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

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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...
and Steiness 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; Yazdankah 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 Gabbrilli 1999; Lopez-Millañ 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/exefflux/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) as well as 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 (Töörnen 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 physicochemistry 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 30.46 ± 7.19 and 30.46 ± 7.23, respectively. Other general features of the soil metagenomes are indicated in Table 2.

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

| Parameter                      | 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

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 Nitrospira. In contrast, there is a massive upsurge in the population of members of the phyla Euryarchaeota (an archaeal phylum), Chlamydiae, Spirochaetes, and Deferrribacteres 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, Acidimicrobiidae and Nitrospira while there is a huge upscale in the population of members of the classes Methanomicrobia, Chlamydiida and Spirochaeta (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, there is preponderance of Acidimicrobiaceae (8.70%), Alcaligenaceae (7.10%), and Sphingobacteriaceae (6.12%) dominates in SL5 while Enterobacteriaceae (7.94%), Alcaligenaceae (7.45%) and Methylocystaceae (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 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 | 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; arabinofuranosyltransferase; 2-deoxyglucosyltransferase), nitrogen metabolism (CFP/FNR family transcriptional regulator, nitrogen oxide reductase effector subunit), polyketide synthases (nogalonic acid methyl ester cyclase), xenobiotic degradation (carboxylesterase 1, alkene monooxygenase, effector subunit), polyketide synthases (nogalonic acid methyl ester cyclase), and vitamin B₁₂, porphyrin and chlorophyll metabolism (adenosylcobinamide-phosphate synthase).

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), 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 (diacylglycerol diphosphate phosphatase/phosphatidate phosphatase).

**Fig. 1** Comparative taxonomic profile of SL4 and SL5 metagenomes at phylum level, computed by EDGE Bioinformatics. Unclassified reads were not used for the analysis. All the phyla detected in SL4 and SL5 metagenomes were used.
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 Mn²⁺/Fe²⁺ transporter, etc.) and mercury, silver, molybdenum, lead, arsenic, tungsten, tellurium and antimony (merA, merB, merR, merH, merP, bphA, 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, czaA, czeD, 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 czaA, czeD, czrA, czrB, manganese transport protein, and manganese/iron superoxide dismutase (MnSOD, sodA; FeSOD, sodB) which were detected in Cd-amended SL5 metagenome were conspicuously
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.
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, 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; apoprotein 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; penicillin repressor/transcription regulator, copP/A; two-component sensor, copS; lipoprotein involved with copper homeostasis and adhesion, cutF; blue copper oxidase CueO; copper tolerance protein; heavy metal transport/detoxification protein, copE; putative laccase | Frankia sp. Cc13; Shigella dysenteriae 1012; Haloalkalicoccus jeotgali B3; Intrasporangium calum DSM 43043; Methanosaeta harundinacea 6Ac; Rhodococcus pyridinivorans AK37; Haloarcula latus profundi ATCC 49239; Azospirillum brasilense Sp245; Geobacillus thermolithotrophicus NGB-2; Pseudomonas aeruginosa 2192; Citrobacter rodentium ICC168; Citrobacter koseri ATCC BAA-899; Acetoacetobacter pomorum DM001; Kyotococcus sedentarius DSM 20547; Azohydrinium cauludinum ORS 571; Nitrosomonas europaea C91; Corynebacterium ammoniagenes DSM 20306; Salmonella enterica subsp. enterica serovar Weltevreden str. HL_N05-337; Oceaniscola sp. S124; Polymorphus galvum SL-038-26A1; Xanthobacter autotrophicus Py2; Bradyrhizobium sp. ORS 375; Delftia acidovorans SPH-1; Achromobacter sp. AO22; Vibrio sp. RSC86; Oxalobacter formigenes OKC-13; Mycobacterium paratuberculosis ATCC BAA-614; Lactobacillus pentosus MP-10; Lactobacillus plantarum JI1; Lactobaclillus plantarum JMD1; Pseudomonas aeruginosa PA01; Pseudomonas aeruginosa M18; Pectobacterium carotovorum subsp. brasiliensis PBR1692; Rhizobium species DSM 4541; Rhodobacteraceae bacterium Y41; Thermomonospora curvata DSM 43183; Verrocomicrobiaceae bacterium CMC16 |
