenomes consisted of 21,042,303 and 12,428,339 bp, mean sequence length of 286.67 ± 59.44 and 268.47 ± 86.22 bp, and mean GC contents of

$55.08\% \pm 12.49$ and $54.20\% \pm 10.61$, respectively. After trimming, dereplication, and quality control, sequence reads in SL4 and SL5 reduced to 69,514 (94.70%) and 40,658 (87.83%) with 20,902,030 (99.33%) and 12,216,171 (98.29%) bp, mean sequence lengths of 300.69 ± 4.38 and 300.46 ± 7.23 bp, and mean GC contents of $57.49\% \pm 4.94$ and $55.70\% \pm 4.49$, respectively. Other general features of the soil metagenomes are indicated in Table 2.

Taxonomic characterization of the metagenomes

Taxonomic characterization of the agricultural soil (SL4) revealed 29 phyla with the preponderance of the phyla *Proteobacteria* (37.38%), *Actinobacteria* (35.26%), *Bacteroidetes* (13.45%), and *Firmicutes* (9.47%). In cadmium-contaminated SL5 microcosm, 25 phyla were recovered with the predominance of *Proteobacteria* (50.50%), *Actinobacteria* (17.17%), *Firmicutes* (16.42%), and *Bacteroidetes* (10.70%). In SL5, 68.05% of members of *Actinobacteria* were lost while there is massive reduction in the population of members of the phyla *Candidatus Saccharibacteria*, *Chloroflexi*, and *Nitrospirae*. In contrast, there is a massive upsurge in the population of members of the phyla *Euryarchaeota* (an archaeal phylum), *Chlamydiae*, *Spirochaetes*, and *Deferribacteres* in SL5 microcosm (Fig. 1).

In class delineation, 42 and 38 classes were retrieved from SL4 and SL5 metagenomes with the dominance of *Actinobacteria* (35.02%), *Alphaproteobacteria* (12.31%), *Betaproteobacteria* (10.93%), and *Gammaproteobacteria* (8.99%) in SL4 and *Alphaproteobacteria* (22.28%), *Actinobacteria* (18.36%), *Gammaproteobacteria* (15.54%), and *Bacilli* (11.34%) in SL5. In SL5, Massive decline was observed in the population of members of the classes *Actinobacteria*, *Rubrobacteridae*, *Negativicutes*, *Acidimicrobiales* and *Nitrospira* while there is a huge upscale in the population of members of the classes *Methanomicrobia*, *Chlamydia* and *Spirochaetia* (Fig. 2).

In order classification where 94 and 78 orders were recovered in SL4 and SL5 metagenomes, there is preponderance of *Actinomycetales* (25.81%), *Burkholderiales* (8.01%) and *Bacteroidales* (7.19%) in SL4 while *Actinomycetales* (17.18%), *Rhizobiales* (8.51%) and *Burkholderiales* (8.35%) dominates in SL5 (Additional file 1: Figure S1). In family delineation, 158 and 126 families were retrieved from SL4 and SL5 metagenomes. *Caulobacteraceae* (8.70%), *Alcaligenaceae* (7.10%), and *Sphingobacteriaceae* (6.12%) dominates in SL4 while *Enterobacteriaceae* (7.94%), *Alcaligenaceae* (7.45%) and *Methyobacteriaceae* (6.61%) were preponderant in SL5 (Additional file 1: Figure S2).

In genus delineation, 270 and 205 genera were recovered in SL4 and SL5 metagenomes. The genera with the highest representation in SL4 include *Prevotella* (6.93%),

Table 1 Physicochemistry and heavy metals content of agricultural soil (SL4) and cadmium-contaminated agricultural soil (SL5)

	SL4	SL5
Physicochemical parameters		
pH	6.87 ± 0.28	6.60 ± 0.06
Moisture (%)	6.75 ± 0.01	4.32 ± 0.01
Total organic matter (%)	73.21 ± 0.21	64.79 ± 1.90
Total nitrogen (%)	53.48 ± 0.69	36.08 ± 2.13
Phosphorus (mg/kg)	29.41 ± 0.82	22.15 ± 1.39
Potassium (mg/kg)	17.880 ± 0.002	12.160 ± 0.003
Heavy metals content		
Lead (mg/kg)	0.020 ± 0.001	ND
Chromium (mg/kg)	5.910 ± 0.003	3.580 ± 0.002
Cadmium (mg/kg)	0.150 ± 0.001	62.800 ± 0.002
Zinc (mg/kg)	14.080 ± 0.003	7.760 ± 0.004
Iron (mg/kg)	13.940 ± 0.003	7.230 ± 0.005
Copper (mg/kg)	12.580 ± 0.001	8.220 ± 0.004
Selenium (mg/kg)	0.006 ± 0.001	ND

ND not detected

Table 2 General characteristics of SL4 and SL5 metagenomes

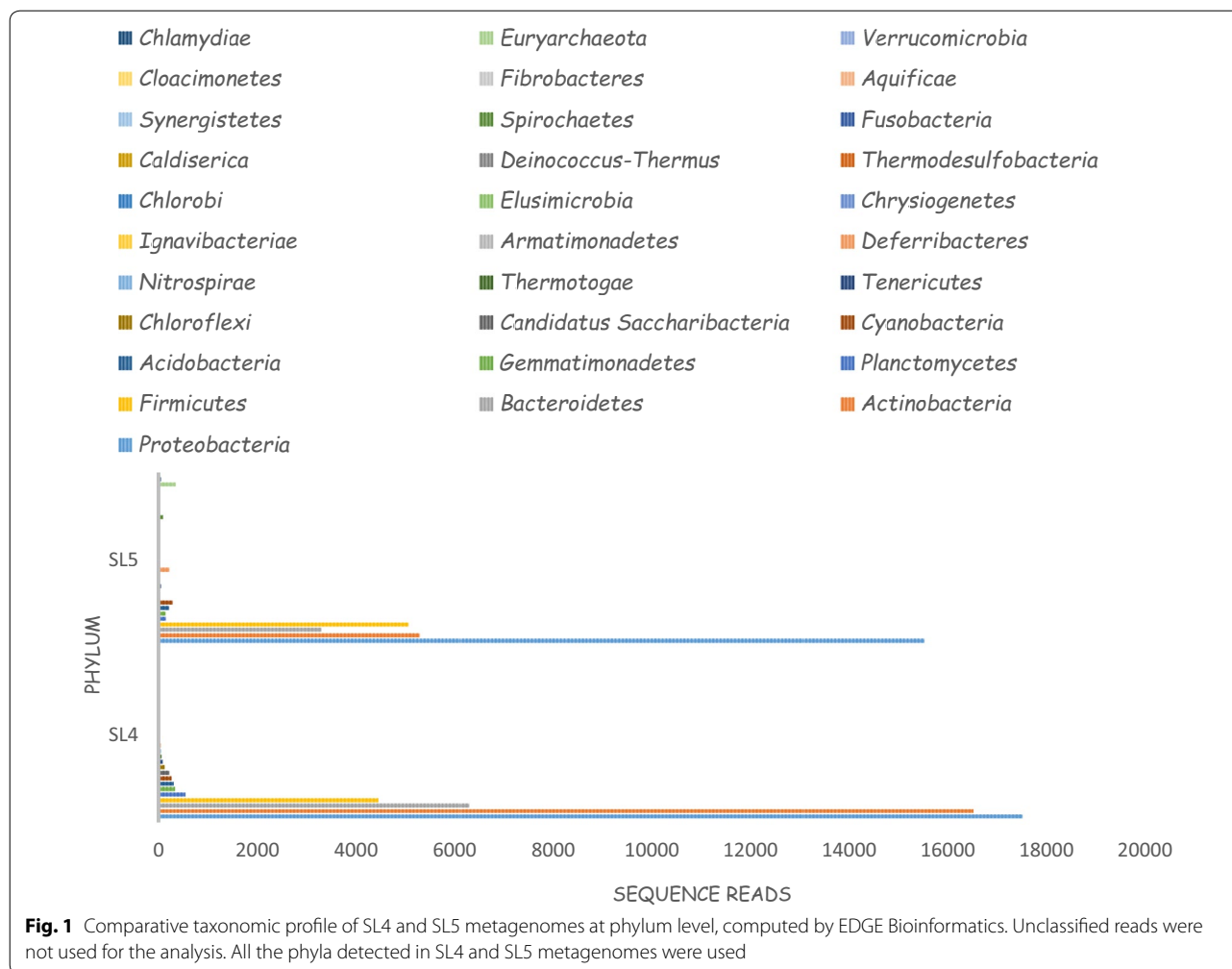
	SL4	SL5
1. Pre-processing		
a. Raw reads		
Reads	73,402	46,294
Total bases (bp)	21,042,303	12,428,339
Mean read length (bp)	286.67 ± 59.44	268.47 ± 86.22
Mean GC content (%)	55.08 ± 12.49	54.20 ± 10.61
b. Quality trimming		
Trimmed reads		
Reads	69,514 (94.70%)	40,658 (87.83%)
Total bases (bp)	20,902,030 (99.33%)	12,216,171 (98.29%)
Mean read length (bp)	300.69 ± 4.38	300.46 ± 7.23
Mean GC content (%)	57.49 ± 4.94	55.70 ± 4.49
Paired reads	69,494 (99.97%)	40,604 (99.87%)
Paired total bases	20,896,965 (99.98%)	12,200,818 (99.87%)
Unpaired reads	20 (0.03%)	54 (0.13%)
Unpaired total bases	5065 (0.02%)	15,353 (0.13%)
2. Assembly and annotation		
a. De novo assembly by idba_ud		
Number of contigs	117	76
N50 (bp)	420	424
Max contig size (bp)	458	462
Min contig size (bp)	255	270
Total assembly size (bp)	47,020	30,607
b. Assembly validation by read mapping		
Number of mapped reads	40,629	23,795
% of total reads	58.45%	58.52%
Number of unmapped reads	28,885	16,863
% of total reads	41.55%	41.48%
Average fold coverage	214.34 X	204.18 X

