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ORIGINAL ARTICLE

Assessment of functional diversity of heterotrophic microbial communities in polluted environments through community level physiological profiles

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Abstract

The community level physiological profiles (CLPP) of bacterial communities inhabiting polluted environments (acid mine drainage water and sediments, soils contaminated with tannery waste and oil refinery effluents) were assessed using the Biolog Ecoplates™ in comparison to non-contaminated sites (water and sediments from a recreational lake and soil from anopen space). Although the polluted sites were characterized by typically high metal concentrations, CLPP fingerprints of the different bacterial communities from these sites were indicative of versatile metabolic potentials. These microbial communities could differentially utilize all the different groups of carbon substrates. However, the rates of utilization were significantly lower, and the number of utilized substrates were fewer than those of microbial communities from non-contaminated sites. This was confirmed by cluster analysis in which the dendrogram showed two clusters of microbial communities from contaminated environments and another for those from non-contaminated sites. Nonetheless, the indices of diversity calculated did not show a reduction of diversity or evenness in the microbial communities from contaminated sites. This study confirms the usefulness of the CCLP method in untangling the functional diversity of microbial diversity in contaminated environments.

Keywords Biolog Ecoplates™ · Carbon substrate utilization · Functional diversity · Microbial community

Abbreviations

CLPP	community level physiological profile
NGS	next generation sequencing
AMD	Acid mine drainage
AMDW	Acid mine drainage water
AMDS	Acid mine drainage sediments
ORE	Oil refinery effluents
RLW	Recreational lake water
RLS	Recreational lake sediments
TDSS	Tannery dump site soil
TDC	Tannery dumpsite control
AWCD	Average well color development
SAWCD	Substrate average well color development
SR	Substrate richness
H	Shannon index of diversity
E	Shannon Evenness
EC	Electrical conductivity
TDS	Total dissolved solids
SAL	Salinity
COD	Chemical oxygen demand
rpm	revolutions per minute
PVDF	Polyvinylidene fluoride
AA	Amino acids
CA	Carboxylic acids
CAR	Carbohydrates
POL	Polymers
PHE	Phenolic compounds
AM	amines
PCA	Principal component analysis
$\mu S\ cm^{-1}$	MicroSiemens per centimeter
$\mu g\ L^{-1}$	Micrograms per litre

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Introduction

The global rise in urbanization and industrialization results in the release of large quantities of various contaminants (such as acid mine drainage, municipal waste, and industrial effluents) into the environment (Stamps et al. 2016; Thiebault et al. 2017). The presence of contaminants in any environment creates "special" habitats with distinct physicochemical parameters which often induce physiological and morphological changes to indigenous microbial communities, with ultimate changes in their biodiversity (Emmanuel et al. 2014; Gümral et al. 2016; Li et al. 2016; Siddiqee et al. 2013). Partly these exert selective pressure on indigenous microbial communities in favor of populations with the tenacity to tolerate and use the introduced contaminants to their advantage. For instance, heavy metal contamination was identified as a major cause for reduced microbial biomass and enzyme activity in soil microbial communities (Fazekaš et al. 2019). While the heavy metal contaminated soils depicted reduced microbial activity and changes in microbial community structure, a high microbial evenness index was observed. This suggests that some populations of the microbial community were 'enriched' by the presence of the very same pollutants which diminish the population sizes of the less tolerant microorganisms (Brito et al. 2013). Similarly, long-term PAH contamination in soils has been linked to reduced microbial diversity and species richness (Markowicz et al. 2016a).

The realization that contaminated environments may harbor specialized microbial communities with possible applications in environmental and industrial biotechnology has sparked research interests in understanding their physiological properties and their adaptive strategies (Sibanda et al. 2017; Singh et al., 2015; Stamps et al. 2016). Therefore, there has been a drive to understand the role of contaminants in shaping microbial diversity and functionality in contaminated environments, as well as how ecological functions respond to pollution stress. The advent of high throughput Next Generation Sequencing (NGS), such as targeted gene amplicon sequencing, has been very beneficial in the field of microbial systematics as it allows comprehensive taxonomic and predictive functional profiling of microbial communities (De Mandal et al. 2015). Nonetheless, despite their obvious advantages, NGS based platforms are limited in providing insights into the *in situ* metabolic potential of microbial communities, which is also crucial for possible exploitation of microbial communities. Consequently, culture-based techniques remain relevant for studying the *in situ* metabolic potentials of microbial communities. The Community-Level Physiological Profiling (CLPP) remains a common and meaningful approach to studying the metabolic potentials and diversity of microbial communities in different environments (Frąc et al. 2012). The Biolog Ecoplate™ is a versatile tool used to assess the metabolic diversity of microbial communities in environmental samples by evaluating their utilization of different carbon sources (Feigl et al. 2017). By means of biological indices such as the Average Well Colour development (AWCD), Substrate Average Well Colour Development (SAWCD), Substrate Richness (SR), Shannon index of diversity (H) and Shannon Evenness (E), it allows quick characterization of the ecological status of environmental samples (Feigl et al. 2017). These have been successfully used to evaluate the metabolic diversity in soil and a range of other environments including aquaculture ponds (Kurten and Barkoh 2016), constructed wetlands (Lv et al. 2017), sediment-water interface (Oest et al. 2018), and compost microbial communities (Huang et al. 2015). Biolog Ecoplates™ have been particularly instrumental in studying the changes in microbial diversity along contamination gradients (Kuźniar et al. 2018; Liang et al. 2017; Oest et al. 2018). This study was aimed at evaluating the functional diversity in acid mine drainage (AMD), oil refinery effluent (ORE) and tannery dumpsite soils (TDSS) as examples of contaminated environments using Biology Ecoplates™.

Materials and methods

Sampling

Sampling was done from three sites identified as model metal polluted sites, an AMD dam, a tannery dumpsite, and an oil refinery plant wastewater treatment plant. For comparison, samples were also collected from non-contaminated sites, a recreational freshwater lake and non-contaminated open space around the location of a tannery dumpsite. AMD water and sediment samples were collected from an abandoned AMD tailings dam, in the Gauteng Province of the Republic of South Africa (GPS coordinates S 26° 07 39.9′ E 27° 46 46.2′). Three sampling points were identified around the dam from which water was collected into sterile 1 L bottles and sediments into sterile 50 mL centrifuge tubes. For chemical analysis water was collected into 1 L polypropylene bottles. In the laboratory, equal volumes of water or sediments from each of the three sampling points were combined to obtain a composite water or sediment sample. From a tannery dumpsite, located in the Limpopo Province of the Republic of South Africa, (GPS coordinates S 23° 54 00′ E 29° 27 00′), topsoil (0–20 cm depth) was collected from six points within the dumpsite, selected based on easy accessibility. Soil was collected into sterile polyethylene bags using standard microbiology procedures. Two sets of samples were collected for microbiological and

chemical analysis. Wastewater samples (effluents) were collected from a petrochemical refinery plant in Sasolburg in the Mpumalanga Province of South Africa. Samples for microbiological work were collected aseptically into 50 mL sterile centrifuge tubes and those for chemical analysis were collected into 500 mL bottles. Two non-contaminated sites, a recreational lake, and an open space within the vicinity of the tannery dumpsite were also considered as control sites. From the recreational lake, located in Rooderpoort in the Westrand area of the Gauteng Province of South Africa, water and sediments were collected. Soil samples were collected from a non-contaminated open space in the vicinity of the tannery dumpsite. In all sampling sites sampling was conducted twice over a period of 1 month.