| Chromium, nickel, cobalt | NADPH-dependent FMN reductase, transcriptional regulator NikR, CopG family (Ni); integral membrane sensor signal transduction histidine kinase msrS; 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-dicarboxylate transporter subunit, membrane component of ABC superfamily (Ni/C); chromate transporters, 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 nickel transporter (Ni/Co); permease of the major facilitator superfamily (Ni/Co); binding-protein-dependent transport system inner membrane, nikB; cadmium-translocating P-type ATPase (Co/Ni) | Bacillus coagulans 2-6; Methanococcus voltae A3; Acaryochloris sp. CCME 5140; Peptoniphilus sp. oral taxon 375 str. FO436; Burkholderia multivorans CGD1; Burnhalidiana multivorans CGD2M; Candidatus Desulfuris audaxviator MP104C; Synergistes bacterium SGP1; Escherichia coli S88; leptothrix chlorodun SP-6; Cellulomonas fimi ATCC 484; Jonesia denitrificans DSM 20603; Azospirillum sp. BS10; Cupriavidus metallidurans CH34 (plasmid); Methylbacterium nodulans ORS 2060; Methylbacterium extorquens DMA; Sphingomonas melloti CCNW001020; Candidatus Desulfuris audaxviator MP104C; Streptomyces greisoroseus Tu4000 |
| Mercury, silver, cadmium, lead | Copper-translocating ATPase Ran1, merR; mercuric ion reductase, merA; mercuric reductase merB1; right orgin-binding protein robA (Ag/Cd); mercuric translocon periplasmic protein, merP; Pb-fflux 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 Zra3 (Pb, Zn); mercurocys transporter, merH | Verticillium dahliae Vd s. 17; Verticillium albo-atrum Vam s. 102; Pseudomonas fluorescens; Xanthobacter autotrophicus Py2; Yersinia ruckeri ATCC 29473; Delftia acidovorans SPH-1; Ralstonia metallidurans CH34 (plasmid); Xanthobacterium sp. Marseille (plasmid); Pseudomonas putida (plasmid); Halomonas ochracea DSM 14366; Vibrio caribbenthicus ATCC BAA-2122; Tolumonas aurum DSM 9187; Methylobacterium amorphae CCNW00123; Citrobacter youngae ATCC 29220; Mycobacterium sp. |
| Zinc, manganese, cadmium | Zinc resistance protein, zraP; Fix family transcriptional regulator; periplasmic soluble-ubinding protein (Mn/Cd); RND family efflux transporter, MFP subunit (Zn); DSBA Oxidoreductase (Cd); membrane fusion protein (MFP-RND) heavy metal cation translocating efflux HmbR (Zn); cation translocating ATPase, P-type; high-affinity zinc transporter periplasmic protein, DSBA gene product (Cd/Zn/Hg); cadmium-translocating P-type ATPase (Zn); sulfide-disulfide isomerase (Cd), heavy-metal-translocating P-type ATPase (Zn); zinc/manganese/iron ABC transporter, periplasmic zinc/manganese/iron-binding protein | Escherichia sp. TW09308; Citrobacter freundii 4_7_47CFDA; Citrobacter youngae ATCC 29220; Salmonella enterica subsp. enterica serovar Hadar str. RS05P66; Desulfovibrio vulgaris str. ‘Muyazaki F’; Thermobacillus composti KWC4; Pseudomonas putida S16; Pseudomonas maculiginis KNP414; Cupriavidus metallidurans CH34 (plasmid); Pyrococcus abyssi GES; Didyella dadaensis Ech703; Candidatus Blochmannia pensylvanicae str. BPEN; Thermobacterium subterraneum DSM 13965; Rhodococcus equi 1035; Bacillus cytotoxicus NVH 391-98; Rhodobacteraceae bacterium HTCC2083 |
| Heavy metals               | Enzyme/genes                                                                 | Taxonomic affiliation                                                                 |
