Fungal community diversity of heavy metal contaminated soils revealed by metagenomics

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Abstract
The inappropriate disposal of toxic compounds generated by industrial activity has been impacting the environment considerably. Microbial communities inhabiting contaminated sites may represent interesting ecological alternatives for the decontamination of environments. The present work aimed to investigate the fungal diversity and its functionality contained in stream sediments with industrial waste contaminated with heavy metals by using metagenomic approach. A total of 12 fungal orders were retrieved from datasets and, at phylum level, Ascomycota was the most abundant, followed by Basidiomycota, Chytridiomycota and Blastocladiomycota. Higher abundance of sequences was encountered within the less contaminated site, while the lower abundance was found in the sample with the higher contamination with lead. Gene sequences related to DNA repair and heavy metals biosorption processes were found in the four samples analyzed. The genera Aspergillus and Chaetomium, and Saccharomycetales order were highly present within all samples, showing their potential to be used for bioremediation studies. The present work demonstrated the importance of using the metagenomic approach to understand the dynamics and the possible metabolic pathways associated with fungal communities related to environmental samples containing heavy metals, as well as evidenced the importance of improving culturomics techniques for isolating strains with potential application in bioremediation processes of environments contaminated with heavy metals.

Keywords Omics technique · Bioremediation · Hazardous wastes · Uncultured fungal · Heavy metal metabolism · Heavy metal tolerance

Introduction

With the increase of human industrial activities, a major impact on the environment has been occurring through the disposal of waste containing heavy metals. Industrial wastewater and sewage sludge mainly from electroplating, leather tanning, wood and pulp processing and steel manufacturing are the main sources of contamination of water bodies and soil by heavy metals which may adversely affect soil health, freshwater resources, and groundwater quality (Iram et al. 2012). These industrial processes may generate residues containing organic matter and a wide variety of heavy metals. The prolonged exposure to excessive heavy metals concentration from industrial effluent may be highly harmful for both animal and human health, and has been associated to the occurrence of carcinogenesis, respiratory cancer, mutagenesis, and cardiovascular and gastrointestinal
The importance of exploring the metagenomic approach to the isolation and culture of microorganisms in soil samples (Feng et al. 2018). Isolation and culture recover a small proportion (0.1–1%) of information as traditional microbiological techniques mostly based on cultured microorganisms, which limits the knowledge around fungal communities’ diversity and their ability to metabolize or adsorb heavy metals from contaminated sediments by using information provided by metagenomics accessed through Next Generation Sequencing (NGS) remains underexplored. The metagenomic datasets have been the object of research that aim to identify the mechanisms of adaptation of the microbial communities to inhospitable environments (Costa et al. 2015), reached due to the metabolic flexibility of these organisms inherited from specific functional genes including heavy metal resistance genes in bacterial communities (Feng et al. 2018; Hiraoka et al. 2016; Yin et al. 2015; Zhang et al. 2016).

Microbial communities present in heavy metals contaminated environments have developed detoxification strategies such as bioaccumulation, to protect the microbial cell wall, in an attempt to survive in these adverse environments (Dopson et al. 2003; Fidalgo 2011; Qayyum et al. 2016). Interaction between heavy metals and microbial communities can be classified into different categories such as extracellular, exocellular and intracellular, in which microbial cells can mobilize, immobilize, transform, precipitate, accumulate, coordinate, exchange and absorb the metal, as well as forming complexes (Joshi 2014). According to Iram and co-workers (2012), melanin (a dark pigment) located in the fungal cell wall and an extracellular polymer, have the ability to reduce the toxic effect of heavy metals. Due to this property, fungi may grow in contaminated environments, binding with heavy metals and accumulating these substances in their cells, representing an efficient alternative in the removal of these toxic compounds in waste waters and other terrestrial and aquatic sites in natural environments.