Analysis of physical and chemical parameters in water, sediments, and soil

To ascertain prevailing environmental conditions under which microbial communities survive in the contaminated and non-contaminated environments, physical and chemical parameters were measured using different analytical techniques. Measurements included temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity (SAL), chemical oxygen demand (COD), sulfates, nitrates, and metals. Where possible measurements such as pH, temperature, EC, TDS, SAL were measured on site using a field multiparameter meter. Other parameters such sulfates, nitrates, and COD, were quantified spectrophotometrically in a Spectroquant Pharo 300 (Merck, South Africa). Prior to any analysis soil and sediment samples were dried in a vacuum freeze-drying equipment (Labconco, USA). Dry samples were ground to a fine powder in a pestle and mortar and homogenized by passing through a 200-mesh sieve. To measure physicochemical parameters soil and sediment samples were mixed with deionized water at the ratio of 1:5 and the mixture shaken at 150 rpm for 2 h. The suspension was allowed to settle overnight, the supernatant was carefully decanted, filtered, and used to measure pH, sulfates, nitrates, and COD.

Metal analysis

For metal analysis, AMD and water from the recreational Lake were first filtered through 0.45 µm polyvinylidene fluoride (PVDF) syringe filters, acidified to pH 2, and analyzed. The refinery wastewater, dried soil and sediments were digested prior to metal analysis in a microwave (SINEO MDS-6G). For soil and sediment samples, 0.5 g sample was weighed and placed in microwave vessels and mixed with 9 mL nitric acid, and 3 mL hydrochloric acid. Thereafter digestion was performed at 175 °C for 60 min at 6 watts power. For the oil refinery wastewater, a 20 mL volume of wastewater was measured into microwave bombs and mixed with 4.5 mL of nitric acid and 1.5 mL of hydrochloric acid. The mixture was digested at 220 °C for 30 min at 6 watts power. After digestion the samples were filtered through qualitative filter paper, the filtrate transferred into volumetric flasks, and quantitatively made up to 50 mL. Metals were measured in an Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer, Nexion 350D).

Assessment of microbial community functional diversity using the Biolog Ecoplate™

Biolog Ecoplates™ (Biolog, Hayward, California) were used to evaluate the functional diversity of the soil, sediment, and water bacterial community from polluted sites comparatively with those from non-polluted sites.

Culturing

For sediment and soil sample analysis, 3 g sample was suspended in 27 mL of sterile 0.85% sodium chloride solution and vortexed for 5 min at maximum speed. After settling for 10 min, 180 µL of the supernatant was inoculated (pipetted) into each of the wells. For water samples, 180 µL of water was directly inoculated into each of the wells without pretreatment. All plates were sealed with parafilm and incubated at 25 °C in the dark. Absorbance was read at 590 nm wavelength with a VarioSkan Flash (Thermoscientific) plate reader at 0, 24, 48, 72, 96, 120, and 144 h.

Data analysis

Prior to analysis absorbance values from each well were corrected by blanking against the corresponding absorbance at 0 h (Insam and Goberna 2004). Furthermore, negative absorbance values were coded as zero (Frąc et al. 2012). For each sample, absorbance values obtained where the growth curve reached an asymptote were used for data evaluation and statistical analysis. To assess the functional diversity of microbial communities, several indices were calculated. The AWCD was calculated for all carbon containing wells at all incubation times using the equation $AWCD = \Sigma ODi/31$ where OD*i* is the corrected absorbance value of each carbon containing well. To calculate the SAWCD, carbon sources were grouped into six biochemical categories (amino acids, carboxylic acids, carbohydrates, polymers, phenols, and amines). The SAWCD was calculated from the equation $SAWCD = \Sigma ODi/N$, where OD*i* is the corrected absorbance value of the substrates within the substrate category and N is the number of substrates within that category. The Shannon index of diversity was calculated from the equation

$H = -\Sigma Pi(\ln Pi)$, where P*i* is the proportional color development of the well over total color development of all wells of a plate. P*i* is given by the equation $Pi = ODi/\Sigma ODi$. SR was calculated as the sum of the number of cells where absorbance reached the threshold value set at 0.15 at the asymptote of the AWCD against time graph (Feigl et al. 2017). E was calculated from the Shannon index and substrate richness using the equation $E = H/logSR$

Results

Physicochemical parameters

The chemical and physical properties of water, soil and sediment samples were evaluated using a broad suite of analytical techniques. For the water samples pH, EC, TDS, SAL, COD, sulfates, nitrates, and metals were quantified (Table 1). AMD water samples (AMDW) were characterized by low pH values that are below 3. The EC, TDS, SAL, COD, and SO_4^{2-} concentrations were also higher than those recorded for the non-contaminated water. In these samples aluminium (Al) was by far the most prevalent dissolved metal quantified, with concentrations exceeding 500 mg L^{-1}. This was followed by iron (Fe), magnesium (Mg), sodium (Na), and manganese (Mn), all with concentrations above 100 mg L^{-1}. Other metal ions detected in substantial amounts are zinc (Zn), calcium (Ca), potassium (K), and copper (Cu). Whilst the oil refinery effluents (ORE) were alkaline (pH > 8), they were also characterized by high levels of SAL, EC, and SO_4^{2-} which were relatively higher than those of the non-contaminated water collected from the recreational lake (RLW). Metal concentrations in oil refinery effluents were also high with their concentrations decreasing in the order Na > Mg > Li > Cr > Fe > Zn > Cu > Al > Mn. The non contaminated water from the recreational lake had a neutral pH (≈ 7) and besides nitrates (> 40 mg L^{-1}), the concentrations of all the other measured parameters were lower than those recorded for AMD and the oil refinery effluents.

For the soil and sediment samples pH, sulfates, nitrates, and metals were quantified (Table 2). The sediment samples from the AMD dam (AMDS) were also acidic with high concentrations of sulfates (1267–2775 mg kg^{-1}) and low concentrations of nitrates. High concentrations of metals were also recorded, with Fe detected as the most abundant metal with concentrations ranging between 35,619 and 65,314 mg kg^{-1}. Al, Ca, Mg, K, Mn, were also detected in substantial amounts (above 500 mg kg^{-1}), whilst Zn, Cr, and Cu were detected in trace amounts. Non contaminated sediment samples collected from the recreational lake (RLS) were neutral (pH 6.47 and 7.36) and rich in nitrates (> 450 mg kg^{-1}). The concentrations of all other parameters including sulfates and metals were lower than those recorded for the AMD sediments. The soil samples collected from a dumpsite receiving solid tannery waste (TDSS) were also slightly acidic (pH 5.99 and 5.43) with high concentrations of sulfates and different metal ions quantified. Fe, Al and Na were detected as the most abundant (concentrations exceeding 1000 mg kg^{-1}). Li, Zn, Mg, Cr, and Ca were also quantified