|---------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Iron, cobalt, nickel, gallium, cadmium, magnesium, manganese, zinc | 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, idER; heavy metal-translocating P-type ATPase (Co/Ni); aciontase A (Fe); ABC transporter; ATP-binding protein YbbL (Fe); TonB-dependent siderophore receptor; zinc/iron ZIP family permease (Zn/Fe/Ni/Cu/Cd); nickel–cobalt-cadmium resistance protein incc (plasmid); acenate hydratase (Fe); ferritin (Fe/Cu/Mn); fpuA2 gene product; predicted divalent heavy-metal cations transporter (Zn/Fe/Cu/Cd); ferrc 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); ferripyoverdine receptor 2 | Pseudomonas aeruginosa C3719; Pseudomonas aeruginosa M18; Pseudomonas aeruginosa PA01; Azospirillum sp. BS10; Vibrio parahydrusolicus RIDM 221063; Pectobacterium atrosepticum SCRI1043; Klebsiella pneumoniae 342; Klebsiella varicola At-22; Pseudomonas aeruginosa 138244; Kineococcus radiotolerans SR30216; Actinosynsema mirum DSM-43827; Bifidobacterium bifidum NCIMB 41171; Bifidobacterium bifidum SI 7; Escherichia fergusonii ATCC 35469; Pseudomonas fulva 12-X; Comamonas testosteroni S44; Varioroxar paraadoxus EPS; Methanosetae |
| Molybdenum, tungsten, tellurium | modE gene product; tellurite resistance protein TgB; KlaB 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 | Xenorhabdus bovienii SS-2004; Roseobacter sp. GA101; uncultured bacterium (plasmid); Serratia odorifera DSM 4582; Serratia marcescens str. ‘mosstructis’; Citrobacter koseri ATCC BAA-895; Thermobacterium tenereum ATCC BAA-798; Enterobacter cloacae subsp. cloacae ATCC 13047; Achromobacter xylooxidans A8 |
| Arsenic, antimony        | 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; arsenite reductase; arsenate reductase; glutathione/glutaredoxin type; arsenic resistance protein arsB; arsenite oxidase small subunit, AoxA; ABC thiol transporter; methyltransferase, ArsM; methyltransferase type II; arsenite S-adenosylmethionine transferase; arsenate reductase (azurin); arsenical resistance protein ArsH; arsenical resistance protein arsB | Leishmania major strain Friedlin; Roseobacter litoralis and 149; Nitrooccus marina sp. l57943; Acidovorax aenescens subsp. aenescens ATCC 18960; Sphaerootherix thermophilus DSM 20745; Staphylococcus saprophyticus subsp. saprophyticus; Staphylococcus aureus; Bacillus subtilis subsp. subtilis RO-NN-1; Micococcus chthonoplastes PCC 7420; Thauera sp. MZIT; Rhodococcus rhodochrous var. scottii ATCC 17100; Burkholderia multivorans ATCC 17616; Leishmania infantum; JPCMS; Mticoccus marina subphosphorus NM-1; Leptonema illini DSM 21528; Deferribacterium retboense DSM 5692; Rhodotherax ferrireductus T118; Gluconacetobacter sp. SKCC-1; Peptidiphilus harei ACS-146-V-Sch2b; Actinobacter cellulolyticus CD2 |
| Heavy metals                  | Enzyme/genes                                                                                                      | Taxonomic affiliation                                                                 |
|------------------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Cadmium, cobalt, mercury, lead, zinc | Heavy metal-translocating P-type ATPase, Cd/Ca/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 CzeA (Zn/Cd); membrane-bound cation-proton-antiporter Zra gene (Zn/Cd); cobalt-zinc-cadmium resistance protein; cation diffusion facilitator family transporter; CzrB gene | Mycobacterium sp. Spy1; Saccharomonospora viridis DSM 43017; Mycobacterium vanbaalenii PYR-1; Mycobacterium gilvum PYR-GCK; Shewanella sediminis HAW-EB3; Senata sambiotica str. 'Cimara cedri'; Candidatus Blochmannia pensylvanicus str. BPEN; Pseudomonas putida DSM 43043; Pseudomonas aeruginosa PA7; Pseudomonas aeruginosa 39016; Pseudomonas aeruginosa 138244; Pseudomonas putida; Pseudomonas longis Guantanamo ATCC 19865; Pseudomonas aeruginosa |