Works have been developed to investigate the sensitivity or resistance of fungi to heavy metals from different habitats and their mechanisms of adaptation to these toxic substances. In the work carried out by Joshi (2014), fungi isolates derived from effluent samples collected from contaminated sites in India were screened for heavy metals resistance for lead (Pb), nickel (Ni), cobalt (Co), chrome (Cr), copper (Cu), manganese (Mg) and zinc (Zn). The authors found one isolate belonging to Neurospora genera which showed tolerance to manganese (60 mg mL⁻¹), copper (5 mg mL⁻¹), cobalt (5 mg mL⁻¹), lead (4.5 mg mL⁻¹), nickel (6 mg mL⁻¹) and chrome (3.9 mg mL⁻¹). Iskandar et al. (2011) screened 41 isolates of filamentous fungi obtained from the sediment of the Langat River, Selangor, Malaysia for their tolerance and capability to uptake copper and lead. In this work, three Aspergillus, one Trichoderma and three Penicillium strains were able to survive at 1000 mg L⁻¹ of copper. Regarding lead, only A. niger survived at 5000 mg L⁻¹ concentration.

Current knowledge about mechanisms of metal resistance is mostly based on cultured microorganisms, which limits the information as traditional microbiological techniques of isolation and culture recover a small proportion (0.1–1%) of the microorganisms in soil samples (Feng et al. 2018). The importance of exploring the metagenomic approach to access information from most microbial communities present in the most diverse environments has been demonstrated by several searches (Barone et al. 2014; Beale et al. 2015; Hiraoka et al. 2016; Mosier et al. 2016). As the methods used in metagenomics have evolved through time, the use of massive sequencing of the microbial community contained in a sample represent an optimum tool to recover information from those communities. However, the knowledge around fungal communities’ diversity and their ability to metabolize or adsorb heavy metals from contaminated sediments by using information provided by metagenomics accessed through Next Generation Sequencing (NGS) remains underexplored. The metagenomic datasets have been the object of research that aim to identify the mechanisms of adaptation of the microbial communities to inhospitable environments (Costa et al. 2015), reached due to the metabolic flexibility of these organisms inherited from specific functional genes including heavy metal resistance genes in bacterial communities (Feng et al. 2018; Hiraoka et al. 2016; Yin et al. 2015; Zhang et al. 2016).

Hemme and co-workers (2010) analyzed the metag- enome of samples from acidic groundwater (pH 3–7) highly contaminated with heavy metals such as uranium and technetium, as well as nitric and organic acids. The authors verified that such conditions resulted in reduction of micro- bial species and allelic diversity, as well as significant loss of metabolic diversity. Zhao et al. (2015) investigated the genes associated with Cd stress in the endophytic fungal Cd-tolerant strain Exophiala pisciphila isolated from the roots of the plant Arundinella bengalensis. Using the RNA-seq approach, authors found 228 unigenes involved in different pathways associated with Cd-tolerance. Lehembre et al. (2013) performed a study by screening soil eukaryotic metatranscriptomes containing RNA from the main eukaryotic phyla organisms. In this work, the authors concluded that some genes categories may be used to probe new pathways involved in homeostasis and resistance to several heavy metals including Cd or Zn. In this way, the present work aimed to perform a comprehensive analysis of the taxonomic and functional composition of fungal communities present in sediment samples contaminated with heavy metals of Macacos Creek in the municipality of northern Juazeiro, Ceará, Brazil, through the metagenomics approach.

**Materials and methods**

**Sampling**

Samples were collected from the edge of four different creeks in the municipality of northern Juazeiro, Ceará, Brazil. To explore the microbial community composition and function, approximately 150 g of soil were collected in the
superficial layers, in the 0–20 cm profile, from areas adjacent to the reek, using a non-metal tool. The chosen depth of the layer, of up to 20 cm, contains the highest levels of chemical elements and organic matter, as well as the interaction between them, according to Aguiar et al. (2007). The samples were properly conditioned and sent to the Laboratory of Sanitation of the Federal University of Cariri (UFCA).