Table 1 Physicochemical properties of water samples collected from an AMD dam, oil refinery wastewater treatment plant and a recreational lake

Parameter	AMDW-1	AMDW-2	ORE-1	ORE-2	RLW-1	RLW-2
pH	3.04 ± 0.08	2.93 ± 0.01	9.47 ± 1.55	8.35 ± 0.72	7.13 ± 0.21	6.73 ± 4.89
EC	5355.33 ± 156.31	5948 ± 186.95	6800 ± 3.20	5200 ± 1.30	156.7 ± 3.32	153.4 ± 2.57
TDS	4391.67 ± 9.71	4413.5 ± 6.5	2100 ± 0.01	2000 ± 0.01	128.7 ± 1.13	143.15 ± 9.06
SAL	3.74 ± 0.01	3.74 ± 0.01	3.74 ± 0.01	3.92 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
COD	129.22 ± 6.59	154.22 ± 22.30	190 ± 4.67	277 ± 8.90	77.89 ± 1.45	80 ± 3.00
SO_4^{-2}	920.00 ± 280.10	1416.67 ± 113.91	165.00 ± 13.73	139.40 ± 14.56	1.51 ± 1.04	2.42 ± 1.22
NO_3^-	1.93 ± 0.70	4.09 ± 0.30	0.75 ± 0.31	0.00 ± 0.00	53.67 ± 1.80	45.56 ± 1.24
Al	550.54 ± 24.49	570.96 ± 12.88	1.42 ± 0.31	1.82 ± 0.03	1.17 ± 1.82	0.14 ± 0.01
Ca	17.62 ± 0.46	17.93 ± 0.34	0.44 ± 0.007	1.48 ± 0.47	4.61 ± 1.45	4.33 ± 2.33
Cr	1.15 ± 0.11	1.17 ± 0.34	8.17 ± 5.09	8.98 ± 5.44	0.00 ± 0.05	0.00 ± 0.00
Cu	2.92 ± 0.11	3.04 ± 0.07	1.96 ± 0.43	0.66 ± 0.27	0.01 ± 0.00	0.00 ± 0.00
Fe	274.68 ± 13.34	288.71 ± 5.71	64.59 ± 9.72	9.06 ± 5.11	1.59 ± 0.08	0.37 ± 0.43
K	4.90 ± 0.11	4.90 ± 1.32	1.96 ± 1.43	0.66 ± 0.27	2.21 ± 0.08	2.48 ± 0.64
Li	0.69 ± 0.14	0.87 ± 0.09	106.54 ± 10.03	182.65 ± 9.74	0.04 ± 0.13	0.05 ± 0.01
Mg	171.62 ± 5.17	175.98 ± 3.55	2121 ± 457	1299.85 ± 110.26	5.53 ± 1.02	4.54 ± 0.04
Mn	65.05 ± 2.71	54.22 ± 24.16	0.25 ± 0.14	0.16 ± 0.01	0.22 ± 0.30	0.11 ± 0.07
Na	112.86 ± 4.34	117.21 ± 1.18	141.26 ± 36.33	94.92 ± 24.88	12.24 ± 1.98	11.61 ± 1.54
Zn	20.73 ± 0.91	22.01 ± 0.49	2.63 ± 2.89	0.56 ± 0.20	0.05 ± 0.07	0.00 ± 0.00

Each value is a mean of 3 measurements ± standard deviation; pH has no units; T in °C, EC in (µS cm^{-1}); SAL (µg L^{-1}); all other measurements in mg L^{-1}. AMDW = acid mine drainage water, ORE = oil refinery effluent, RLW = recreational lake water

Table 2 Physicochemical properties of sediment and soil samples collected from contaminated and non-contaminated environments

Parameter	AMDS-1	AMDS-2	RLS-1	RLS-2	TDSS-1	TDSS-2	TDC-1	TDC-2
pH	3.07 ± 0.21	3.33 ± 0.32	6.47 ± 0.72	7.36 ± 1.72	5.99 ± 1.39	5.24 ± 0.87	6.97 ± 0.42	7.01 ± 0.56
SO_4^{-2}	5166.67 ± 151.61	1483.00 ± 61.71	27.72 ± 1.75	29.4 ± 7.72	432.50 ± 184.95	316.30 ± 190.41	25.23 ± 424	45.18 ± 721
NO_3^{-1}	22.50 ± 0.87	13.83 ± 2.75	465 ± 148.56	615 ± 579.83	15.38 ± 4.37	16.37 ± 1.98	8.50 ± 1.47	8.35 ± 0.72
Al	16129.32 ± 739.93	10513.63 ± 823.21	65.28 ± 3.55	63.16 ± 2.58	7365.28 ± 473.55	3613.16 ± 562.58	657.41 ± 62.58	568.92 ± 1.45
Ca	3518.15 ± 332.57	1922.29 ± 257.47	1.51 ± 0.11	1.62 ± 0.01	746.50 ± 45.7	750.18 ± 62.24	7.66 ± 5.65	20.46 ± 1.35
Cr	126.67 ± 80.65	95.85 ± 9.79	15.04 ± 1.91	15.5 ± 0.04	85.57 ± 77.42	195.06 ± 14.90	1.53 ± 1.09	1.58 ± 1.45
Cu	88.58 ± 13.51	59.65 ± 74.36	2.46 ± 1.35	1.8 ± 0.04	4.33 ± 2.50	7.87 ± 4.90	0.00 ± 0.00	0.00 ± 0.00
Fe	65344.97 ± 1436.91	37979.80 ± 572.43	50.18 ± 8224	82.41 ± 86.71	2750.18 ± 82.24	3182.41 ± 86.71	243.73 ± 86.72	121.10 ± 81.47
K	1604.39 ± 436.39	829.99 ± 127.80	0.65 ± 0.00	0.23 ± 0.04	2.82 ± 0.60	1.96 ± 0.41	0.80 ± 0.46	0.77 ± 0.32
Li	63.86 ± 0.43	64.14 ± 0.34	8.47 ± 2.58	4.78 ± 1.47	136.85 ± 98.16	401.94 ± 72.89	0.00 ± 0.00	0.00 ± 0.00
Mg	2027.87 ± 152.33	816.07 ± 44.77	193.36 ± 10.20	458.22 ± 14.85	837.23 ± 72.80	581.00 ± 76.81	47.72 ± 14.86	68.73 ± 7.37
Mn	473.81 ± 80.09	171.29 ± 35.48	142.02 ± 5.78	251.30 ± 6.10	12.54 ± 3.51	17.57 ± 5.01	6.99 ± 2.10	5.62 ± 1.76
Na	273.91 ± 37.23	290.25 ± 81.73	25.81 ± 5.21	36.51 ± 5.55	1814.03 ± 176.98	2018.50 ± 188.86	132.59 ± 5.05	140.44 ± 1.02
Zn	194.50 ± 148.28	112.32 ± 102.73	7.07 ± 1.13	2.36 ± 0.29	7.07 ± 1.13	2.36 ± 0.29	0.26 ± 0.13	0.15 ± 0.00

Each value is a mean of 6 measurements ± standard deviation; pH has no units; EC in (μS cm^{-1}); all other measurements in mg kg^{-1}. AMDS = acid mine drainage sediments, RLS = recreational lake sediments, TDSS = tannery dumpsite soil, TDC = tannery dumpsite control

with concentrations between 100 and 1000 mg kg^{-1}. As the least abundant metals, Cu, K and Mn were quantified with concentrations below 100 mg kg^{-1}. The non-contaminated soils collected as a control (TDC) had a neutral pH (6.97–7.01) with comparatively lower concentrations of sulfates, nitrates,and metal ions.

