Iran. J. Biotechnol. April 2021;19(2): e2955 DOI: 10.30498/IJB.2021.2955

Research Article

A Comparative Taxonomic Profile of Microbial Polyethylene and Hydrocarbon-Degrading Communities in Diverse Environments

Faeze Hesami Zokaei[1], Sara Gharavi[1]*, Ezat Asgarani[1], Mahboobeh Zarrabi[1], Mohammad Reza Soudi[2]

[1] Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2] Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran

**Corresponding author:* Sara Gharavi, Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, Tel/Fax: +98-2188035187, E-mail: sgharavi@alzahra.ac.ir

Background: Polyethylene (PE) is one of the most abundant plastic wastes which accumulates in marine and terrestrial environments. As microbial degradation has been a promising approach for the bioremediation of polluted environments, identification of the microbial community profile where these pollutants accumulate, has recently been in focus.

Objective: We have investigated the taxonomic and functional characteristics of polyethylene- degrading microorganisms in a plastic waste recycling site in Tehran, Iran.

Materials and Methods: We have analyzed and compared a 16S rRNA dataset from this study with 15 datasets from 4 diverse plastic and oil polluted habitats to identify and evaluate bacterial communities involved in bioremediation.

Results: Our findings reveal that *Proteobacteria, Actinobacteria, Acidobacteria and Cloroflexi* were the dominant phyla and *Actinobacteria, Alphaproteobacteria, Gammaproteobacteria and Acidimicrobia* were dominant classes in these samples. The most dominant Kegg Orthology associated with PE bioremediation in these samples are related to peroxidases, alcohol dehydrogenases, monooxygenases and dioxygenases.

Conclusions: Long-term presence of contaminants in soil could lead to changes in bacterial phyla abundance, resulting in metabolic adaptations to optimize biological activity and waste management in a diverse group of bacteria.

Keywords: 16S rRNA, Bioremediation, Bioinformatic analysis, Microbial community, Plastic wastes, Polyethylene

1.Background

One of the challenges of our planet in recent years has been the accumulation of plastic debris in terrestrial and marine ecosystems through anthropogenic activities leading to extensive contamination of the environment (1). Plastic is a broad name for different types of synthetic polymer with high molecular weight and is generally derived from various petrochemical and hydrocarbon sources (2, 3).

Since polyethylene (PE) consists of long chains of hydrocarbon with very balanced charge and various degrees of branching and also lacks chemical groups (4), it has become a stable polymer with widespread uses in different industries and agriculture (5). The most consumed synthetic polymer is PE with a current global production of 140 million tons per annum in 2001 (6). Global demand for polyethylene resins was 99.6 million metric tons in 2018 (www.freedoniagroup.com). Since 2015, approximately 8.3 billion metric tons of plastic have been produced out of which 6.3 billion metric tons have become waste. Of this waste 12% was incinerated, 79% was stored in landfills and only 9% has been recycled (7).

Microorganisms have important and key roles in their various ecosystems (8) and are involved in the degradation of natural and man-made polymers (9). Standard culturing techniques can be used to isolate and identify only less than 1% of microorganisms in many environments. Therefore, culture-independent techniques are now a better tool in the detection of millions of unknown species in different environments with applications in biotechnology (10) using molecular ecological methods to overcome this limitation (11).

Marker gene amplification metagenomics using PCR amplification and sequencing marker genes such as the 16S rRNA genes, provide extensive information

about community and taxonomic distribution profile of microorganisms and their probable functions (12-14).

2. Objectives

In this study, taxonomic characteristics of polyethylene-degrading microorganisms in a plastic waste recycling site in Iran was investigated. A 16S rRNA dataset from this study as well as 3 contaminated petroleum sites and 12 samples of PE contaminated soils from SRA databases for comparison were used.

3. Materials and Methods

3.1. Sample Collection and Enrichment

Soil samples were collected by random sampling method (15) from plastic waste recycling site at a Tehran city suburb in Iran (35°30'22.1"N 51°22'51.3"E) (16) with a semi-arid climate. The samples were transferred to the laboratory on ice and processed within 24 hours. The enrichment procedure was carried out to assist in the survival of microorganisms capable of consuming and degrading PE as the sole carbon source. Therefore, 10 g of each soil samples was placed in a flask containing 20 mL Bushnell Hass (BH) broth medium (per liter of distilled water): 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g NH_4NO_3, 0.05 g $FeCl_3$ with a final pH of 7. Four flasks for each sample contained, as the sole carbon source, 1% LDPE powder (Low Density Polyethylene), 1% LDPE powder and 0.1% (yeast extract + glucose), 1% hexadecane (as model substrate) (17, 18) or 1% hexadecane and 0.1% (yeast extract + glucose). The flasks were incubated in a shaker at 37ºC, with an rpm of 120 for 30 days and subsequently mixed for metagenomic DNA extraction.