Soils from four different sites were sampled, including: (a) Granjeiro River (RG—control—Rio Granjeiro), reserved area less likely to be contaminated by metals, located in the municipality of Crato-CE (7° 28′ 02.27″ S 39° 43′ 81.50″ W); (b) Salesiano Creek (RS—Riocho Salesiano) which is highly contaminated by heavy metals, mainly Cr and Pb, in the municipality of Juazeiro do Norte-CE (7° 21′ 16.25″ S 39° 19′ 31.15″ W); (c) APUC Lagoon (RA—Lagoa APUC), with moderate contamination of all heavy metals, in the municipality of Juazeiro do Norte-CE (7° 14′ 45.02″ S 39° 18′ 58.85″ W); and (d) Macacos Creek (RM—Riacho dos Macacos), containing great contamination of heavy metals studied, mainly Cu, Ni and Zn, in the municipality of Juazeiro do Norte-CE (7° 21′ 29.77″ S 39° 30′ 38.83″ W) (Fig. 1). The Macacos Creek is closely located to manufacturing industries that work with corrosive, flammable, and toxic products, such as jewelry, aluminum, and leather dyeing factories. Soils were sampled in triplicate at one meter from the margin of sampling sites at a depth of 5 cm by using polyvinyl chloride (PVC) pipes of 10 cm diameter previously sterilized. After sampling, the material was taken to the laboratory and maintained at −20 °C until processing to perform the analyzes.

Soil analysis for heavy metals

The samples were dried at room temperature and particles were manually reduced to a fine powder, which were sifted to obtain a fine fraction (<0.074 mm), required for chemical analysis. For the determination of the heavy metals, the methodology of digestion in microwaves in the presence of concentrated nitric acid was used, followed by reading in inductively coupled plasma-optical emission spectroscopy—ICP/OES (method 3051-16), recommended by United States Environmental Protection Agency (US EPA 1994). The analysis was performed by an outsourced company.

DNA extraction

Total soil community DNA was extracted from 0.25 g of each soil sample by using the MoBio PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer’s instructions. Purity of the extracted DNA was checked using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and DNA concentration was determined using Qubit® 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Integrity of the DNA was confirmed by electrophoresis in a 0.8% agarose gel. All DNA samples were stored at −20 °C until downstream analysis.

Metagenomic DNA sequencing and in silico data analysis

Shotgun metagenome libraries were constructed by using a Nextera XT DNA Sample Preparation Kit (Illumina) as recommended by the manufacturer and then sequenced by Illumina MiSeq (Illumina, San Diego, CA) technology at the Center for Genomics and Bioinformatics (CeGenBio) of the Drug Research and Development Center (NPDM) at the Federal University of Ceará, Brazil.

Obtained sequences from Illumina sequencing of the four metagenomic samples were trimmed for quality control (QC) using the FastQC software (Andrews 2010). Libraries from all samples had low-quality sequences removed according to the preprocessing tool Trimmomatic (v 0.36) (Bolger et al. 2014) to select high quality sequences and for the removal of OTUs (Operational Taxonomic Units) shorter than 50 pb or of low quality (Phred score < 20). OTUs were assembled into contigs with the aid of metaSPAdes v3.10.1 (Nurk et al. 2017) and, to improve assembly to generate scaffolds and to fill gaps, Assembly Improvement v1.7.0 (Page et al. 2016) was used, having as input the assembly obtained in the previous stage with their respective trimmed OTUs. The Redundans (v0.12c) tool (Pryszcz and Gabaldón 2016) was used for final assembly of sequences, reducing fragmentation and eliminating redundant scaffolds.

The resulting four pooled datasets (RS, RA, RM and RG) were submitted to the MG-RAST (MetaGenome Rapid Annotation using Subsystem Technology, v3.1) server for annotation (http://metagenomics.nmpdr.org) (Meyer et al. 2008). Functional annotation of assembled contigs was performed using BLAST against the M5NR protein...
database and the pipeline mapped the uploaded high-quality OTUs (unassembled) against the annotated contigs to create quantitative (abundance) profiles. Our DNA dataset is available on MG-RAST under the following MG-RAST ID: mgm4765758.3, mgm4765759.3, mgm4765760.3 and mgm4765761.3. The Principal Component Analysis (PCA) graphic was built using PAST 3.22 program (Hammer et al. 2001).

**Gene function classification**

To retrieve information about gene function of the taxonomic groups, genes were annotated with clusters of orthologous groups (COG), KEGG Orthology (KO), nonsupervised orthologous group (NOG) and subsystem database including RefSeq and Subsystems inside MG-RAST that compared the homology of functional genes against the database. The total DNA datasets at a maximum e value cut-off was of $10^{-10}$, with a minimum percent identity cut-off of 60% and a minimum alignment length cut-off of 15 base pairs (Pfister et al. 2010). Materials and methods were performed according to Xavier et al. (2019).