Conexibacter (5.91%), *Brevundimonas* (5.02%), and *Bifidobacterium* (4.46%). In Cd-contaminated SL5 metagenome, the predominant genera include *Methylobacterium* (9.14%), *Streptococcus* (4.29%), *Paenibacillus* (3.74%), and *Prevotella* (3.67%). Massive decline was observed in the population of *Caulobacter*, *Acinetobacter*, *Megasphaera*, *Conexibacter*, *Burkholderia*, *Prevotella* and several others in SL5. In contrast, massive enrichment in the population of *Methylobacterium*, *Paenibacillus*, *Modestobacter*, *Methanosaeta*, *Flexistipes*, *Desulfomicrobium*, *Arcobacter* and few others were observed in the Cd-contaminated SL5 metagenome (Fig. 3). Statistically significant ($P < 0.05$; $P = 0.0016$) difference in genus delineations was observed between SL4 and SL5 metagenome.

In species delineation, 310 and 230 species were retrieved from SL4 and SL5 metagenomes. The preponderant species in SL4 metagenome are *Conexibacter woesei* (8.93%), *Brevundimonas subvibrioides* (7.58%),

Sphingobacterium sp. 21 (6.47%), and *Pedobacter saltans* (4.59%). In Cd-amended SL5 metagenome, the dominant species are *Methylobacterium radiotolerans* (12.80%), *Sphingobacterium* sp. 21 (4.86%), *Modestobacter marinus* (4.60%) and *Sphingomonas wittichii* (3.60%), respectively. Population of *C. woesei*, *Acinetobacter baumannii*, *Megasphaera elsdenii*, *Acidimicrobium ferrooxidans* and several others massively nosedived in SL5 while species such as *M. radiotolerans*, *M. marinus*, *Methanosaeta concilii*, *Flexistipes sinusarabici* and many others were massively enriched (Fig. 4). Statistically significant ($P < 0.05$; $P = 0.01$) difference in species delineations was observed between SL4 and SL5 metagenome.

Contig-based classification of the metagenomes (SL4 and SL5) conducted by aligning the SL4 and SL5 contigs to NCBI's RefSeq database using the BWA-mem aligner is indicated in Additional file 1: Figs. S3 to S8.



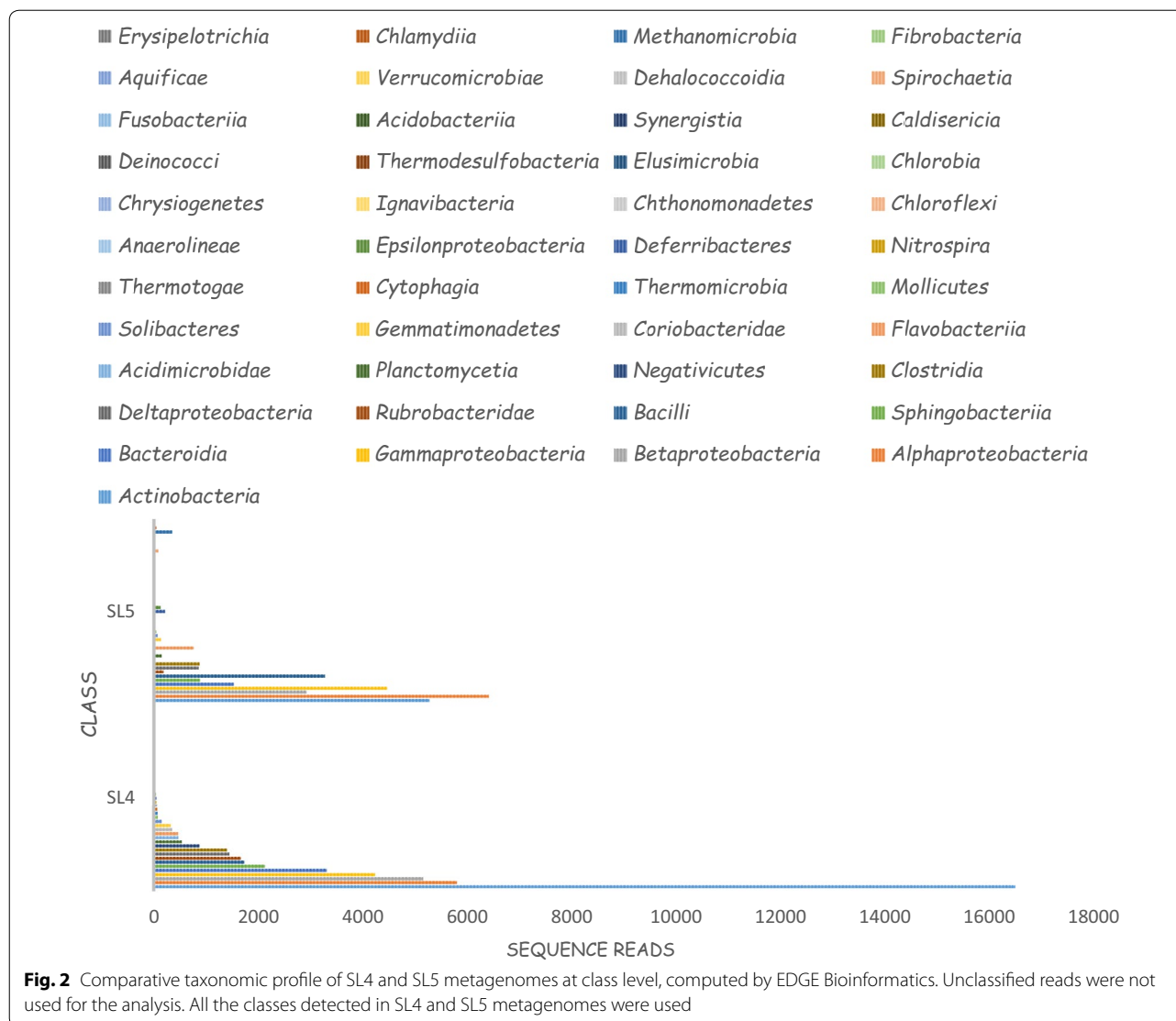
Functional annotation of the metagenomes

Functional characterization of the metagenomes revealed significant differences. In SL4 metagenome, putative genes for carbohydrate metabolism (fructose-6-phosphate aldolase 2; arabinoxylan arabinofuranohydrolase; 2-dehydro-3-deoxygluconokinase/2-dehydro-3-deoxygalactonokinase), nitrogen metabolism (CFP/FNR family transcriptional regulator, nitrogen oxide reductase regulator), sulphur metabolism (sulphite oxidase), methane metabolism (Ni-sirohydrochlorin a,c-diamide reductive cyclase, play a key role in methanogenesis and anaerobic methane oxidation), and autotrophic CO₂ assimilation (energy-converting hydrogenase B) were detected. Other putative genes detected include genes responsible for biosynthesis of bioactive compounds and antibiotics (fumagillin biosynthesis methyltransferase, nocardicin N-oxygenase, trigonelline monooxygenase, oxygenase component), xenobiotic degradation (cyanamide hydratase, cytochrome P450 RapN, poly(3-hydroxyoctanoate) depolymerase), and stress response

(diacylglycerol diphosphate phosphatase/phosphatidate phosphatase).

In SL5 metagenome, putative genes and enzymes were detected for carbohydrate metabolism (2,3-bisphosphoglycerate-independent phosphoglycerate mutase, UDP-glucose-4 epimerase), amino acid metabolism (cysteine desulfurase, tryptophan synthase beta chain), xenobiotic degradation (carboxylesterase 1, alkene monooxygenase, effector subunit), polyketide synthases (nogalonic acid methyl ester cyclase/aklanonic acid methyl ester cyclase), and vitamin B₁₂, porphyrin and chlorophyll metabolism (adenosylcobinamide-phosphate synthase).