Fifteen 16S rRNA datasets publicly available in SRA (Sequence Read Archive) from 3 different environments were used as control and for comparison to the sample from this study. Six samples (ISP; Italy Soil PE) were collected at dump site in Fiorenzuola d'Arda in Northern Italy with temperate climate and average temperature of 12.8ºC and also mean annual rainfall of 774 mm (19) . Six samples (CSP; China Soil PE) were collected from cotton field in Shihenzi city with a desert climate and an average temperature of 7.8ºC and also mean annual rainfall of 225 mm (20). Three samples (PSO; Poland Soil Oil well) were collected at an oil well site in Weglowka near Krosno Poland with cold and temperate climate with the average temperature of 6.9 °C and mean annual rainfall of 816 mm (21). Our sample (TSP; Tehran Soil PE) was collected in a waste recycling workshop at Kahrizak suburb in Tehran with cold-desert climate and average temperature of 16.9 ºC and 156 mm average annual rainfall (https://en.climate-data.org/).

3.2. Total DNA Extraction and Illumina Sequencing

In order to extract total metagenomic DNA from soil samples, the following extraction protocol was used: 4 flasks of each sample were mixed following which 1 g sludge was added to 200 µL extraction buffer (NaCl 1.5 M, Tris-HCl 100 mM, EDTA 100 mM, pH=8), 30 µL NaH_2PO_4 (0.12 M, pH=7.4) (22), 0.1 g sterile glass powder (23), 30 µL SDS 25% and 20 µL Tween 20. The sample was vortexed for 5 min and incubated at 65ºC for 15 min (with invert mixing after every 1-2 min). The tubes were centrifuged at 15000 x g for 10 min at 4ºC to collect the supernatants which were then mixed with 10 $mg.mL^{-1}$ lysozyme, 20 $mg.mL^{-1}$ proteinase K followed by incubation at 37ºC for 1 h and inverting the tubes every 10 min. In the next step, equal volume of chloroform: isoamylalcohol (24:1 v/v) was added to every tube and centrifuged at 12000 x g for 15 min at 4ºC to collect the upper phase. DNA was precipitated by adding ice-cold absolute ethanol and centrifugation at 2000 g for 20 min at 4ºC. Supernatant was discarded and the pellet was washed with 70% ethanol and centrifuged at 2000 g for 20 min at 4ºC. Finally, the pellet was air-dried and dissolved in 50 µl TE buffer. Concentration and quality of the extracted DNA were determined using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) and 1% agarose gel electrophoresis, respectively.

The V3-V4 variable regions of 16S rRNA genes were amplified and sequenced by Illumina Miseq Paired-end Technology (Macrogen, Korea). The raw sequence reads were deposited in to the NCBI sequencing read archive (SRA) under Accession No PRJNA597282.

3.3. Data Analysis

Raw sequences were analyzed using QIIME2-2019.7 (24). The sequence reads were demultiplexed and denoised with DADA2 (25) based on quality scores and filter <Q10 and truncated reads. After removing chimeric sequences, paired end reads of each sample were merged. Alpha and beta diversity analysis were carried out through the q2-diversity. PCoA Plot based on weighted and unweighted unifrac was used to compare the similarity between the samples for beta diversity. Sequences were clustered with 99% similarity cutoff to Operational Taxonomic Unit (OTU). The GREENGENES reference database (13_8) was used for classification and taxonomic assignment (26).

3.4. Functional Metagenomic Prediction Using 16S rRNA Datasets

Prediction of functional profile was carried out using PICRUSt (27). After OTU data generation for all 16S rRNA data based on the GREENGENES reference database, biome file format was prepared as input for PICRUSt2 software.