**Results and discussion**

**Quantification of heavy metals from soil samples**

Little is known about the profiles of microbial community composition and structure in these metal contaminated soils. Since a small number of eukaryotes have been investigated with respect to heavy metal resistance so far, the present work presents a solid investigation on the microbial communities from soils contaminated by heavy metal. As part of this investigation, it was carried out the quantification of the metals contained in the samples analyzed, to robustly relate the presence of gene sequences and heavy metal contamination. The RG and RM samples presented the large amount of organic carbon found in these samples. Similarly, a high concentration of nitrogen could be observed in the RM sample. These soil compositions may have influenced the microbial community biosorption among the studied samples. The results are disposed in Table 1.

In 2016, Khamesy and co-workers identified two isolates from *Aspergillus* genus that were able to tolerate the presence of high concentrations of lead and cadmium, between 0 and 2500 mg L$^{-1}$ in waste deposits. According to Qayyum and co-workers (2016), three strains isolated from sites under a slag heap at the Smelting Industry located in Weifang city, Shandong Province, China, related to genera *Aspergillus* and one related to genera *Neosartorya* were considered resistant to heavy metals including 50 and 100 mg L$^{-1}$ of lead and chromium.

**Fungal microbial community and functional diversity**

After quality control performed by FastQC software, the metagenomes sequenced by Illumina MiSeq platform yielded approximately 674 thousand high-quality short DNA sequences. Annotation was performed using MG-RAST for taxonomic affiliation and putative protein-coding sequences related to heavy metal metabolism were searched against the databases including COG, KEGG Orthology and NOG. Principal properties of metagenomes, statistical analysis, and the total of significant BLAST OTUs obtained are available in Supplementary material—Supp 1.

High fungal abundance was found in all samples, totaling 2,583 OTUs (10.5%) from Ascomycota and 273 OTUs (1.1%) from Basidiomycota phylum. However, low sequences from Mortierellomycotina (RM—4 OTUs; RG—8 OTUs) and from Entomophthoromycotina (RG-3 OTUs; RM—1 OTU) subphylla were found. Among the four samples, the greater fungal abundance was revealed in RG site, i.e., the less contaminated sampled spot, with 964 and 116 sequences from Ascomycota and Basidiomycota,

| Samples | Heavy metals concentration (mg Kg$^{-1}$) | TOC (mg Kg$^{-1}$) | pH | N (g Kg$^{-1}$) |
|---------|------------------------------------------|------------------|----|---------------|
| RG      | 0.3                                      | 11.1             | 11.5764 | 6.61 | 0.8960         |
| RS      | 1.2                                      | 42.9             | 224.5 | 1.9321 | 8.40 | 0.2800         |
| RA      | 0.9                                      | 50.2             | 5.0475 | 8.30 | 0.5040         |
| RM      | 1.4                                      | 176.4            | 297.3 | 13.1178 | 8.19 | 3.5280         |

FAO/WHO maximum values (µg g$^{-1}$): Cd = 20; Cr = 100; Pb = 100; Cu = 100; Ni = 50; Zn = 300 (FAO—Food and Agricultural Organization/WHO—World Health Organization) (WHO 1989)

RG Granjeiro River (control), RS Salesiano Creek (great contamination of all heavy metals; mainly Cr, Pb and Zn), RA APUC Lagoon (moderate contamination of all heavy metals), RM Macacos Creek (great contamination of all heavy metals; mainly Cu, Ni and Zn), TOC total organic carbon, N nitrogen

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respectively. Within RA sample, 759 sequences related to Ascomycota and 56 assigned to Basidiomycota phylum were found, representing the second most abundant site sampled. The RM sample, highly contaminated with heavy metals, presented 716 and 65 Ascomycota and Basidiomycota related sequences, respectively. From RS sample, 144 and 36 OTUs from Ascomycota and Basidiomycota were recovered, respectively. In the present work, the fungal communities from all analyzed samples exhibited similar relative species richness in accordance with the rarefaction curves, and the profile of these curves suggests that the sampling was able to cover the fungal diversity of these samples (Fig. 2).