Functional annotation of the predicted genes in SL4 and SL5 metagenomes for heavy metals resistance genes using the BacMet database revealed interesting findings. Diverse protein families responsible for transport, uptake and efflux of heavy metals were detected in the two metagenomes (Tables 3, 4). In agricultural soil SL4 metagenome, putative genes for transport, uptake, and efflux of copper (*copA*, *copB*, *copC*, *copP*, multicopper



oxidase type 2 and 3; *CueO*, *cutC*, *cutE*, etc.), chromium, cadmium, nickel, cobalt (*chrA*, *chrB*, *nikA*, *nikB*, *nikR*, cadmium-translocating P-type ATPase, nickel-cadmium-cobalt resistance protein *nccC*, etc.) were detected. Other putative genes detected include resistance genes for iron, zinc, magnesium, manganese (*furA*, *BasS/PmrB*, zinc/iron ZIP family permease, *mgtB*; magnesium-translocating P-type ATPase; NRAMP family Mn^{2+}/Fe^{2+} transporter, etc.) and mercury, silver, molybdenum, lead, arsenic, tungsten, tellurium and antimony (*merA*, *merB*, *merR*, *merH*, *merP*, *pbrA*, *modA*, *modB*, *modC*, *TrgB*, *TehA*, *WtpA*, arsenite oxidase, *arsB*, *arsC*, *arsM*, etc.) (Table 3).

In Cd-contaminated SL5 metagenome, putative genes were detected for cadmium, cobalt, nickel, zinc (heavy metal-translocating P-type ATPase, *czcA*, *czcD*, *czrA*,

czrB, *zraR*, *zraP*, *znuA*, cobalt-zinc-cadmium resistance protein, *nikA*, *nikR*, *nikD*, *nike*, etc.), and copper, magnesium, and silver (*copA*, *copB*, *copC*, magnesium-transporting ATPase, *corA*, copper/silver efflux P-type ATPase, etc.). Also detected are putative resistance genes for iron, lead, chromium, manganese, tellurium, selenium (*fpvA2* gene, *fur*, *fbpC*, ferroxidase, *ctpC* gene, *tehB*, *chrA*, *chrC*, *trgB*, etc.), and mercury, arsenic, molybdenum and tungsten (*merA*, *merR*, *merT*, *merB1*, *arsB*, *arsC*, *arsH*, arsenite oxidase, *arsM*, *modB*, *wtpA*, etc.) (Table 4).

It was observed that putative genes, responsible for cadmium homeostasis, transport, efflux and detoxification such as *czcA*, *czcD*, *czrA*, *czrB*, manganese transport protein, and manganese/iron superoxide dismutase (MnSOD, *sodA*; FeSOD, *sodB*) which were detected in Cd-amended SL5 metagenome were conspicuously

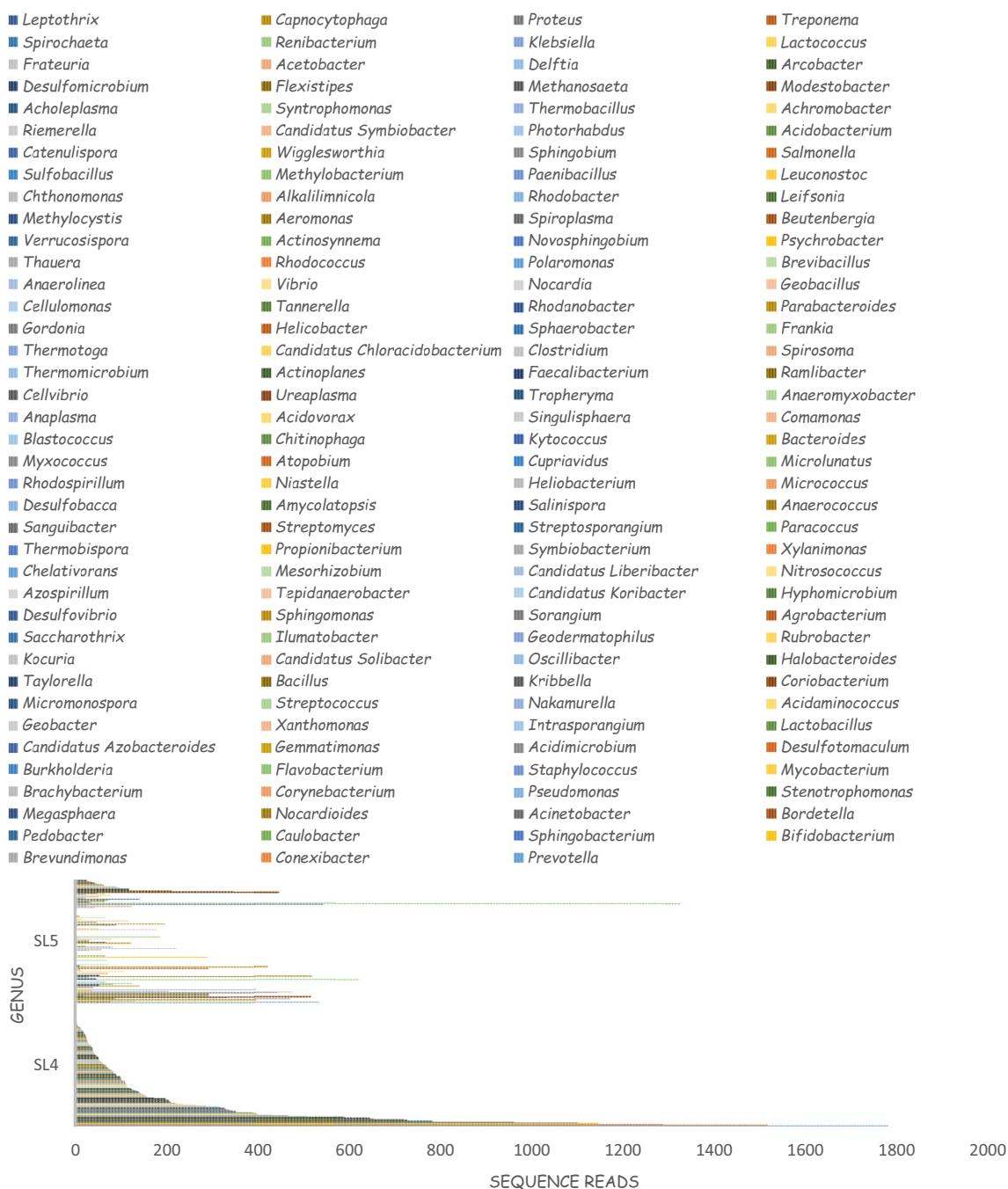


Fig. 3 Comparative taxonomic profile of SL4 and SL5 metagenomes at genus level, computed by EDGE Bioinformatics. Unclassified reads were not used. Only genera with ≥ 10 sequence reads were used for the analysis

absent in SL4 metagenome. It was also observed based on functional annotation of protein sequences in Cd-amended SL5 metagenome using PANNZER2 that one thousand four hundred and forty (1440) of the sequences were annotated for alkyl hydroperoxide reductase (AhpC), an organic hydroperoxide detoxification enzyme.

However, the AhpC gene was not detected in the protein sequences of SL4 metagenome.