Assessment of functional diversity

The metabolic potentials of microbial communities from both contaminated and non-contaminated environments were quantified in terms of the AWCD, which is the average absorbance (A_{590}) of all the wells at a particular time. These were plotted against time for all the communities (Fig. 1). For the non-contaminated sites (recreational lake water, recreational lake sediments and tannery dumpsite control soil) the plot of AWCD against time is a sigmoid curve, depicting the distinct phases of a typical bacterial growth curve. These curves are characterized by remarkably low AWCD values between 0 and 24 h (lag phase), gradually increasing to reach the maximum at 96 or 120 h. On the contrary, for microbial communities inhabiting contaminated sites, the plot of AWCD against time generally showed rapid growth between 0 and 48 h followed by gradual/slow growth until the end of incubation. For these communities the optimal AWCD values were observed at either 24 or 48 h. Notably, the AWCD values for contaminated environments are significantly lower than those of the non-contaminated sites. For example, the maximum AWCD values recorded for AMD water are 0.11 and 0.12 which is significantly lower than 2.67 and 1.64 recorded for recreational lake water which is treated as a non-contaminated control. Similarly, for the AMD sediments the maximum recorded AWCD values are 0.40 and 0.18 compared to 1.17 and 1.28 for the recreational lake sediments.

SAWCD

The rate of utilization of carbon sources in six different categories is presented in Fig. 2 as a percentage contribution of each substrate guild towards the overall activity pattern. Like the AWCD values, SAWCD values calculated for the different microbial communities indicate some variations in their potential to catabolize the six groups of carbon substrates. The SAWCD values calculated for microbial communities inhabiting non-contaminated environments are higher than those calculated for microbial communities from contaminated environments. For example, for the AMD water the SAWCD values for AA, CA, CAR, PHE, POL, AM were 0.17, 0.16, 0.06, 0.09, 0.07, 0.08 respectively compared to 1.75, 1.75, 2.04, 2.11, and 0.05 for the recreational lake water. The microbial communities show

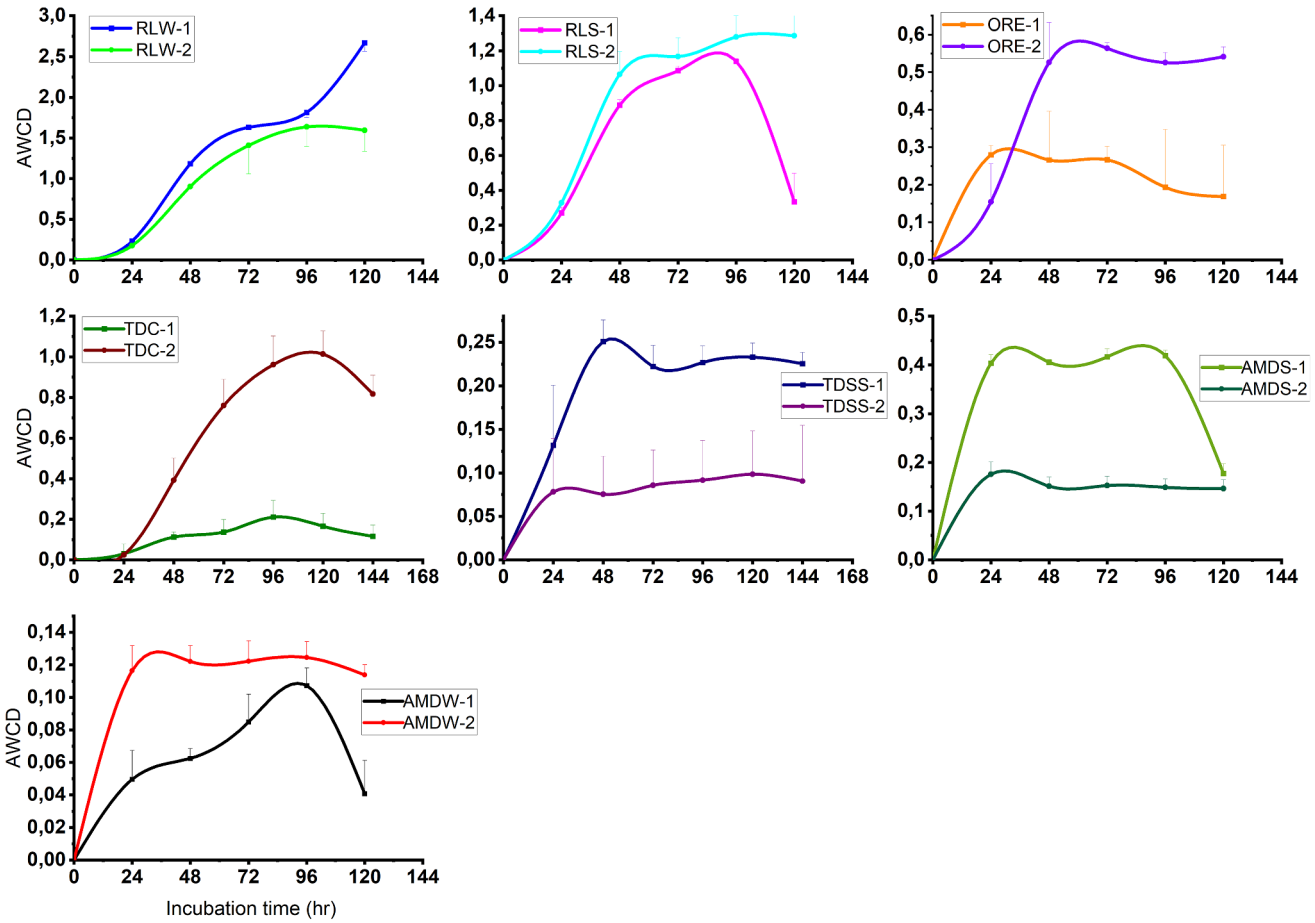


Fig. 1 Plot of AWCD of metabolized single carbon substrates in Biolog Ecoplates. Error bars represent standard deviation ($n = 3$)

wide variation in their preference to catabolize the different groups of carbon substrates. For example, for the tannery dumpsite soils the level of substrate utilization decreased in the order AM > AA > CA > CAR > PHE > POL with SAWCD values ranging between 0.50 and 0.16. Comparatively, the pattern of substrate utility for the control soil was POL > PHE > CAR > CA > AM > AA with the SAWCD values ranging between 0.99 and 0.53.