The great fungal diversity was verified by the presence of representatives of 12 orders including Capnodiales, Pleosporales, Eurotiales, Onygenales, Helotiales, Pezizales, Saccharomycetales, Schizosaccharomycetales, Hypocreales, Magnaporthales, Phyllachorales and one unclassified taxonomic group from the phylum Ascomycota, suggesting a new sequence yet to be classified. On the other hand, five orders belonging to the phylum Basidiomycota were found: Agaricales, Polyporales, Malasseziales, Tremellales and Ustilaginiales. One (Mortierellales) and two (Entomophthorales and Mucorales) orders were found from Mortierellomycotina and Entomophthoromycotina subphyla, respectively. Blastocladiales order was found from Blastocladiomycota phylum and Chytridiomycota phylum was represented by Cladochytriales, Chytridiales, Spizellomycetales and Monoblepharidales orders (Fig. 3).

The majority of Ascomycota OTUs in all samples were related to the classes Eurotiomycetes (910 OTUs) and Sordariomycetes (1,030 OTUs) (Fig. 4). From Eurotiomycetes class, the order Eurotiales was the most diverse, containing

![Fig. 2](image_url) Rarefaction curves exhibit the recovered species richness from all samples. Evolutionary distance used was 0.03 (built by MG-RAST)

![Fig. 3](image_url) Taxonomic distribution of fungal orders present in all samples. The main groups of sequences are divided in bars below x axis: Ascomycota (red bar), Basidiomycota (blue bar), Blastocladiomycota (grey point) and Chytridiomycota (grey bar) phyla; Entomophthoromycotina (black bar) and Mortierellomycotina (black point) subphyla.
sequences from genera *Aspergillus, Emericella, Neosartorya* (the most abundant—282 OTUs), *Penicillium* and *Talaromyces*. The greatest diversity was found in RG sample (control), showing that the absence of heavy metals preserves the characteristics of the environment, allowing the development of different classes of these microorganisms, thus increasing fungal diversity. The sample RS, highly contaminated with heavy metals, presented the lowest microbial diversity belonging to these two classes, revealing that the presence of such substances has undermined the survival and/or the development of some classes which were not able to adapt to the contaminated environment. Presence of species belonging to Eurotiales order, such as *Penicillium* and *Aspergillus*, in places impacted with heavy metals, occurs due to the ability of individuals from these species to remove heavy metals from the environment, including chromium and nickel (Iram et al. 2012).

From Sordariomycetes class, the orders Sordariales (RA sample with 552 OTUs) and Hypocreales (RG sample with 334 OTUs) were observed. From Sordariales order, the genera *Chaetomium, Neurospora* (the most abundant-222 OTUs), *Bombardia* and *Podospora* were found. Concerning to Hypocreales order, the genera *Gibberella* (the most abundant-238 OTUs), *Nectria, Cordyceps* and *Hypocrea* were observed. In these two locations, the higher frequencies of sequences OTUs related to those genera are possibly due to the lower concentration of heavy metals, which allowed these microbial communities to thrive. Considering that it was possible to observe a reduction in the number of sequences related to the same genera found in the samples contaminated with greater concentration of heavy metals (RS and RM), it is possible to infer that the presence of these contaminants interfered in the homeostasis of the environments, culminating in the reduction of these microbial communities. A considerable number of OTUs related to Saccharomycetes class was observed in all sample sites, reaching 54, 43, 57 and 52 OTUs from RM, RS, RG, and RA, respectively. Yeasts have robust strategies of resistance to heavy metals including detoxification of the cytoplasm by the transporting the metals to the outside of the cell by specific carriers, or by accumulation of metals, normally complexed with thiolated peptides, in organelles (Fidalgo 2011; Tsai et al. 2009).