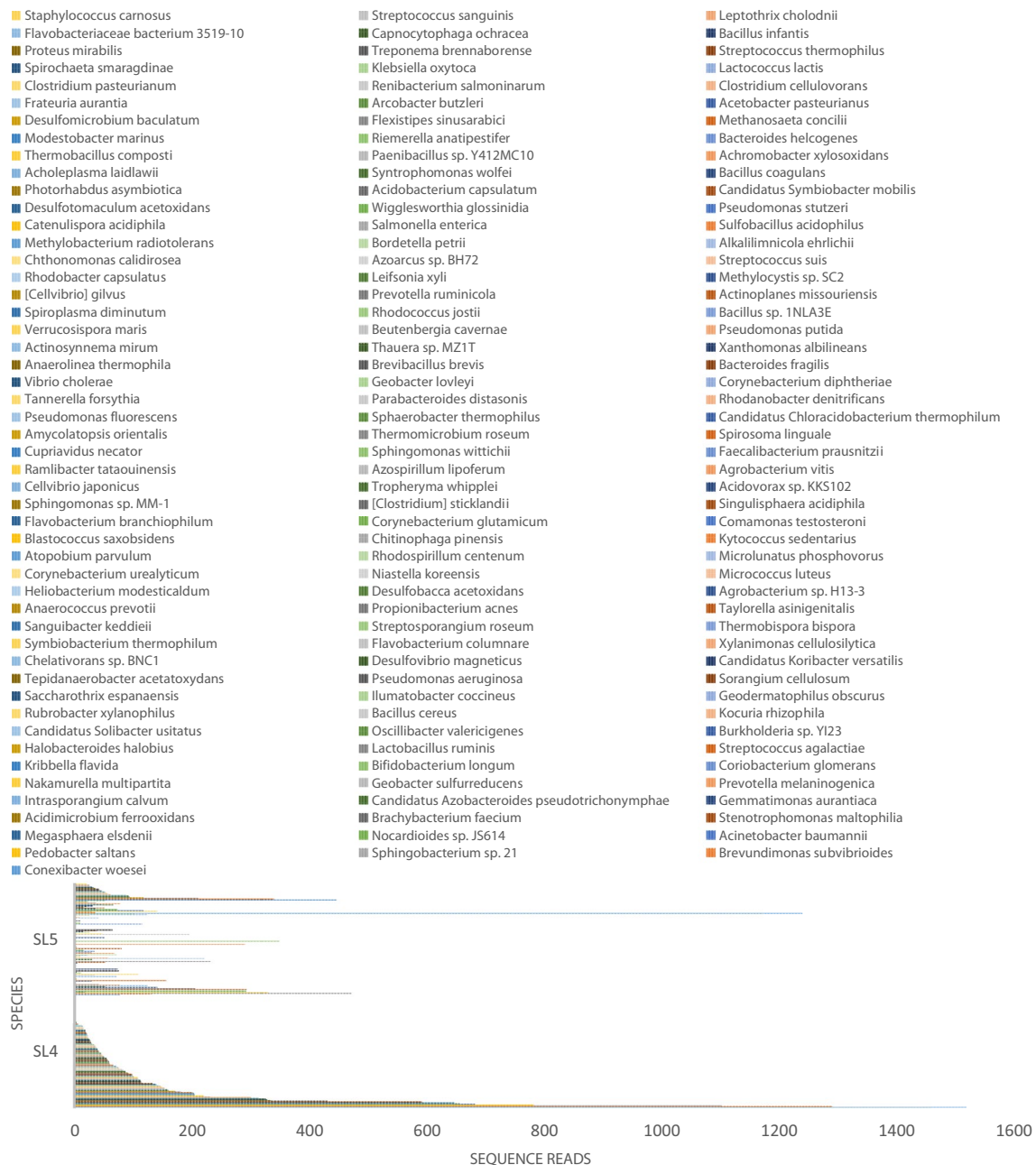


Fig. 4 Comparative taxonomic profile of SL4 and SL5 metagenomes at species level, computed by EDGE Bioinformatics. Unclassified reads were not used. Only species with ≥ 10 sequence reads were used for the analysis

Discussion

Point and non-point release of heavy metals and metalloids into soil environments via atmospheric deposition and diverse agricultural activities have negatively impacted soil ecological balance, alter soil physicochemistry and biogeochemistry, reduce soil microbial diversity and pose serious health risk to animals and humans (Feng et al. 2018; Rai et al. 2019; Salam et al. 2019). In

this study, all the physicochemical parameters considerably reduce in Cd-amended SL5 microcosm, though not as profound as those reported in our previous study on mercury (Salam et al. 2019). This may be attributed to Cd contamination. Previous reports have indicated that increase in soil pH increases Cd sorption to soil organic matter (Gray et al. 1998, 1999). The decrease in soil pH observed in SL5 microcosm may thus be indicative of

Table 3 Predicted heavy metals resistance genes detected in SL4 metagenome and their taxonomic affiliations

Heavy metals	Enzyme/genes	Taxonomic affiliation
Copper	Copper resistance protein CopC; multicopper oxidase type 3; copper exporting ATPase; putative multicopper oxidase (laccase-like); copper resistance protein B precursor; copper homeostasis protein, cutC; copper resistance protein A, copA; twin-arginine translocation pathway signal; putative copper binding protein; copper-translocating P-type ATPase, copB; multicopper oxidase type 2; apolipoprotein N-acyltransferase; two-component heavy metal transcriptional regulator; apolipoprotein N-acyltransferase/Copper homeostasis protein, cutE; heavy-metal transporting P-type ATPase; P-ATPase superfamily P-type ATPase copper transporter; penicillinase repressor/transcription regulator, copY/tcrY; two-component sensor, copS; lipoprotein involved with copper homeostasis and adhesion, cutF; blue copper oxidase CueO; copper tolerance protein; heavy metal transport/detoxification protein, copP; putative laccase	<i>Frankia</i> sp. Cc13; <i>Shigella dysenteriae</i> 1012; <i>Haloalkalicoccus jeotgali</i> B3; <i>Intrasporangium calvum</i> DSM 43,043; <i>Methanoseta harundinacea</i> 6A; <i>Rhodococcus pyridinivorans</i> AK37; <i>Halorubrum lacusprofundi</i> ATCC 49239; <i>Azospirillum brasilense</i> Sp245; <i>Geobacillus thermodenitrificans</i> NG80-2; <i>Pseudomonas aeruginosa</i> 2192; <i>Citrobacter rodentium</i> ICC168; <i>Citrobacter koseri</i> ATCC BAA-895; <i>Acetobacter pomorum</i> DM001; <i>Kytococcus sedentarius</i> DSM 20547; <i>Azorhizobium caulinodans</i> ORS 571; <i>Nitrosomonas eutropha</i> C91; <i>Corynebacterium ammoniagenes</i> DSM 20306; <i>Salmomella enterica</i> subsp. enterica serovar Weltevreden str. HJ_N05-537; <i>Oceanicola</i> sp. S124; <i>Polymorphum gilvum</i> SL-003B-26A1; <i>Xanthobacter autotrophicus</i> Py2; <i>Bradyrhizobium</i> sp. ORS 375; <i>Delftia acidovorans</i> SPH-1; <i>Achromobacter</i> sp. A022; <i>Vibrio</i> sp. RC586; <i>Oxalobacter formigenes</i> OXCC13; <i>Mycobacterium parascrofulaceum</i> ATCC BAA-614; <i>Lactobacillus pentosus</i> MP-10; <i>Lactobacillus pentosus</i> G1; <i>Lactobacillus plantarum</i> JDM1; <i>Pseudomonas aeruginosa</i> PA01; <i>Pseudomonas aeruginosa</i> M18; <i>Pectobacterium carotovorum</i> subsp. brasiliensis PBR1692; <i>Rahnella</i> sp. Y9602; <i>Providencia rustigianii</i> DSM 4541; <i>Rhodobacterales bacterium</i> Y4i; <i>Thermomonospora curvata</i> DSM 43183; <i>Verrucumicrobiaceae bacterium</i> CNC16
Chromium, nickel, cobalt	NADPH-dependent FMN reductase, transcriptional regulator NikR, CopG family (Ni); integral membrane sensor signal transduction histidine kinase nrsS; nickel ABC transporter, nickel/metallophore periplasmic binding protein; chromate resistance protein, chrB; binding-protein-dependent transport system inner membrane; nickel ABC transporter, periplasmic nickel-binding protein, nikA; iron-dicitrate transporter subunit, membrane component of ABC superfamily (Ni/Co); chromate transporter, chrA; major facilitator superfamily protein (Ni/Co); ArsR family transcriptional regulator (Co/Ni); peptide/nickel transport system substrate-binding protein, regulatory protein, chrB1; cation diffusion facilitator family transporter (Ni/Co); permeases of the major facilitator superfamily (Ni/Co); binding-protein-dependent transport system inner membrane protein, nikB; cadmium-translocating P-type ATPase (Co/Ni)	<i>Bacillus coagulans</i> 2-6; <i>Methanococcus voltae</i> A3; <i>Acaryochloris</i> sp. CCME5 5410; <i>Peptoniphilus</i> sp. oral taxon 375 str. F0436; <i>Burkholderia multivorans</i> CGD1; <i>Burkholderia multivorans</i> CGD2M; <i>Candidatus Desulfuridus audaxviator</i> MP104C; <i>Synergistes</i> bacterium SGP1; <i>Escherichia coli</i> S88; <i>Leptothrix cholodnii</i> SP-6; <i>Cellulomonas fimi</i> ATCC 484; <i>Jonessia denitrificans</i> DSM 20603; <i>Azospirillum</i> sp. B510; <i>Cuptriavidus metallidurans</i> CH34 (plasmid); <i>Methylobacterium nodulans</i> ORS 2060; <i>Methylobacterium extorquens</i> DM4; <i>Sinorhizobium meliloti</i> CCNWSX0020; <i>Candidatus Desulfuridus audaxviator</i> MP104C; <i>Streptomyces griseoflavus</i> Tu4000
Mercury, silver, cadmium, lead	Copper-transporting ATPase RAN1, merP; mercuric ion reductase, merA; mercuric reductase merB1; right origin-binding protein roba (Ag/Hg/Cd); mercuric transport protein periplasmic protein, merP; Pb-efflux ATPase pbrA (Pb); organomercurial lyase, merB; MerR family transcriptional regulator; disulfide bond formation protein B (Cd/Hg); Pb/Cd/Zn/Hg transporting ATPase; sensor protein ZraS (Pb, Zn); mercuric transporter, merH	<i>Verticillium dahliae</i> VdLs. 17; <i>Verticillium albo-atrum</i> VaMs. 102; <i>Pseudomonas fluorescens</i> ; <i>Xanthobacter autotrophicus</i> Py2; <i>Yersinia ruckeri</i> ATCC 29473; <i>Delftia acidovorans</i> SPH-1; <i>Ralstonia metallidurans</i> CH34 (plasmid); <i>Janthinobacterium</i> sp. Marseille (plasmid); <i>Pseudomonas stutzeri</i> (plasmid); <i>Haliangium ochraceum</i> DSM 14365; <i>Vibrio caribbenthicus</i> ATCC BAA-2122; <i>Tolumonas auensis</i> DSM 9187; <i>Mesorhizobium amorphae</i> CCNWS0123; <i>Citrobacter youngae</i> ATCC 29220; <i>Mycobacterium</i> sp.
Zinc, manganese, cadmium	Zinc resistance protein, zraP; Fis family transcriptional regulator; periplasmic solute-binding protein (Mn/Cd); RND family efflux transporter, MFP subunit (Zn); DSBA Oxidoreductase (Cd); membrane fusion protein (MFP-RND) heavy metal cation tricomponent efflux HmxB (Zn); cation transporting ATPase, P-type; high-affinity zinc transporter periplasmic protein; DSBA gene product (Cd/Zn/Hg); cadmium-translocating P-type ATPase (Zn); dithiol-disulfide isomerase (Cd); heavy metal-translocating P-type ATPase (Zn); zinc/manganese/iron ABC transporter, periplasmic zinc/manganese/iron-binding protein	<i>Escherichia</i> sp. TW09308; <i>Citrobacter freundii</i> 4_7_47CFAA; <i>Citrobacter youngae</i> ATCC 29220; <i>Salmomella enterica</i> subsp. enterica serovar Hadar str. RL_05P066; <i>Desulfivibrio vulgaris</i> str. Miyazaki F; <i>Thermobacillus composti</i> KW4; <i>Pseudomonas putida</i> S16; <i>Paenibacillus mucilaginosus</i> KNP414; <i>Cuptriavidus metallidurans</i> CH34 (plasmid); <i>Pyrococcus abyssi</i> GE5; <i>Dickeya dadantii</i> Ech703; <i>Candidatus Blochmannia pennsylvanicus</i> str. BPEN; <i>Thermaerobacter subterraneus</i> DSM 13965; <i>Rhodococcus equi</i> 1035; <i>Bacillus cytotoxicus</i> NVH 391-98; <i>Rhodobacterales bacterium</i> HTCC2083