Single substrate utilization

The potential of the microbial communities in contaminated and non-contaminated sites to utilize each of the 31 carbon substrates was measured as a percentage of the overall potential to utilize all 31 carbon substrates. The substrate utilization patterns (Fig. 3) further highlight subtle differences between microbial communities from contaminated and non-contaminated sites. Clearly, microbial communities in non-contaminated environments could utilize (% substrate utilization > 3) more substrates as compared to the contaminated sites. For instance, for the two microbial communities in tannery dumpsite soils 12 and 11 substrates had their substrate utilization percentage exceeding 3%. Comparatively, for the corresponding control soils 13 and 17 substrates recorded a substrate utility percentage of more than 3%. The patterns of substrate utility also showed a high level of uniformity for microbial communities in non-contaminated environments. These communities depicted an almost comparable level of substrate utilization for all the different substrate guilds. Also, the range between the most utilized and the least utilized substrates is small or minimal. On the other hand, the microbial communities from contaminated environments showed a high level of variation in their patterns of substrate utility, the difference between the most utilized substrate and the least used is high. For instance, the most utilized carbon substrate in the tannery dumpsite soil community is α-ketobutyric acid at 21.77% compared to 0.36% for Glycyl-L-glutamic acid which is the least utilized.

Cluster analysis

Cluster analysis was performed based on the AWCD and SAWCD values using the Wards method (Fig. 4). Based on

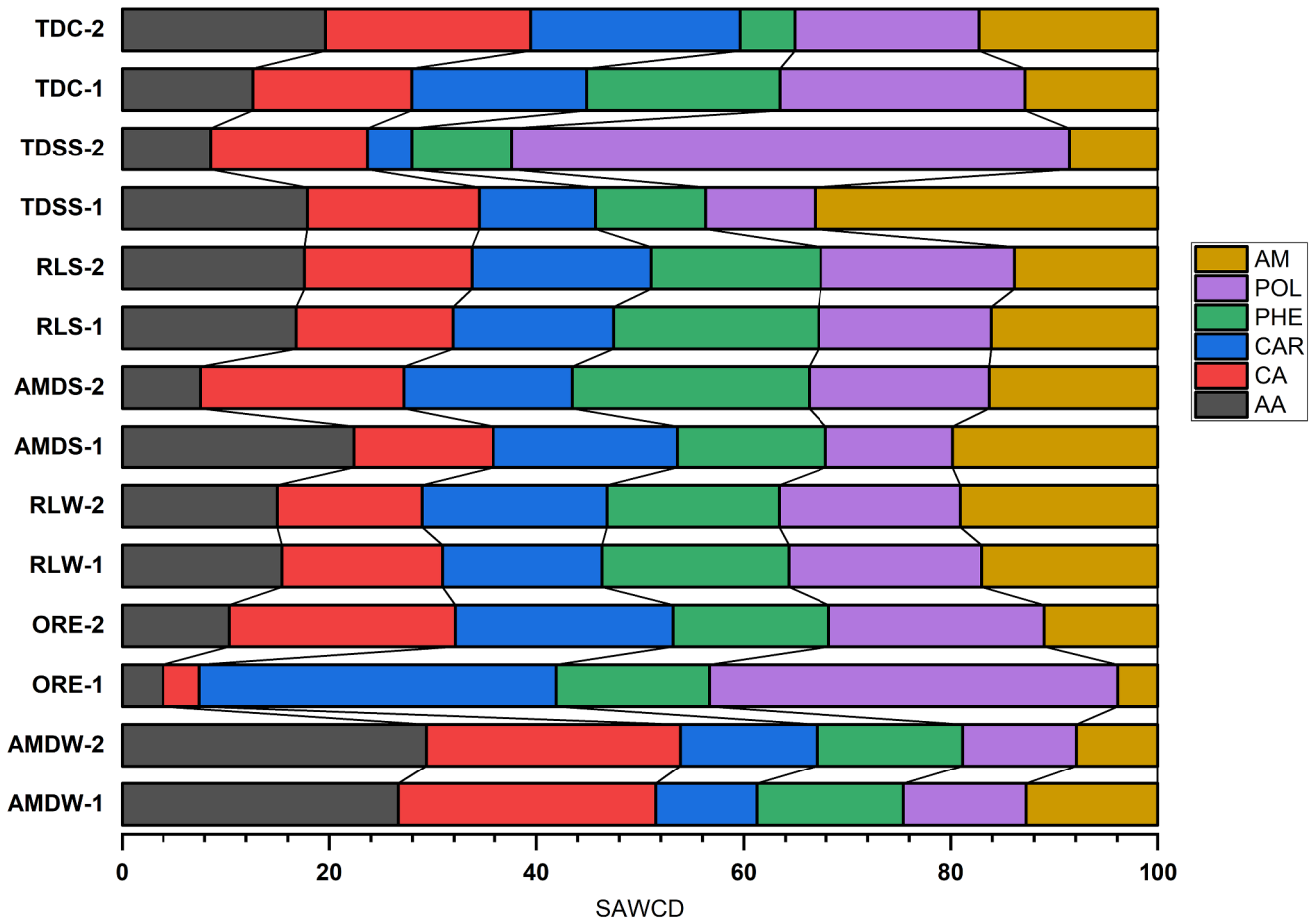


Fig. 2 Metabolic activity of heterotrophic bacterial populations inhabiting water, sediment, or soil samples from contaminated and non-contaminated sites expressed as the utilization rate of different carbon sources presented as percentage SAWCD. AA = amino acids, CA = carboxylic acids, CAR = carbohydrates, PHE = phenolics, POL = polymers, AM = amines

their potential metabolic activity the microbial communities can be grouped into two clusters, one for the microbial communities in contaminated sites and another for microbial communities in non-contaminated sites.

Furthermore, the Principal Component Analysis (PCA) was performed to identify the extent to which different physicochemical parameters influence the potential metabolic activity of various bacterial communities. From the PCA scatter diagram for water samples (Fig. 5a) the first axis accounted for 62% of the correlation. Along this axis pH and nitrates showed a positive correlation to microbial community functional diversity whilst all other parameters (sulfates, Al, Ca, Cr, Fe, K, Li, Mg, Mn, Na, and Zn) depicted a negative correlation. The second axis accounted for 34% of the correlation. Along this axis positive correlations were noted with sulfate, Al, Ca, Fe, K, Mn, and Zn whilst pH, nitrates, Li, Mg, Cr, and Na showed negative correlations. For the sediments and soils PCA biplot (Fig. 5b), the first axis accounted for 53% of the variation with positive correlations shown by pH, nitrates, and Mn. Negative correlations were shown by sulfates and the metals Al, Ca, Cr, Fe, K, Li, Mg, Na and Zn. The second axis accounted for 29% of the variation and components that showed positive correlations were pH, and nitrates. Negative correlations were noted with sulfates, and the metals K, Fe, Mg, Al, Ca, Zn, Cr, Li, Na.