The higher Basidiomycota abundance found in this work was related to the Agaricomycetes class, derived from samples RA, RG, RM and RS, totaling 26, 53, 37 and 18 OTUs, respectively (Fig. 4). The majority of OTUs sequences from these groups were recovered from RG sample (control), the less heavy metal-contaminated site (data shown on Table 1). The orders Agaricales and Tremellales were the most abundant taxonomic groups, with 122 and 67 OTUs respectively. Agaricales order was represented by sequences of *Coprinopsis, Moniliophthora, Schizophyllum* and *Laccaria* (the most abundant-44 OTUs) genera. Concerning Tremellales, only sequences from *Filobasidiella* genus were found (65 OTUs). Sequences related to Blastocladiomycota and Chytridiomycota phyla were found from samples RM, RG and RA. Chytridiomycota was represented by sequences from genera *Cladochytrium, Olpidium, Spizellomyces* and *Harposporium*, while Blastocladiomycota was represented by sequences from *Allomyces* and *Blastocladiella* genera. These two last phyla were not represented by
any sequence of RS sample, probably due to the great contamination by all heavy metals, mainly Cr, Pb and Zn. However, as the other contaminated sites presented sequences from Chytridiomycota and Blastocladiomycota phyla, it is possible to affirm that these taxonomic groups are able to thrive under certain concentrations of heavy metals, and may not tolerate high concentrations of lead. Some representatives of these phyla are cosmopolitan found in different environments and terrestrial ecosystems (Jerônimo et al. 2015). However, the tolerance to heavy metals by genus from Blastocladiomycota and Chytridiomycota remains poorly known (Georg and Gomes 2007; Jia et al. 2018).

Filamentous fungi are known to be resistant to heavy metals due to their ability to detoxify these compounds by several mechanisms such as (1) valence transformation; (2) active uptake; (3) impermeability and sequestration; (4) extra and intracellular precipitation; (5) biosorption to cell wall; (6) transformation of metals; (7) defense mechanisms including immobilization of heavy metals using extracellular and intracellular chelating compounds and (8) presence of transporter systems for the uptake of essential metals in the cell membrane (Islam et al. 2008; Qayyum et al. 2016). In the environment, heavy metals can interact with extracellular enzymes of fungi and must be taken up by the fungus to promote a physiological response (Baldrian 2003).

Alpha diversity (α) is the total number of species in a habitat (Nogueira et al. 2008; Magurran 2004). In our work, a great species richness (alpha diversity) was found in all samples. The greatest diversities were found in RG sample, which totaled 68 distinct species (55, 8, 2, 1, 1 and 1 sequence from Ascomycota, Basidiomycota, Chytridiomycota, Blastocladiomycota, Entomophthoromycota and Mortierellomycota, respectively). In the RM sample, 68 species (52, 8, 4, 1, 1 and 2 from Ascomycota, Basidiomycota, Chytridiomycota, Blastocladiomycota, Entomophthoromycota and Mortierellomycota, respectively) was recovered. Samples RA and RS recovered, respectively, 50 and 40 distinct species of Ascomycota, and 8 Basidiomycota species each. From RA sample, only 3 species from Chytridiomycota were recovered (Table 2).

In general, the RS sample showed a considerable reduction (about 90%) in the number of genera representatives of the Ascomycota phylum (n = 57), mainly due to the presence of lead, chromium, and zinc, when compared to the control sample (n = 559), followed by RA (n = 515) and RM (n = 489) with a reduction of 7.8% and 12.5%, respectively. In the same way, the genera belonging to the phylum Basidiomycota also suffered a reduction in numbers, totaling RS (n = 25; 66.2%), RA (n = 40; 46%) and RM (n = 48; 35%) compared to the control (n = 74).

Despite the higher contamination with Cd, Cu, Ni and Zn metals in the RM sample in comparison to RS sample, RS site presented lower α-diversity (48 distinct species), and, comparing the contaminations of all samples, the possible responsible for the lower diversity was the higher concentration of lead in this area (Table 1), as the presence of other heavy metals such as Zn and Cr did not interfere in the diversity of the other samples. Lead is considered a non-essential metal and an environmental micro-contaminant, being more harmful to the fungal communities (Rasool and Irum 2014; Woldeamanuale 2017). The total alpha-diversity of RM and RG samples had the same number of species recovered, while RA sample had only about 10% of the loss of alpha-diversity (Table 2). Observing the PCA (Supplementary material–Supp 2), it is possible to observe that all the variables, including all the evaluated heavy metals, N and pH, had greater influence on the diversity of the RM and RS samples. These results may be explained by the difference in the number of OTUs related to some genera found in the four samples studied. The genera that emphasize this finding are Aspergillus and Chaetomium. In the RG sample, 87 OTUs (Aspergillus) and 27 (Chaetomium) were found. Meanwhile, in the RM sample, were recovered about 25% and 51% more OTUs from Aspergillus and Chaetomium, respectively. These two genera may be acting as the main groups involved with the expression of genes related to heavy metal tolerance, since the other genera showed a smaller abundance. In the same way, in RA sample (moderate contamination of all heavy metals), were recovered about 3.5 and 1.4 times more sequences affiliate to Chaetomium and Podospora, respectively and about 74% more sequences affiliate to Neosartorya, compared to RG sample. The number of sequences assigned to genus Neosartorya in RG and RM samples was practically the same, showing that the concentration of metals found within RM sample did not affect the prevalence of this group of organisms. From these results, it is possible to affirm that the genera Neosartorya, Aspergillus, Podospora, Chaetomium and Neosartorya are tolerant to high levels of heavy metals. Representatives of these genera have already been reported in the literature involved in some metal tolerance process (Iram et al. 2009; Joshi 2014; Abdel-Azeen et al. 2015; Oladipo et al. 2016; Qayyum et al. 2016; Haruma et al. 2018).