Table 3 (continued)

Heavy metals	Enzyme/genes	Taxonomic affiliation
Iron, cobalt, nickel, gallium, cadmium, magnesium, manganese, zinc	<p>Fe(III)-pyochelin outer membrane receptor precursor; peptide/nickel transport system substrate-binding protein; ferrichrome ABC transporter permease (Ni/Co); mgtB gene product (Mg/Co); magnesium-translocating P-type ATPase (Mg/Co); iron-dependent repressor, ideoR; heavy metal-translocating P-type ATPase (Co/Ni); aconitase A (Fe); ABC transporter, ATP-binding protein YbbL (Fe); TonB-dependent siderophore receptor; zinc/iron ZIP family permease (Zn/Fe/Co/Ni/Cu/Cd); nickel-cobalt-cadmium resistance protein nccc (plasmid); aconitase hydratase (Fe); ferritin (Fe/Cu/Mn); fvpA2 gene product; predicted divalent heavy-metal cations transporter (Zn/Fe/Co/Ni/Cu/Cd); ferric uptake regulator family protein, furA (Fe); DNA-binding transcriptional regulator BasR (Fe); iron-dicitrate transporter ATP-binding subunit; NRAMP family Mn^{2+}/Fe^{2+} transporter (Mn/Fe/Cd/Co/Zn); sensor protein BasS/PmrB (Fe); ferrityoverdine receptor 2</p>	<p><i>Pseudomonas aeruginosa</i> C3719; <i>Pseudomonas aeruginosa</i> M18; <i>Pseudomonas aeruginosa</i> PA01; <i>Azospirillum</i> sp. BS10; <i>Vibrio parahaemolyticus</i> RIMD 2210633; <i>Pectobacterium atrosepticum</i> SCR11043; <i>Klebsiella pneumoniae</i> 342; <i>Klebsiella variicola</i> At-22; <i>Pseudomonas aeruginosa</i> 138244; <i>Kineococcus radiotolerans</i> SRS30216; <i>Actinosynnema mirum</i> DSM 43827; <i>Bifidobacterium bifidum</i> NCIMB 41171; <i>Bifidobacterium bifidum</i> S17; <i>Escherichia fergusonii</i> ATCC 35469; <i>Pseudomonas fulva</i> 12-X; <i>Comamonas testosteroni</i> S44; <i>Variovorax paradoxus</i> EPS; <i>Methanosarcina</i></p> <p><i>Harundinacea</i> 6Ac; <i>Corynebacterium glucuronolyticum</i> ATCC 51867; <i>Streptomyces violaceusniger</i> Tu 4113; <i>Proteus mirabilis</i> H14320; <i>Azoarcus</i> sp. BH72; <i>Soilbacterilus silvestris</i> StL.B046; <i>Segniliparus rigosus</i> ATCC BAA-974; <i>Sodalis glossinidius</i> str. 'moisitans'; <i>Citrobacter koseri</i> ATCC BAA-895; <i>Thermobaculum terrenum</i> ATCC BAA-798; <i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047; <i>Achromobacter xylosoxidans</i> A8</p>
Molybdenum, tungsten, tellurium	<p>modE gene product; tellurite resistance protein TrgB; K1aB protein (Te); molybdate ABC superfamily ATP-binding cassette transporter, ABC protein, modC; molybdate ABC transporter periplasmic molybdate-binding protein modA; tungstate ABC transporter binding protein WtpA; molybdenum ABC transporter permease protein modB; tellurite resistance protein TehA</p>	<p><i>Xenorhabdus bovienii</i> SS-2004; <i>Roseobacter</i> sp. GA1101; uncultured bacterium (plasmid); <i>Serratia odorifera</i> DSM 4582; <i>Sinorhizobium melloti</i> 1021; <i>Desulfurobacterium thermolithotrophum</i> DSM T11699; <i>Gemmatimonas aurantiaca</i> T-27; <i>Neisseria mucosa</i> ATCC 25996</p>
Arsenic, antimony	<p>ABC transporter; multidrug resistance protein, p-glycoprotein; arsenite oxidase, large subunit, AoxB; phosphate ABC transporter permease (As); phosphate ABC transporter substrate-binding protein; protein-tyrosine phosphatase, low molecular weight, arsc; arsenate reductase; arsenate reductase, glutathione/glutaredoxin type; arsenic resistance protein ardB; arsenite oxidase small subunit, AoxA; ABC thiol transporter; methyltransferase, arsm; methyltransferase type II; arsenite S-adenosylmethyltransferase; arsenate reductase (azurin); arsenical resistance protein Arsh; arsenical resistance protein arisB</p>	<p><i>Leishmania major</i> strain Friedlin; <i>Roseobacter litoralis</i> och 149; <i>Nitrosomonas</i> sp. IS79A3; <i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860; <i>Sphaerobacter thermophilus</i> DSM 20745; <i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i>; <i>Staphylococcus aureus</i>; <i>Bacillus subtilis</i> subsp. <i>subtilis</i> RO-NN-1; <i>Microcoleus chthonoplastes</i> PCC 7420; <i>Thauera</i> sp. MZ11; <i>Rhodospirillum rubrum</i> ATCC 17100; <i>Burkholderia multivorans</i> ATCC 17616; <i>Leishmania infantum</i> JPCMS; <i>Microbacterium phosphovorans</i> NM-1; <i>Leptonema illini</i> DSM 21528; <i>Desulfotolobium retbaense</i> DSM 5692; <i>Rhodolferax ferrieducens</i> T118; <i>Glucanacetobacter</i> sp. SKCC-1; <i>Peptoniphilus harei</i> ACS-146-V-Sch2b; <i>Acetivibrio cellulolyticus</i> CD2</p>

Table 4 Predicted heavy metals resistance genes detected in cadmium-amended SL5 metagenome and their taxonomic affiliations