Indices of diversity

The measurement of richness, diversity, and evenness in substrate utilization were inferred from the Substrate richness, Shannon diversity and Shannon evenness indices (Table 3) and used to delineate subtle differences between the bacterial microcosms from contaminated and non-contaminated environments. SR values for microbial communities from contaminated sites are lower/ smaller than those of communities from non-contaminated sites. However, the Shannon diversity and the evenness index do not discriminate the microbial communities into those from contaminated and non-contaminated sites. Low values of H and E

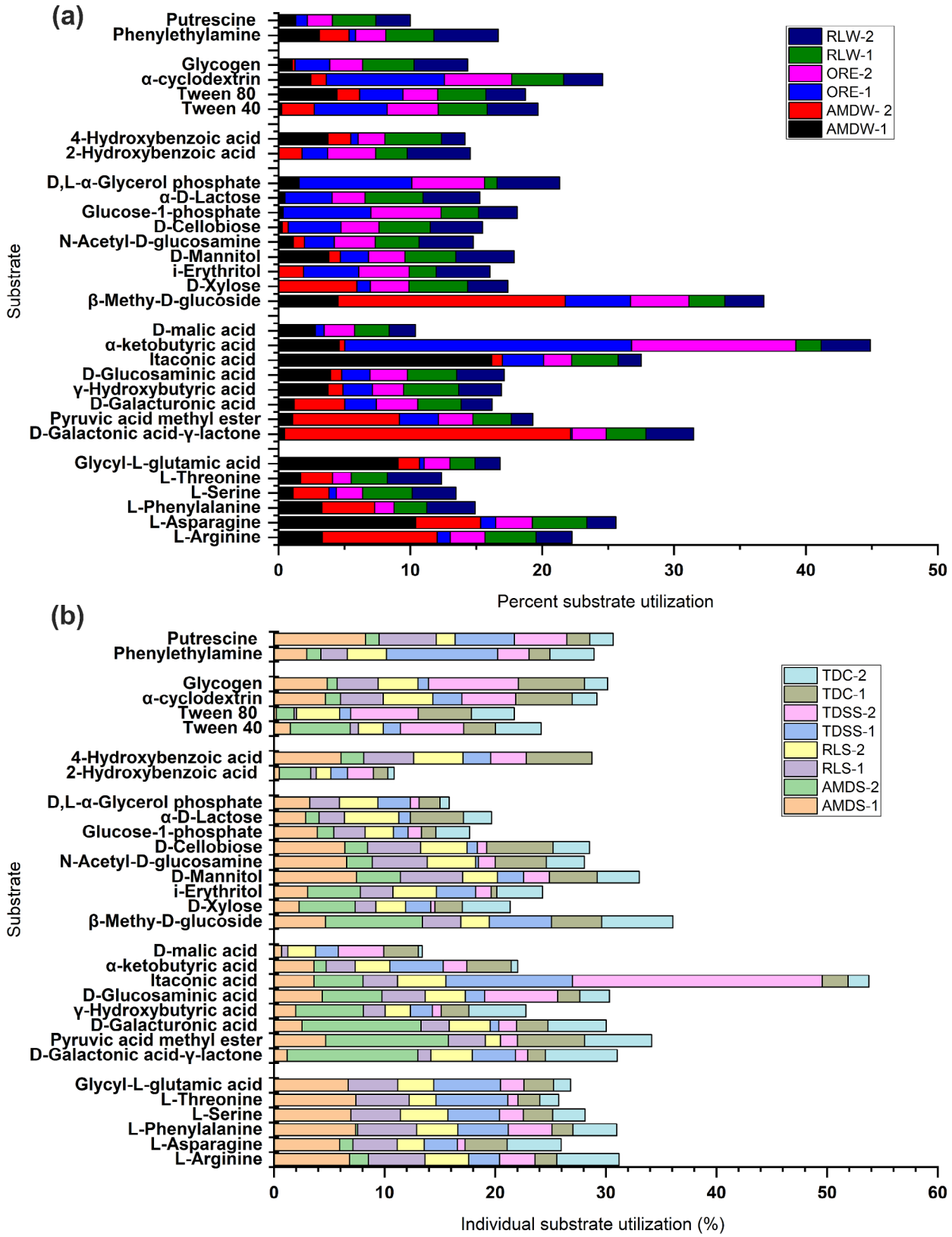


Fig. 3 Patterns of substrate utilization of each of 31 organic carbon substrates in Biolog Ecoplates by microbial communities in contaminated and non-contaminated sites