### Table 2 α-Diversity from four samples studied, considering the recovered OTUs richness

| Phylum                  | RG/% | RM/% | RA/% | RS/% |
|-------------------------|------|------|------|------|
| Ascomycota              | 55/87| 52/86.6| 50/84| 40/80|
| Basidiomycota           | 8/13 | 8/13.3| 8/16 | 8/20 |
| Blastocladiomycota      | 1/50 | 1/50  | 0/0  | 0/0  |
| Chytridiomycota         | 2/22.2| 4/44.4| 3/33.3| 0/0 | 0/0 |
| Entomophthoromycota     | 1/50 | 1/50  | 0/0  | 0/0  |
| Mortierellomycota       | 1/33.3| 2/66.6| 0/0  | 0/0  |
| Total                   | 68   | 68    | 61   | 48   |
Regarding Entomophthoromycotina and Mortierellomycotina subphyla, the number of sequences assigned to these taxonomic groups were higher in the RG sample, in comparison to the RM sample, with representatives from Mortierellales order (Mortierella genus) and Entomophthorales order (Basidiobolus genus). From Mucorales order, sequences assigned to this group were only found in the less contaminated site (RG), showing the sensitivity of these organisms to heavy metal contamination. Regarding Blastocladiomycota and Chytridiomycota subphyla, no sequences assigned to these two groups were found within RS sample, showing that lead contamination may have been the most striking for the survival of these organisms in the environment. However, members of Chytridiomycota subphylum, i.e., Cladochytridium, Olpidium and Harpochytrium, were only found in RM sample, considered highly contaminated. It was not possible to find available information in the literature on resistance to heavy metals by Cladochytrium and Harpochytrium.

Concerning tolerance, functional metabolism, and biodegradation of heavy metals, analysis of the Ascomycota sequences of the four samples utilizing the KO (KEGG Orthology) database revealed a high number of OTUs related to genes responsible for the metabolic pathways of the target compounds, comprising a total of 635 OTUs, being 161, 264, 157 and 53 sequences from RA, RG, RM and RS, respectively. The sequences were shown to be associated mainly to genes related to metabolism (307 OTUs), genetic information processing (174 OTUs), cellular processes (67 OTUs), environmental information processing (23 OTUs) and organismal systems (6 OTUs) gene (Fig. 5A). In the Basidiomycota sequences analysis, genes related to metabolism (17 OTUs) and genetic information processing (12 OTUs) were found within all samples (Fig. 5B). Functional analyzes performed by COG database revealed sequences related to predicted divalent heavy-metal cations transporter and to copper chaperone in RA sample. The copper chaperone is a metalloprotein responsible for the delivery of Cu to Superoxide dismutase that constitutes a very important antioxidant defense against oxidative stress (Younus 2018). Zhang et al. (2016) reported the lead- and cadmium—induced oxidative stress on the activities of catalase enzyme in Phanerochaete chrysosporium, a species from the Basidiomycota phylum. In the same way, in our study were found the same sequences related to predicted Zn-dependent proteases, predicted Zn-dependent hydrolases of the beta-lactamase fold, predicted metal-dependent protease of the PAD1/JAB1 superfamily and cytokine deaminase and related metal-dependent hydrolases, data suggesting the use of heavy metals for cellular activity. The results, especially from COG database, showed the recovery of a large amount of potential unknown functional genes sequences.