| 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-translocating 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, ctpB; Int gene product (Cu/Co), Cull-responsive transcriptional regulator, MerR family transcriptional regulator (Cu), MerR family transcriptional regulator (Cu), lipoprotein involved with copper homeostasis and adhesion, cutF/nlpE; heavy metal transport/detoxification protein, copP; copper/silver efflux P-type ATPase | Acidobacterium capsulatum ATCC 51196; Acetobacter pomorum DM001; Achromobacter xylosoxidans A8; Anaeromyxobacter sp. Fwi O9-5; Haloalkalococcus geotolygi B3; Stenotrophomonas maltophilia R513; Frankia sp. Cc15; Haloarcula lacusprofundi ATCC 49239; Corynebacterium ammoniagenes DSM 20306; Corynebacterium lipophilum DSM 44291; Xanthobacter autotrophicus Py2; Haladaptatus paucihalophilus DX253; Rhodococcus jostii RHA1; Intrasporangium calvins DSM 43043; Streptomyces sp. S4; Streptomyces albus J1074; Halo bacterium sp. DL1; Achromobacter xylosoxidans C54; Cupriavidus metallidurans CH3; Methanosaeta harundinacea 6Ac; Rhodothermus ferrireducens; Erwinia sp. Eps617; Micrococcus luteus NCTC 2665; Kytococcus sedentarius DSM 20547; Mycobacterium parascrofulaceum ATCC BAA-614; Proteus mirabilis ATCC 29906; Shewanella sediminis HAW-EB3; Bacillus amylyoliquefaciens TA208; Pseudomonas syringae pv. glycinea str. B076; Klebsiella pneumoniae 342; Gardneria polysaccharovorans NBRC 16320; Methyllobacterium chloromethanum CMA; Shigella dysenteriae 10112; Acaryochloris sp. CCME 5410; Hyphomicrobium neptunium ATCC 15444; Caulobacter sp. K31; Pseudomonas syringae pv. aeruginosa str. M30223P; Pseudomonas entomophila L8; Pseudomonas putida S16, Rahmella sp. Y9602; Pseudonocardia sp. P1; Citromicrobium bathyomarinum 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, nikE | Azospirillum sp. BS10; Streptococcus sanguinis SK1056; Methanococcus voltae A3; Methanospirillum hungatei JF-1; Pseudomonas sp. TJu-51; Synergistes bacteriae SG1P1; Rhodospirillum rubrum 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) | Pseudomonas putida KT2440; Citrobacter freundii 4_7_AFCFA; Citrobacter youngae ATCC 29220; Salmonella enterica subsp. enterica serovar. Johannesburg str. SS-703; Salmonella enterica subsp. enterica serovar Typhi str. CT18; Sodalis glossinidius str. 'morristans'; Oxalobacter formigenes OXCC13; Roseobacter litoralis Och 149; Roseobacter denitrificans Och 114; Desulfovibrio magneticus RS-1; Escherichia coli 83972; Hyphomonas neptunium ATCC 15444; Ralstonia solanacearum CMR15; Photobacterius luminescens subsp. laumondii TTO1; Haemophilus pittmanae HK 85; Yersinia ruckeri ATCC 29473; Dickeya dadiesi Ech703; Erwinia pyrifoliae DSM 12163; Erwinia amylovora CFBP 1430 |
| Arsenic | Arsinite oxidase, large subunit (aoxB); arsenite S-adenosylmethyltransferase; arsenite methyltransferase arsM; arsenical resistance protein, arsB; protein-tyrosine phosphatase, low molecular weight, arsC; arsenite oxidase, small subunit (aoxA); arsenical resistance protein ArsH; phosphate ABC superfamily ATP-binding cassette transporter, binding protein | Burkholderia oklahomensis G6786; Roseobacter litoralis och 149; Pseudomonas sp. JEO62; Desulfobulbus retbaense DSM 55629; Desulfuromonas alcaliphilus AHT2; Desulfuromonas salexigens DSM 2638; Magnetospirillum gryphiswaldense MSR-1; Rubrobacter xylanophilus DSM 9941; Mycobacterium paradoxum lacrimale ATCC BAA-614; Sphaerothrix thermophila DSM 20745; Desulfoveliibacter acidothermus DSM 11109; Burkholderia multivorans ATCC 17616; Acidiphilium multivorans AIU301; Mannheimia haemolytica PHL 213 |
| Cadmium, cobalt, nickel, manganese, magnesium | Cadmium-translocating P-type ATPase; periplasmic solute-binding protein, czcD gene product; heavy metal-translocating P-type ATPase, Co/Ni; putative nrbE-like protein (Ni/Co); major facilitator transporter, nrsD/mneB (Ni/Co); magnesium and cobalt transport protein, orA (Mg/Co/Ni/Mn); probable NrbE protein (Ni/Co); manganese transport protein (Mn/Fe/Gd/Cu/Zn); manganese-translocating P-type ATPase (Co/Mg); ApaG gene product (Co/Mg) | Streptomyces flavogriseus ATCC 33331; Thermobacillus composti KWC4; Alkanivorax borkumensis SK2; Actinomycena mirum DSM 43827; Hoeflea phototrophica DFL-43; Roseiflexus sp. RS-1; Micromonospora sp. ATCC 39149; Paenibacillus alcaliphilus V453; Photobacterium leiognathi subsp. mandapamensis svers.1.1.; Photobacterium sp. SKA34; Vibrio angustus S14; Oceanospirillum sp. MED92; Pantoea stewartii subsp. stewartii; Serratia marcescens ATCC 1.12; Vibrio carlisiensis ATCC BAA-2122; Sphingobacterium degradans 2-40 |