Heavy metals	Enzyme/genes	Taxonomic affiliation
Cadmium, cobalt, mercury, lead, zinc	Heavy metal-translocating P-type ATPase, Cd/Co/Hg/Pb/Zn-transporting; ApaG protein; protein disulphide isomerase I, dsbA gene product (Cd/Zn/Hg); DSBA oxidoreductase (Cd); RND divalent metal cation efflux transporter Czca (Zn/Cd); membrane-bound cation-proton-antiporter CzrA gene (Zn/Cd); cobalt-zinc-cadmium resistance protein; cation diffusion facilitator family transporter; CzrB gene	<i>Mycobacterium</i> sp. Splyr1; <i>Saccharomonospora viridis</i> DSM 43017; <i>Mycobacterium vanbaalenii</i> PYR-1; <i>Mycobacterium gilvum</i> PYR-GCK; <i>Shewanella sediminis</i> HAW-EB3; <i>Serratia symbiotica</i> str. 'Cimara cedri'; <i>Candidatus Blochmannia pennsylvanicus</i> str. BPEIN; <i>Paenibacillus mucilaginosus</i> KNP414; <i>Pseudomonas aeruginosa</i> PA7; <i>Pseudomonas aeruginosa</i> 39016; <i>Pseudomonas aeruginosa</i> 138244; <i>Pseudomonas putida</i> ; <i>Pseudomonas</i> sp. 2_1_26; <i>Xanthomonas gardneri</i> ATCC 19865; <i>Pseudomonas aeruginosa</i>
Copper, magnesium, silver	Copper-resistance protein A (copA); multicopper oxidase type 3 (cutO); copper resistance protein C (copC); putative copper binding protein; multicopper oxidase; multicopper oxidase type 2; molecular chaperone DnaK; copper-translocating P-type ATPase; magnesium-transporting ATPase; copper exporting ATPase; heavy metal-translocating P-type ATPase; blue copper oxidase cueO precursor; P-ATPase superfamily P-type ATPase copper transporter, ctpV; apolipoprotein N-acyltransferase; ApaG protein; cation-transporting ATPase; twin-arginine translocation pathway signal; copper resistance protein; cation efflux system protein CusB (Cu/Ag); heavy metal-translocating P-type ATPase, copB; int gene product (Cu); Cu(I)-responsive transcriptional regulator; MerE family transcriptional regulator (Cu); MerR family transcriptional regulator (Cu); lipoprotein involved with copper homeostasis and adhesion, cutF/mlpE; heavy metal transport/detoxification protein, copP; copper/silver efflux P-type ATPase	<i>Acidobacterium capsulatum</i> ATCC 51196; <i>Acetobacter pomorum</i> DM001; <i>Achromobacter xylosoxidans</i> A8; <i>Anaeromyxobacter</i> sp. Fw109-5; <i>Halalkalicoccus jeotgali</i> B3; <i>Stenotrophomonas maltophilia</i> R5513; <i>Frankia</i> sp. Cc13; <i>Halorubrum lacusprofundi</i> ATCC 49239; <i>Corynebacterium ammoniagenes</i> DSM 20306; <i>Corynebacterium lipophiloflavum</i> DSM 44291; <i>Xanthobacter autotrophicus</i> Py2; <i>Haladaptatus paucihalophilus</i> DX253; <i>Rhodococcus jostii</i> RHA1; <i>Intrasporangium calvum</i> DSM 43043; <i>Streptomyces</i> sp. S4; <i>Streptomyces albus</i> J1074; <i>Halobacterium</i> sp. DL1; <i>Achromobacter xylosoxidans</i> C54; <i>Cupriavidus metallidurans</i> CH34; <i>Methanoseta harundinacea</i> 6Ac; <i>Rhodolax ferrereducens</i> ; <i>Erwinia</i> sp. Ejp617; <i>Micrococcus luteus</i> NCTC 2665; <i>Kytococcus sedentarius</i> DSM 20547; <i>Mycobacterium parascrofulaceum</i> ATCC BAA-614; <i>Proteus mirabilis</i> ATCC 29906; <i>Shewanella sediminis</i> HAW-EB3; <i>Bacillus amyloliquefaciens</i> TA208; <i>Pseudomonas syringae</i> pv. glycinosa str. B076; <i>Klebsiella pneumoniae</i> 342; <i>Gordonia polyisoprenivorans</i> NBRC 16320; <i>Methyllobacterium chloromethanicum</i> CM4; <i>Shigella dysenteriae</i> 1012; <i>Acaryochloris</i> sp. CCME5 5410; <i>Hyphomonas neptunium</i> ATCC 15444; <i>Caulobacter</i> sp. K31; <i>Pseudomonas syringae</i> pv. <i>aceris</i> str. M302273PT; <i>Pseudomonas entomophila</i> L48; <i>Pseudomonas putida</i> S16; <i>Rahnella</i> sp. Y9602; <i>Pseudonocardia</i> sp. P1; <i>Citromicrobium bathyomarinum</i> JL354
Nickel	Peptide/nickel transport system, substrate-binding protein, nika; transcriptional regulator NIKR, CopG family (nikR); nickel transporter ATP-binding protein, nikD; nickel ABC transporter, periplasmic nickel-binding protein; nickel transporter ATP-binding protein, nika	<i>Azospirillum</i> sp. B510; <i>Streptococcus sanguinis</i> SK1056; <i>Methanococcus voltae</i> A3; <i>Metahnospirillum hungatei</i> JF-1; <i>Pseudomonas</i> sp. TJJ-51; <i>Synergistetes bacterium</i> SGP1; <i>Rhodospirillum rubrum</i> ATCC 11170

Table 4 (continued)

Heavy metals	Enzyme/genes	Taxonomic affiliation
Zinc, tellurium	RND family efflux transporter MFP subunit; zinc resistance-associated protein, zraP; low-affinity inorganic phosphate transport protein; drug efflux pump transmembrane protein, mdtB; tellurite resistance gene, trgB; transcriptional regulatory protein, zraR (Zn); signal transduction histidine-protein kinase, baeS; int gene product (Zn); sensory histidine kinase in two-component regulatory system with BaeR; zinc-responsive transcriptional regulator; high-affinity zinc transporter, periplasmic component znuA; low-affinity inorganic phosphate transporter 1 (Zn/Te)	<i>Pseudomonas putida</i> KT2440; <i>Citrobacter</i> sp. 30_2; <i>Citrobacter freundii</i> 4_7_47CFAA; <i>Citrobacter youngae</i> ATCC 29220; <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Johannesburg str. 55-703; <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhi str. CT18; <i>Sodalis glossinidius</i> str. 'morisans'; <i>Oxalobacter formigenes</i> OXCC13; <i>Roseobacter litoralis</i> Och 149; <i>Roseobacter denitrificans</i> Och 114; <i>Desulfovibrio magnetus</i> RS-1; <i>Escherichia coli</i> 83972; <i>Hyphomonas neptunium</i> ATCC 15444; <i>Ralstonia solanacearum</i> CMR15; <i>Photobacterium luminescens</i> subsp. <i>laumondii</i> TTO1; <i>Haemophilus pitmaniae</i> HK 85; <i>Yersinia ruckeri</i> ATCC 29473; <i>Dickeya dadantii</i> Ech703; <i>Erwinia pyrifoliae</i> DSM 12163; <i>Erwinia amylovora</i> CFBP1430
Arsenic	Arsenite oxidase, large subunit (aoxB); arsenite S-adenosylmethyltransferase; arsenite methyltransferase <i>arsM</i> ; arsenical resistance protein, <i>arsB</i> ; protein-tyrosine phosphatase, low molecular weight, <i>arsC</i> ; arsenite oxidase, small subunit (<i>aoxA</i>); arsenical resistance protein <i>ArsH</i> ; phosphate ABC superfamily ATP-binding cassette transporter, binding protein	<i>Burkholderia oklahomensis</i> C6786; <i>Roseobacter litoralis</i> och 149; <i>Pseudovibrio</i> sp. JE062; <i>Desulfohalobium retbaense</i> DSM 5692; <i>Desulfovibrio alkaliphilus</i> AHT2; <i>Desulfovibrio salexigens</i> DSM 2638; <i>Magnetospirillum glyphiswaldense</i> MSR-1; <i>Rubrobacter xylanophilus</i> DSM 9941; <i>Mycobacterium parascrofulaceum</i> ATCC BAA-614; <i>Sphaerobacter thermophilus</i> DSM 20745; <i>Desulfobacca acetoxidans</i> DSM 11109; <i>Burkholderia multivorans</i> ATCC 17616; <i>Acidiphilium multivorum</i> AU301; <i>Mannheimia haemolytica</i> PHL213
Cadmium, cobalt, nickel, manganese, magnesium	Cadmium-translocating P-type ATPase; periplasmic solute-binding protein; <i>czcD</i> gene product; heavy metal-translocating P-type ATPase, Co/Ni; putative <i>nrbE</i> -like protein (Ni/Co); major facilitator transporter, <i>nrsD/nreB</i> (Ni/Co); magnesium and cobalt transport protein, <i>coiA</i> (Mg/Co/Ni/Mn); probable <i>NreB</i> protein (Ni/Co); manganese transport protein (Mn/Fe/Cd/Co/Zn); magnesium-translocating P-type ATPase (Co/Mg); <i>ApaG</i> gene product (Co/Mg)	<i>Streptomyces flavogriseus</i> ATCC 33331; <i>Thermobacillus composti</i> KWC4; <i>Alkanovorax bokumensis</i> SK2; <i>Actinosynnema mirum</i> DSM 43827; <i>Hoeflea phototrophica</i> DFL-43; <i>Roseiflexus</i> sp. RS-1; <i>Micromonospora</i> sp. ATCC 39149; <i>Paenibacillus vortex</i> V453; <i>Photobacterium leiognathi</i> subsp. <i>mandapamensis</i> svers.1.1.; <i>Photobacterium</i> sp. SKA34; <i>Vibrio angustum</i> S14; <i>Oceanospirillum</i> sp. MED92; <i>Pantoea stewartii</i> subsp. <i>stewartii</i> ; <i>Serratia</i> sp. AS12; <i>Vibrio caribbenticus</i> ATCC BAA-2122; <i>Saccharophagus degradans</i> 2-40
Chromium, tellurium, selenium	ATP-dependent DNA helicase <i>RecG</i> ; tellurite resistance protein <i>TehB</i> ; chromate transporter; chromate ion transporter family protein; chromate transport protein <i>ChrA</i> ; tellurite resistance gene, <i>trgB</i> ; manganese/iron superoxide dismutase, <i>chfC</i>	<i>Tolunomas auensis</i> DSM 9187; <i>Yersinia pestis</i> KIM10 +; <i>Methylobacterium</i> sp. 4-46; <i>Methylobacterium nodulans</i> ORS 2060; <i>Desulfovibrio</i> sp. A2; Alpha-proteobacterium BAL199; <i>Roseobacter litoralis</i> Och 149; <i>Roseobacter denitrificans</i> Och 114; <i>Beijerinckia indica</i> subsp. <i>indica</i> ATCC 9039; <i>Cupriavidus basilensis</i> OR16; <i>Enterobacteriaceae</i> subsp. <i>cloacae</i> NCTC 9394; <i>Alicyclophillus denitrificans</i> ; <i>Cupriavidus metallidurans</i> CH34 (plasmid); <i>Zunongwangia profunda</i> SM-AB7