Sequences associated with DNA repair genes and affiliated to Ascomycota phylum were retrieved within three samples, including two contaminated with heavy metals (RA and RS). In the RM sample, the most contaminated with metals, no DNA repair genes were found, contrary to what was expected, since the presence of heavy metals such as chromium and cadmium may activate stress-responsive pathways in filamentous fungi (Pócsi 2011; Viti et al. 2014). However, the sample presented the higher number of genes related to heavy metals biosorption processes, which shows that the biosorption is the main tolerance activity executed by the fungal community inhabiting this site. The RA site,
moderately contaminated with heavy metals, presented both DNA repair genes and genes related to heavy metals biosorption processes, which indicates that fungal communities inhabiting this site have developed different adaptation mechanisms to confront the contamination.

The mechanism involved in the biosorption process can range from ion exchange to membrane diffusion, that can be influenced by biomass (carbon) and solution chemistry (nitrogen source). Factors such as cell age, and environmental and nutritional conditions interfere with the preference of living biomass in the bonds with metallic ions. To overcome the toxic effect of metals at high concentrations, dead biomass may be preferred in these biosorption processes (Ahmad et al. 2005). In addition, metal concentration as well as factors such as temperature and pH are known to influence the biosorption process (Ahmad et al. 2005), and according to Chen (2012), Cr (VI) biosorption decreases as the pH of the solution increases. In our work, all samples had pH values above 8.0 (except the control), thus the microbial community could be adsorbing metals due to a relatively high pH range.

The bioremediation of metals is regarding the presence of a high percentage of cell-wall material within fungal biomass, which increases the variety of functional groups involved in metal binding (Dhankhar and Hooda 2011). In this sense, it is possible to establish a relationship between the enzymes or proteins involved in membrane transport processes and the biosorption of heavy metals by filamentous fungi. In our work, we found, in RM and/or RA samples, proteins sequences involved in signal transduction including two-component system and calcium signaling pathway as well as proteins involved in membrane transport such as ABC transport, endorsing the role of these proteins in the adaptation of filamentous fungi in contaminated environments. The properties of environments, especially those heavily contaminated with heavy metals from manufacturing industries of aluminum, leather dyeing and those that utilize toxic, corrosive, and flammable products, may influence, and modify the microbial community composition and functionality. By using metagenomic approach, it was demonstrated the diversity and dynamics of metabolic activities in the microbial communities from banks of stream, conferring these communities the role of recyclers of circulating compounds and making the filamentous fungi a key point in the biosorption of heavy metals, with the possibility of collaborating in the reduction of these compounds in the environment. The environment impacted with heavy metals is a drastically modified habitat, and the microbial communities must adapt to the new toxic conditions. Filamentous fungi could adjust through the expression of genes involved in heavy metal bioremediation process, aiming to survive and thrive in extremely toxic environments surrounding manufacturing industries that disposal toxic products including heavy metals. The results obtained from the metagenomic approach from this work provide valuable and robust information for subsequent studies focused on the development and improvement of selective culture media for the isolation of fungal strains adapted to bioremediation processes from environments contaminated with heavy metals.

**Conclusion**

In our work, the metagenomic approach was able to reveal a phylogenetic as well as metabolic profile of the fungal community associated with soil samples contaminated with heavy metals. The most abundant phyla were Ascomycota and Basidiomycota, known to be resistant to several environments due to their metabolic diversity. However, sequences related to heavy metal metabolism were only found from Ascomycota phylum, demonstrating that the other phyla and subphyla found in all samples may have different mechanisms, genes or complex metabolic pathways for the processes of adaptation, metabolism and tolerance to heavy metals. Concerning heavy metal metabolism, repair of DNA processes sequences was found in three of the four samples, not being present in the RM sample, considered the most contaminated site. Regarding heavy metals biosorption processes, both site RM and RA showed sequences from these processes. These gene sequences were not found in the control site (RG) nor within RS sample, the most contaminated with lead. It was possible to observe that most fungal communities present in sites contaminated with heavy metal were able to thrive except in the presence of high concentration of lead, the metal that most interfered in the abundance of fungal sequences among all sampled sites. The present work provides relevant data about the dynamics of adaptation, survival, and tolerance of fungal communities under stress caused by industrial waste, in addition to bringing references to allow to infer the functions of these organisms in the environment and their potential for bioremediation.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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