| Chromium, tellurium, selenium | ATP-dependent DNA helicase RecG; tellurite resistance protein TehB; chromate transporter; chromate ion transporter family; chromate transport protein ChrA; tellurite resistance gene, trgB; manganese/iron superoxide dismutase, chrC | Tolunomas auensis DSM 9187; Yersinia pestis KIM10-4; Methylobacterium sp. 4-46; Methylobacterium nodulans ORS 2060; Desulfovibrio sp. A2; Alpha-proteobacterium BAL194; Roseobacter litoralis Och 149; Roseobacter denitrificans Och 114; Beijerinckia indica subsp. indica ATCC 9039; Cupiavidus basilensis OR16; Enterobacter cloacae subsp. cloacae NCTC 3934; Alcyonides denitrificans; Cupiavidus metallidurans CHB4 (plasmid); Zunongwanga profundus SM-AB7 |
| Heavy metals | Enzyme/genes                                                                 | Taxonomic affiliation                                                                 |
|--------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Manganese, iron, cobalt, zinc, nickel, copper, cadmium, gallium | TonB-dependent siderophore receptor; ferripyoverdine receptor; ABC transporter-like protein; zinc/iron permease; iron-dependent regulatory protein; idER; DNA protection protein; dpsA; fpvA2 gene product; acconitate hydratase 1; ABC transporter transmembrane region family protein (Fe); lipoprotein inner membrane ABC transporter, Trp6; permease and ATP-binding protein of yersiniabactin-iron ABC transporter YbtP; ABC transporter precursor of the inner membrane lipoprotein; iron(III)-transport ABC-binding protein, fbpC, ferroxidase; zinc/iron ZIP family permease (Zn/Fe/Cu/Ni/Zn); zcnD gene product (Fe/Zn/Cu/Ni/Cd); zinc transporter zupT (Zn/Fe/Cu/Ni/Cd); ferric uptake regulator, Fur family; ctpC gene product (Mn/Zn) | Stenotrophomonas maltophilia R5513; Stenotrophomonas sp. SKA14; Verminephrobacter eiseniae EF01-2; Ethanoligenes herbicola YUAN-3; Pseudomonas fulva 12-K; Kocuria rhizophila DC2201; Synechococcus sp. CC9311; Pochlorochoccus marinus str. MIT 9303; Azoarcus sp. BH72; Pseudomonas fluorescens PF-5; Stenotrophomonas hygroscopicus ATCC 53653; Streptomyces violaceoruber Tu 4113; Escherichia coli DECBC; Yersinia enterocolitica; Escherichia coli S5989; Escherichia coli O104:H4 str. 01-09591; Escherichia coli NA114; Yersinia pseudotuberculosis IP 32953; Yersinia pestis KIM10++; Neisseria meningitidis ATCC 13091; Neisseria meningitidis MC98; Neisseria meningitidis M01-240355; Neisseria meningitidis alpha153; Rhodococcus equi 1035; Methanosarcina halophila M6Ac; Aeromonas salmonicida subsp. salmonicida A449; Erwinia billingiae Eb661; Serratia sp. AS12; Serratia odorifera 4Rx13; Serratia proteamolucans 568; Serratia odorifera DSM 4582; Prevotella dentalis DSM 3688; Mycobacterium ulcerans Ags99; Mycobacterium marinum M |
| Mercury      | Mercuric (Hg(II)) reductase, merA, mercuric transport protein, merR, MerR family transcriptional regulator, Hg(II)-responsive transcriptional regulator; mercuric transporter, merH; merH gene product; mercuric reductase, merB1 | Thiromonas sp. 3As; Endothrix Paraphenyem ’Hot96, 1+Hot96, 2+; Bacillus cellulosilyticus DSM 2522; Agrobacterium tumefaciens F2; Leptospirillum ferrooxidans; Methylthermus silvanus DSM 9946; Thermus thermophilus HB27; Thermus thermophilus HB8; Oceanithermus profundus DSM 14977; Enterobacter cloacae subsp. cloacae ATCC 13047; Sphingopyxis alaskensis RB2256; Methylocystis versatilis universalis FAMS; Novosphingobium nitritovorans DSM 19370; Burkholderia glumae BGR1; Acidovorax sp. JS42; Mycobacterium sp.; Sphingobium sp. SYK-6; Xanthobacter autotrophicus Py2 | |
| Molybdenum, tungsten | Molybdobium 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 | Methanosarcina acetivorans C2A; Methanosphaerobium estuariim Z-7303; Escherichia coli 83972; Ralstonia solanacearum CMR15; Desulfovibrio sp. A2; Raphidiopsis brookii 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$_2$O$_2$ 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; Mongkolxuk and Helmann 2002; Banjerdkij 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$^{2+}$, 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 crrCBA efflux system, a prototype of the czzCBA 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 Czc-CBA 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 decontamination 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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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.

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