Table 4 (continued)

Heavy metals	Enzyme/genes	Taxonomic affiliation
Manganese, iron, cobalt, zinc, nickel, copper, cadmium, gallium	TonB-dependent siderophore receptor; ABC transporter-like protein; zinc/iron permease; iron-dependent regulatory protein, ideR; DNA protection protein, dpsA; fpwA2 gene product; aconitate hydratase 1; ABC transporter transmembrane region family protein (Fe); lipoprotein inner membrane ABC transporter, Irp6; permease and ATP-binding protein of yersiniabactin-iron ABC transporter YbtP; ABC transporter precursor of the inner membrane lipoprotein; iron(III)-transport ATP-binding protein, fbpC; ferroxidase; zinc/iron ZIP family permease (Zn/Fe/Co/Ni/Cu/Cd); czcD gene product (Fe/Zn/Co/Cd/Ni); zinc transporter zupT (Zn/Fe/Co/Ni/Cu/Cd); ferric uptake regulator, Fur family; ctpC gene product (Mn/Zn)	<i>Stenotrophomonas maltophilia</i> R513; <i>Stenotrophomonas</i> sp. SKA14; <i>Verminephrobacter eiseniae</i> EF01-2; <i>Ethanoligenes herbinense</i> YUAN-3; <i>Pseudomonas fulva</i> 12-X; <i>Kocuria rhizophila</i> DC2201; <i>Synechococcus</i> sp. CC9311; <i>Prochlorococcus marinus</i> str. MIT 9303; <i>Azoarcus</i> sp. BH72; <i>Pseudomonas fluorescens</i> Pf-5; <i>Streptomyces hygroscopicus</i> ATCC 53653; <i>Streptomyces violaceusniger</i> Tu 4113; <i>Escherichia coli</i> DECBC; <i>Yersinia enterocolitica</i> ; <i>Escherichia coli</i> 55989; <i>Escherichia coli</i> O104:H4 str. 01-09591; <i>Escherichia coli</i> NA114; <i>Yersinia pseudotuberculosis</i> IP 32953; <i>Yersinia pestis</i> KIM10 +; <i>Neisseria meningitidis</i> ATCC 13091; <i>Neisseria meningitidis</i> MC58; <i>Neisseria meningitidis</i> M01-240355; <i>Neisseria meningitidis</i> alpha 153; <i>Rhodococcus equi</i> 103S; <i>Methanosarcina harundinacea</i> 6Ac; <i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> A449; <i>Erwinia billingiae</i> Eb661; <i>Serratia</i> sp. AS12; <i>Serratia odorifera</i> 4Rx13; <i>Serratia proteamaculans</i> 568; <i>Serratia odorifera</i> DSM 4582; <i>Prevotella dentalis</i> DSM 3688; <i>Mycobacterium ulcerans</i> Agy99; <i>Mycobacterium marinum</i> M
Mercury	Mercuric (Hg(II)) reductase, merA; mercuric transport protein, merT; MerR family transcriptional regulator; Hg(II)-responsive transcriptional regulator; mercuric transporter, merH; merR gene product; mercuric reductase, merB1	<i>Thiomonas</i> sp. 3As; <i>Endoriftia Persephone</i> Hot96_1 + Hot96_2; <i>Bacillus cellulolyticus</i> DSM 2522; <i>Agrobacterium tumefaciens</i> F2; <i>Leptospirillum ferrooxidans</i> DSM 9946; <i>Meiothermus silvanus</i> DSM 9946; <i>Thermus thermophilus</i> HB27; <i>Thermus thermophilus</i> HB8; <i>Oceanithermus profundus</i> DSM 14977; <i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047; <i>Sphingopyxis alaskensis</i> RB2256; <i>Methyloversatilis universalis</i> FAMS; <i>Novosphingobium nitrogenifigans</i> DSM 19370; <i>Burkholderia glumae</i> BGR1; <i>Acidovorax</i> sp. JS42; <i>Mycobacterium</i> sp.; <i>Sphingobium</i> sp. SYK-6; <i>Xanthobacter autotrophicus</i> Py2
Molybdenum, tungsten	Molybdenum ABC transporter ATP-binding; extracellular solute-binding protein, wtpA; signal transduction histidine-protein kinase, baeS; sensory histidine kinase in two-component regulatory system with BaeR; ABC transporter family protein; molybdate ABC transporter, permease protein modB	<i>Methanosarcina acetivorans</i> C2A; <i>Methanohalobium evestigatum</i> Z-7303; <i>Escherichia coli</i> 83972; <i>Ralstonia solanacearum</i> CMR15; <i>Desulfovibrio</i> sp. A2; <i>Raphidiopsis brookii</i> D9

solubility of cadmium in the soil and its availability in soil solution.

The detection of various heavy metals in SL4 agricultural soil as revealed in the heavy metal content analysis, though at thresholds permitted for soils (WHO/FAO 2001) may be attributed to atmospheric deposition and various agricultural practices, which introduce the heavy metals into the soil. The significant reduction of these metals in Cd-amended SL5 microcosm may be due to several reasons. First, utilization of biologically important heavy metals such as zinc, copper, iron and chromium are tightly linked to the metabolic functioning of soil biota as they are essential micronutrients required by most microorganisms, which possibly cause their reduction (Bruins et al. 2000; Marschner 2012; Rai et al. 2019). Also, addition of Cd to the agricultural soil induces the activation of Cd resistance systems, which are also used by microorganisms for uptake, transport, efflux, and detoxification of other heavy metals detected in this study (Nies 1999, 2003).

The predominance of the phyla *Proteobacteria* and *Actinobacteria* in the agricultural soil is not surprising as the two phyla comprise members that are well adapted to agricultural soils (Cheema et al. 2015; Trivedi et al. 2016; Salam et al. 2017; Yin et al. 2017). The exhibition of filamentous growth, possession of spores that are recalcitrant to various environmental stressors, and secretion of avalanche of enzymes, which degrade various macromolecules that abound in soil provide distinctive edge for members of *Actinobacteria* phylum in soil environments (Larkin et al. 2005; Salam and Obayori 2019). Members of the phylum *Proteobacteria* have diverse morphological, physiological, and metabolic properties. These properties facilitate their preponderance in soils with various environmental conditions (Aislabie and Deslippe 2013; Montecchia et al. 2015; Salam et al. 2019).

While about 11% of proteobacterial members were lost due to Cd contamination in SL5, it still constitutes the most abundant phylum (50.50%). In contrast, though the second most abundant phylum in SL5 (17.17%), the phylum *Actinobacteria* loses 68.05% of its members. This may be due to Cd toxicity to majority of its members, which results in oxidative damage via production of reactive oxygen species, and displacement of Zn and Fe ions from metalloproteins, resulting in their inactivation (Vallee and Ulmer 1972; Stohs and Bagchi 1995; Fortuniak et al. 1996; Stohs et al. 2001; Banjerdkij et al. 2005).