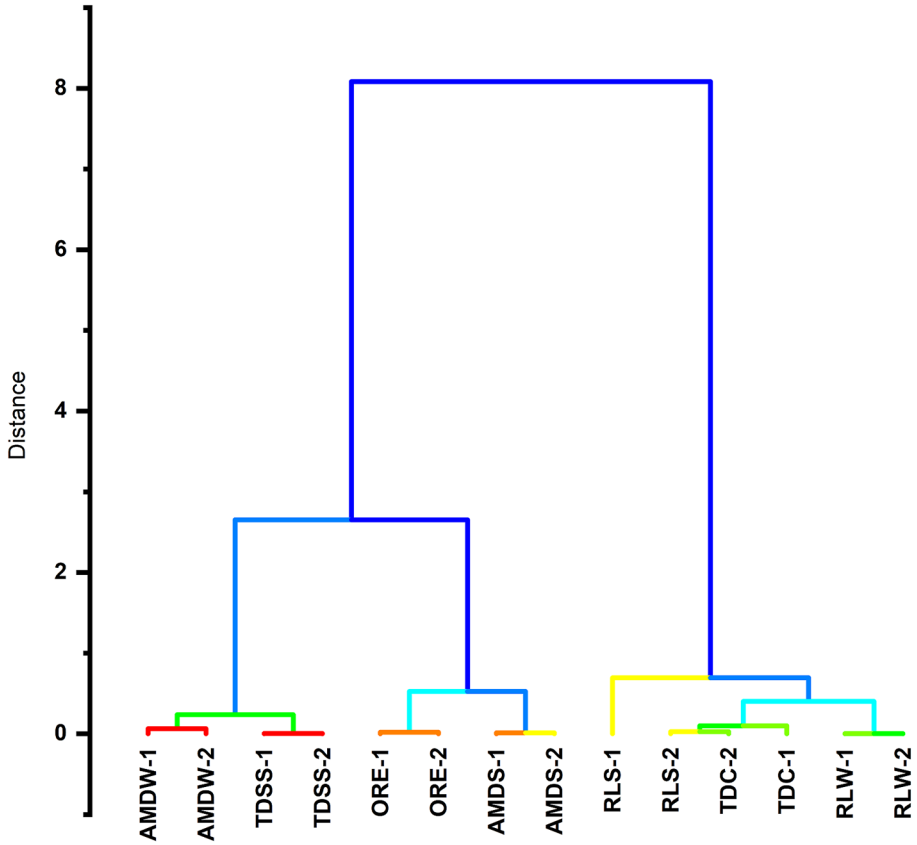


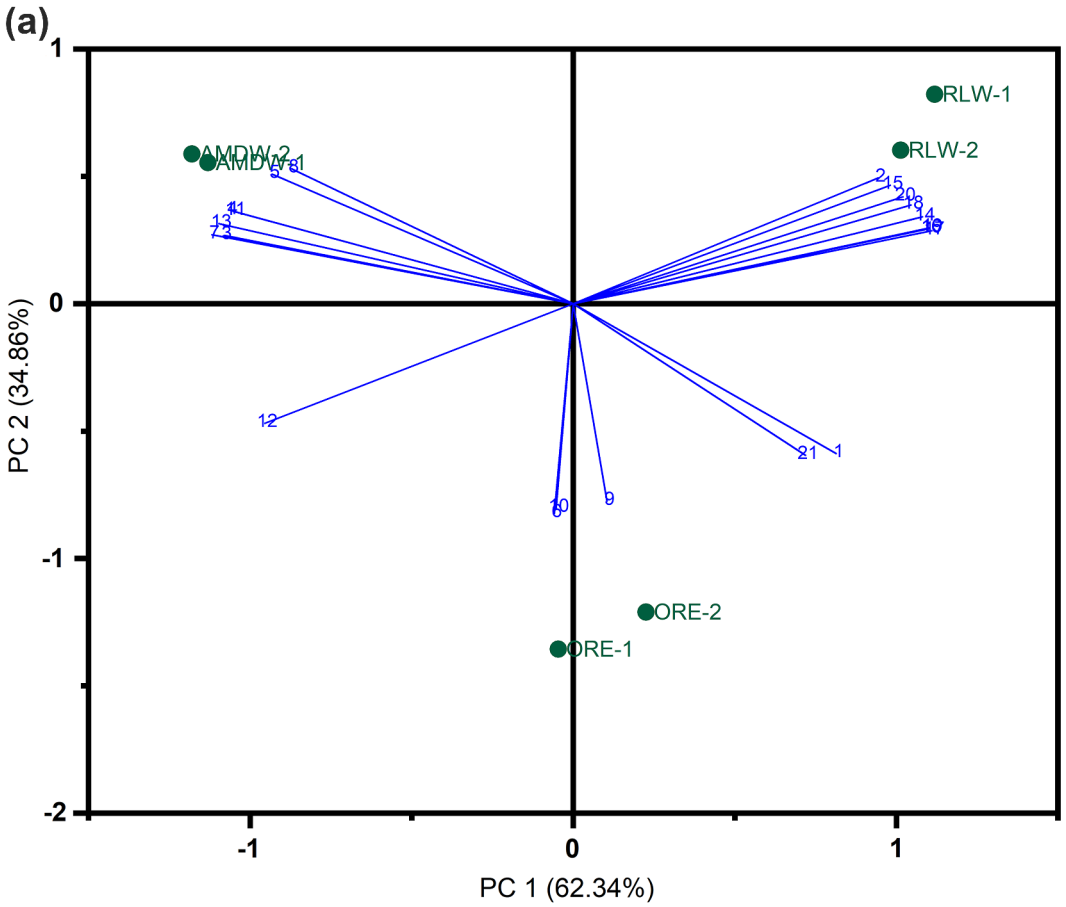
Fig. 4 Dendrogram showing the clustering of microbial communities based on the AWCD and SAWCD of microbial communities from contaminated and non-contaminated environments

were reported for AMD water and sediments, recreational lake water and sediments as well tannery dumpsite control soils. On the contrary the oil refinery effluents, and tannery dumpsite soils recorded high values of H and E.

Discussion

In this study the metabolic potential of microbial communities inhabiting contaminated environments were evaluated comparatively with those from non-contaminated environments. The contaminated environments (AMD water, AMD sediments, oil refinery effluent, tannery dumpsite soils) receive substantial amounts of metals from mining (ongoing or abandoned) and industrial activities. Typically, the analysis of physicochemical parameters revealed significantly high concentrations of EC, TDS, and COD. This ultimately translated to high concentrations of ions including metals. These results on high metal concentrations are congruent to previous findings, of excessive metal concentrations in industrial wastewaters (Hallberg 2010; Khatoon and Malik 2019; Oyetibo et al. 2017). The available literature suggest that high levels of dissolved solids in any environment may be selective to microbial growth. For instance, previous works have attested to the fact that the metal accumulation in soils induces changes in the microbial community structure, diversity, and function (Markowicz et al. 2016a, b). These harsh environmental conditions in the contaminated sites may account for deterioration in the microbial activity and diversity of the heterotrophic microbial community which could be related to reduction in catabolic function (Keshri et al. 2015).

Microbial utilization of the 31 substrates in the BioLog EcoPlate™ occurred in all the water, soil and sediment samples tested, indicating potential for heterotrophic processes at varying levels. Obviously the rate and level of substrate utilization differed from site to site as shown by the significantly different AWCD, SAWCD and SR indices. The AWCD, SAWCD and SR values calculated for all the contaminated sites were significantly lower than those calculated for the non-contaminated sites. For most of these sites the AWCD values were below 0.75 which is accepted as an optimal response of a microbial community that can be seen in most wells and a point at which highly active microbial communities reach the asymptote of color development (Oest et al. 2018). The reduced or lowered catabolic activity in the contaminated sites can be explained by reduced numbers of heterotrophic bacteria, a changed community composition and or inhibited enzyme activity attributed to metal toxicity (Markowicz et al. 2016a, b). The presence of metals in these environments could also be a cause for reduced metabolic functions. Several authors have alluded


(a)
RLW-1
RLW-2
AMDW-2
AMDW-1
ORE-2
ORE-1
PC 2 (34.86%)
PC 1 (62.34%)