Structural analysis of the SL5 metagenome revealed the dominance of the class *Alphaproteobacteria* and the genus *Methylobacterium*. The preponderance of members of the class and the genus may be attributed to several factors. The preponderance of *czrCBA* efflux system and other Cd uptake/transport/efflux systems

among members of the class *Alphaproteobacteria* may have contributed immensely to their abundance in SL5 system. The *czrCBA* efflux system is involved mainly in response to Cd and zinc showing significant induction in their presence (Nies 2003; Braz and Marques 2005; Hu et al. 2005; Valencia et al. 2013). In addition, members of the genus *Methylobacterium* are reputed to be widely distributed in diverse environmental compartments with propensity for detoxification of heavy metals (De Marco et al. 2004; Fernandes et al. 2009; Salam et al. 2015). They are renowned for possession of heavy metal resistance genes such as cation efflux system protein *czcA* gene, ABC transporters involved in metal uptake, copper-translocating P-type and genes encoding arsenic resistance and chromate transport (Madhaiyan et al. 2007; Dourado et al. 2012; Kwak et al. 2014; Dourado et al. 2015).

Functional characterization of the two metagenomes (SL4, SL5) revealed the presence of heavy metal resistance genes (Tables 3, 4). Detection of resistance genes in SL4 agricultural soil metagenome is not surprising as traces of various heavy metals were detected in the soil (Table 1). The survival of some members of the community despite the heavy metals stress indicates the presence of resistance systems that tightly control intracellular concentrations of the heavy metal ions and their attendant toxicities (Nies 1999, 2003; Hu et al. 2005).

One of the toxic effects of Cd is that it causes oxidative stress by depleting glutathione and protein-bound sulfhydryl groups resulting in formation of reactive oxygen species (ROS). The resultant ROS causes enhanced lipid peroxidation, DNA damage and distorted calcium and sulfhydryl homeostasis (Kachur et al. 1998). In this study, thioredoxin-based thiol disulfide oxidoreductase (*dsbA*, *dsbB*) and dithiol disulfide isomerase, which protect microbial cells against oxidative stress were detected in the two metagenomes. However, manganese/iron superoxide dismutase, two superoxide dismutases known to remove superoxide radicals that may be generated upon exposure to heavy metals (Jones et al. 1991; Stohs and Bagchi 1995; Kachur et al. 1998; Nies 1999) were only detected in SL5 metagenome. This is interesting as previous reports have averred that the greatest induction of Mn superoxide dismutase (*sodA*) occurred under Cd and chromium stress, while induction of Fe superoxide dismutase (*sodB*) occurred only under Cd stress (Hu et al. 2005; Ammendola et al. 2014). Thus, the induction of these two intracellular superoxide dismutases required to control Cd-mediated oxidative stress in SL5 metagenome could only be attributed to elevated concentration of Cd in SL5 microcosm.

Another interesting finding is the detection of alkyl hydroperoxide reductase (*ahpC*) gene in 1440 protein

sequences of SL5 metagenome, which is not detected in the protein sequences of SL4 metagenome. The detection of this gene in SL5 metagenome may be attributed to Cd contamination. Previous works have reported cadmium-induced cross-protection against H_2O_2 in *E. coli* cells pre-treated with $CdCl_2$ while others have reported increase in induction of AhpC gene by tenfold after cells were exposed to Cd (Ferianc et al. 1998; Mongkolsuk and Helmann 2002; Banjerdkiy et al. 2005).

The three major families of efflux transporters involved in Cd^{2+}/Zn^{2+} resistance namely the P-type ATPases (Nucifora et al. 1989; Rensing et al. 1997), the CBA transporters (Nies and Silver 1989; Nies 1995; Hassan et al. 1999), and the cation diffusion facilitator (CDF) transporters (Xiong and Jayaswal 1998; Anton et al. 1999; Grass et al. 2001; Nies 2003) were detected in this study. Several P-type ATPases were detected in SL4 (cadmium-translocating P-type ATPase; Pb/Cd/Zn/Hg transporting ATPase; cation transporting P-type ATPase) and SL5 (cadmium-translocating P-type ATPase; heavy metal-translocating P-type ATPase Cd/Co/Hg/Pb/Zn-transporting) metagenomes. The functional features of these pumps include maintenance of homeostasis of essential metals (Cu^+ , Co^{2+} , Zn^{2+}) and mediating resistance to toxic metals (Cd^{2+} , Pb^{2+} , Ag^+) (Rensing et al. 1997, 1999; Lee et al. 2001; Hu and Zhao 2007; Scherer and Nies 2009).

It is instructive to note that while CBA transporters (*czcA*, *czrA*, *czrB*) were detected in Cd-amended SL5 metagenome (Table 4), only the *nccC* (nickel–cadmium–cobalt) protein, which confers resistance to nickel, cadmium and cobalt was detected in the SL4 metagenome (Table 3). The RND protein *CzcA* component of the three-component *CzcCBA* (cadmium–zinc–cobalt) efflux system detected in SL5 metagenome mediates the active part of the transport process, determines the substrate specificity and is involved in the assembly of the trans-envelope protein complex. Its presence in a heavy metal-polluted system is exceptional and indicates high-level resistance to heavy metal ions (Nies et al. 1989; Franke et al. 2003; Nies 2003). Another RND efflux system detected in SL5 metagenome is the *czrCBA* efflux system, a prototype of the *czcCBA* efflux system (Hassan et al. 1999; Valencia et al. 2013). It is an efflux system that showed significant induction in the presence of cadmium and zinc. The detection of *czrA* and *czrB* in SL5 metagenome could only be attributed to Cd amendment, which upregulate the *czr* regulon in the metagenome. CBA transporters mainly carried out outer membrane efflux by removing periplasmic metal ions transported there by ATPases or CDF transporters or expelling the ions before they entered the cytoplasm (Scherer and Nies 2009).

The cation diffusion facilitator (CDF) transporters are represented in Cd-amended SL5 metagenome with the *czcD* gene, the archetype of the family. The gene, first described as a regulator of expression of the *CzcCBA* high-resistance system in *Ralstonia* (now *Cupriavidus metallidurans* strain CH34) can also mediate resistance to small degree of $Zn^{2+}/Co^{2+}/Cd^{2+}$ in the absence of *CzcCBA* system (Nies 1992; Anton et al. 1999; Nies 2003).

The interplay of different transporters in Cd and zinc resistance clearly indicated, as shown in several studies that full resistance to Cd^{2+} requires both the activity of CBA transporter and P-type ATPase (Legatzki et al. 2003; Scherer and Nies 2009). This is because some Cd^{2+} can escape the CBA transporter and enter the cytoplasm. In such instance, they will be exported by the P-type ATPases (Scherer and Nies 2009). This perhaps explains the reason why both P-type ATPases and CBA transporters were upregulated in Cd-perturbed SL5 metagenome.

A cursory look at the taxonomic affiliation of the heavy metal genes detected in SL4 and SL5 metagenome revealed they belong exclusively to the two dominant phyla, *Proteobacteria* and *Actinobacteria*, with *Proteobacteria* members largely dominating. This is in tandem with the structural analysis results, which shows the dominance of *Proteobacteria* and *Actinobacteria* in the two metagenomes. This is interesting as it revealed that the two phyla not only dominate the ‘who is there?’ part of the two microbial community, but were equally responsible for the detoxification of Cd (SL5) and other heavy metals in the communities.

Conclusions

In summary, Illumina shotgun metagenomics and analysis of soil physicochemistry and heavy metals content has revealed the presence of several heavy metals and the effects of Cd contamination on soil physicochemistry and microbial community structure of SL4 agricultural soil. Detection of various heavy metals in the agricultural soil, though at low threshold is concerning as heavy metals are not biodegradable and can bioaccumulate in the food chain over time. Possession of diverse resistance genes by members of the microbial community may be exploited for depuration of agricultural soils inundated with Cd and other heavy metals. The need to embrace environmentally friendly methods for pest and herbage control and to improve crop yield is becoming more profound, due to the negative impacts of current agricultural practices on the general wellbeing of the soil ecosystem and its inhabitants.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s40643-020-00314-w>.

Additional file 1. Additional figures.

Abbreviations

Cd: Cadmium; CDF: Cation diffusion facilitator; RND: Resistance nodulation division; CBA: Capsule biogenesis/assembly; NRAMP: Natural resistance-associated macrophage protein.

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Authors' contributions

LBS conceived the study and performed the experiments. OSO coordinated the study and in consultation with LBS wrote the Materials and Methods and Results. MOI and OOA contributed to the Discussion section. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no competing interest.

Author details

¹ Department of Biological Sciences, Microbiology Unit, Summit University, Offa, Kwara, Nigeria. ² Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria. ³ Department of Microbiology, University of Lagos, Akoka, Lagos, Nigeria.

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