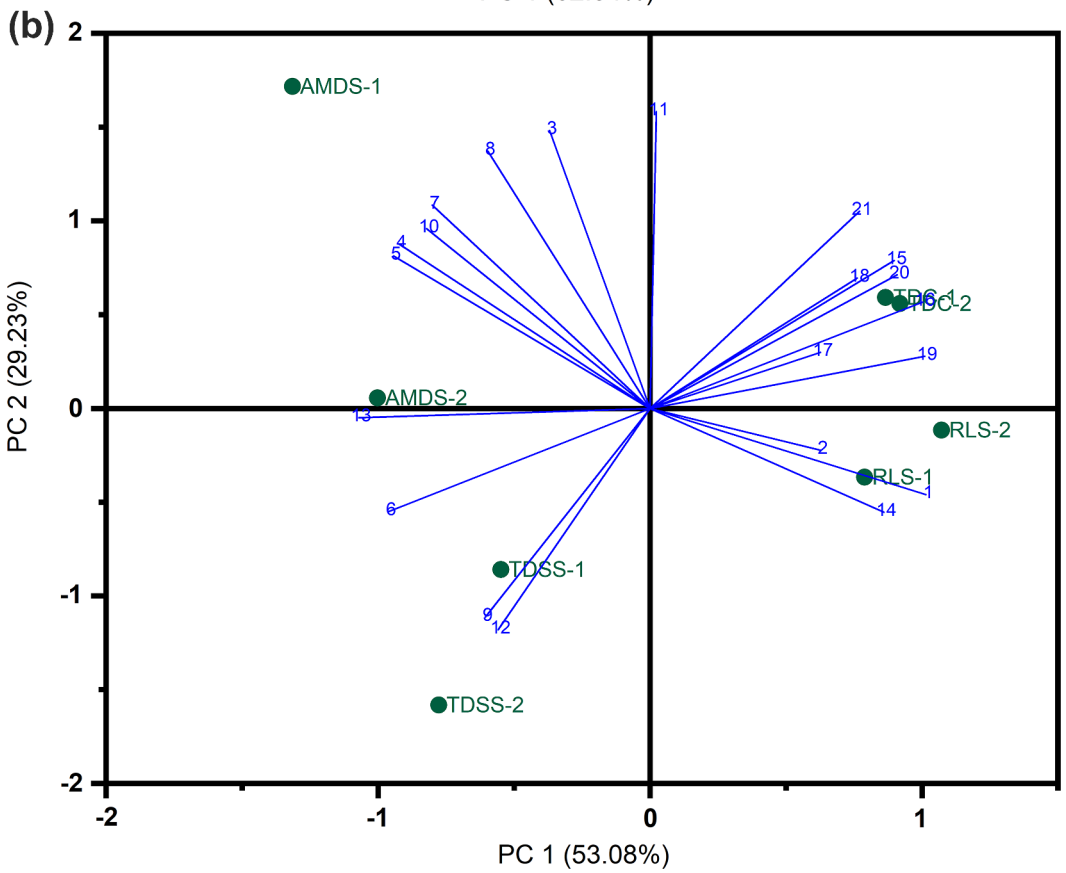

(b)
AMDS-1
AMDS-2
TDC-1
TDC-2
RLS-2
RLS-1
TDSS-1
TDSS-2
PC 2 (29.23%)
PC 1 (53.08%)

Fig. 5 PCA biplot showing correlations between metabolic potential of bacterial communities from contaminated and non-contaminated environments in relation to the utilization of 31 carbon substrates and selected physicochemical parameters. 1 = pH, 2 = NO_3^-, 3 = SO_4^{-2}, 4 = Al, 5 = Ca, 6 = Cr, 7 = Fe, 8 = K, 9 = Li, 10 = Mg, 11 = Mn, 12 = Na, 13 = Zn, 14 = AWCD, 15 = SAWCD AA, 16 = SAWCD CA, 17 = SAWCD CAR, 18 = SAWCD PHE, 19 = SAWCD POL, 20 = SAWCD AM, 21 = SR

to the fact that metal contamination impacts negatively on microbial population size, biodiversity, and physiological activity. Metal contamination has been reported to shift microbial populations towards metal tolerant populations which are often gram negative (Quadros et al. 2016). Several other studies have reported a reduction in the metabolic potential/activity of microbial communities in metal contaminated soils (Fazekaš et al. 2019; Kenarova et al. 2014). The reported mechanism by which metals induce changes in microbial communities is through their toxic effects which reduces microbial viability of certain populations with specific functional attributes, inhibits microbially mediated metabolic activities as well as restrict the availability of carbon sources (Martínez-Toledo et al. 2021). The reduced metabolic activity in contaminated environments can also be explained as a function of the availability of resources in the ecosystem (Díaz Villanueva et al. 2018). The metabolic function of bacterial communities differs as per the pool of available organic substrates in the environment. This has been observed in different environments with different trophic states including streams, lakes, and rivers (Bastidas Navarro et al. 2014; Freixa and Romaní 2014; Sala et al. 2020). In this study microbial communities from contaminated sites recorded lower nitrate concentrations which may be depictive of limited essential resources for bacterial growth. Ultimately, the limited availability of essential resources may be restrictive to the catabolic activity.

Another discrepancy observed between the metabolic potential of contaminated and non-contaminated sites is with the growth patterns of the different microbial communities. The growth on EcoPlate™ is a new stress for bacteria. Therefore, the time taken to recover, as deduced from the lag phase, differs according to the contrasting habitats and is indicative of the microbial community's resistance/tolerance to new stresses in the environment. In this study microbial communities from contaminated sites had shorter lag phases. This could imply that they easily adapt to new stresses attributable to their exposure to different stresses in their respective environments. Alternatively, this could be explained in terms of differences in the microbial community composition with the contaminated sites probably being dominated by fast growing R strategists and the non-contaminated sites being dominated by slow growing K-strategists (Oest et al. 2018).

Table 3 Indices of diversity for microbial communities in contaminated and non-contaminated environments

Sample	Index		
	SR	H	E
AMDW-1	8 ± 1.53	3.40 ± 0.08	1.70 ± 0.13
AMDW-2	9 ± 6.56	2.20 ± 7.00	1.28 ± 0.35
ORE-1	16 ± 6.56	70.14 ± 6.56	23.92 ± 4.22
ORE-2	28 ± 2.08	97.73 ± 3.81	28.62 ± 0.50
RLW-1	31 ± 0.00	4.47 ± 0.01	1.30 ± 0.00
RLW-2	29 ± 1.73	3.31 ± 0.07	0.98 ± 0.00
AMDS-1	26 ± 4.04	4.30 ± 0.02	1.31 ± 0.13
AMDS-2	10 ± 10.79	2.39 ± 0.55	1.76 ± 1.11
RLS-1	31 ± 0.00	4.49 ± 0.03	1.31 ± 0.25
RLS-2	30 ± 0.58	3.36 ± 0.02	0.99 ± 0.01
TDSS-1	15 ± 5.57	22.27 ± 0.86	9.80 ± 1.38
TDSS-2	9 ± 3.51	18.24 ± 1.24	10.91 ± 2.35
TDC-1	28 ± 1.53	5.59 ± 1.48	0.96 ± 1.07
TDC-2	25 ± 2.31	3.18 ± 0.20	0.99 ± 0.05

A closer look at the CLPP patterns indicates limited variability in terms of the number of individual carbon substrates utilized by microbial communities in contaminated sites compared to the non-contaminated sites. The types of substrates used, and the utilization levels exhibited for substrates are indicative of either the presence or absence of specific catabolic potentials within a microbial community (Stefanowicz 2006). Individual carbon substrates with a calculated substrate utility of 3–4% relative absorbance are perceived to be supportive of bacterial activity (Oest et al. 2018). Substrates that produce consistently low % substrate utility are assumed to be poorly degraded. Other studies comparing the metabolic potential of bacterial communities along contamination gradients have identified shifts in metabolic function with those communities from contaminated sites displaying an ability to metabolize the more complex substrates like polymers (Kuźniar et al. 2018; Oest et al. 2018). However, in this study there were no clear patterns separating the microbial communities from contaminated and non-contaminated sites.

Conclusions

The CLPP profiles of microbial communities in contaminated sites (AMD water, AMD sediments, tannery dumpsite soil, oil refinery effluent) indicate a reduction in their catabolic potentials which could be attributed to the presence of high concentrations of metals and an unavailability of adequate resources for microbial growth. Indices of diversity and evenness did not show any trend of reduction in either the microbial diversity or the evenness in both the contaminated and non-contaminated sites. The study provides insights into the versatile nature of physiological responses

by indigenous microbial communities in contaminated environments, in response to the limited availability of essential nutrients and stresses induced by the presence of contaminants in the environment. However, the BioLog EcoPlate™ data is only indicative only of the potential metabolic capability of the samples under controlled environments which may not reflect the actual in situ processes in the natural environment. Therefore, these should be treated simply as a guide to the potential physiological processes in the natural environment.

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Data availability The datasets generated during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Authors declare no conflict of interest with regards to the experimental design, authorship, and publication of this work.

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