Full pipeline for EC number, KEGG Orthology (KO) and MetaCyc pathway abundance prediction was carried out using *picrust2_pipeline.py* script. The Nearest Sequenced Taxon Index (NSTI) was assumed >2 by default. Phylogenetic placement of reads was done by HMMER, EPA-NG (28) and Gappa (14) algorithms, KO prediction by Castor (29) algorithm and relative pathway abundance by MinPath (30) implemented in HUMAnN2 (31).

4. Result

4.1 Bacterial Community Structure

For a comprehensive study of the microbial community that is capable of degradation and PE consumption, enriched samples collected from Tehran were compared with 12 samples of PE contamination at a cotton farm in China, landfill in Italy and 3 samples of oil well soil in Poland that were previously published and deposited in NCBI, SRA (**Table 1**).

Data analysis were performed using Qiime2 software, following which 4202 OTU were obtained consisting of 36 phyla, 90 classes, 140 orders, 184 families, 200 genera and 50 species. Alpha diversity in rarefaction curves based on observed OTU showed acceptable and nearly complete sampling for all of the samples (**Fig. 1**).

Bacterial community profile of samples is shown in the phylum and class level in bar plot (**Fig**. **2A and B**). Dominant phyla based on the mean relative abundance include *Proteobacteria* 27.5% (9.5-49.11%), *Actinobacteria* 27.24% (7.28-67.60%), *Chloroflexi* 9.039% (0.829-17.75%) and *Acidobacteria* 6% (0.511-23.091). The main classes in all samples are *Actinobacteria* 21.5% (2.7-66%), *Alphaproteobacteria* 19.45% (8.9-40.4%), *Gammaproteobacteria* 7.4% (0.06-27.66%) and *Acidobacteria* 3% (0.2-8.1%) mean percentage abundance.

In addition, *Micrococcaceae, Nocardiodaceae, Streptomycetacease* and *Mycobacteriaceae* in the *Actinobacteria* class and *Sphingomonadaceae*, *Rhodospirillaceae* and *Rhodobacteraceae* in the *Alphaproteobacteria* class were the prevailing families. The *Gammaproteibacteria* class was dominant with *Alcanivoracaceae, Pseudomonadaceae* and *Xanthomonadaceae* families in TSP and PSO samples. The bacterial community is mostly represented by members of the genera *Mycobacterium* sp.*, Streptomyces* sp.*, Rhodococcus* sp.*, Nocardia* sp.*, Microbispora* sp. and *Cellulomonas* sp. in the *Actinobacteria* class and *Kaistobacter* sp.*, Sphingomonas* sp.*, Skermanella* sp.*, Rubellimicrobiom* sp.*, Hyphomicrobium* sp.*, Devosia* sp. and *Agrobacterium* sp. in the *Alphaproteobacteria*

Table 1. Summary of 16S rDNA datasets used for analysis

SRA Accession Number	ID*	Treatment	Location	Duration of Exposure	Total Reads	High quality reads	Reference
SRX7433929	TSP	PE Enrichment	Iran	20 years<	97543	13807	This study
SRX4084527	PSO1	Oil Well	Poland		49336	10883	
SRX4084525	PSO2	Oil Well	Poland	30 years<	43910	8346	(20)
SRX4084523	PSO3	Oil Well	Poland		52014	11575	
SRX5439652	CSP1	Soil Around PE	China		67144	17678	
SRX5439651	CSP2	Soil Around PE	China		49933	13291	
SRX5439646	CSP3	Macro PE Enrichment	China	30 years<	40122	10171	(19)
SRX5439645	CSP4	Macro PE Enrichment	China		32687	6349	
SRX5439643	CSP5	Micro PE Enrichment	China		65432	18082	
SRX5439642	CSP6	Micro PE Enrichment	China		34382	4462	
SRX6798559	ISP1	PE Plastic	Italy		23678	5791	
SRX6798558	ISP2	PE plastic	Italy		28467	7391	
SRX6798553	ISP3	Control Soil	Italy	35 years<	17803	5846	(18)
SRX6798550	ISP4	Control Soil	Italy		13422	4423	
SRX6798549	ISP5	Dump Soil	Italy		14674	3842	
SRX6798548	ISP6	Dump Soil	Italy		14803	4099	

*The IDs used in the table are not taken from the relevant articles reference and are as follows: TSP (Tehran Soil PE sample), PSO (Poland Soil Oil well samples), CSP (China Soil PE samples), ISP (Italy Soil PE

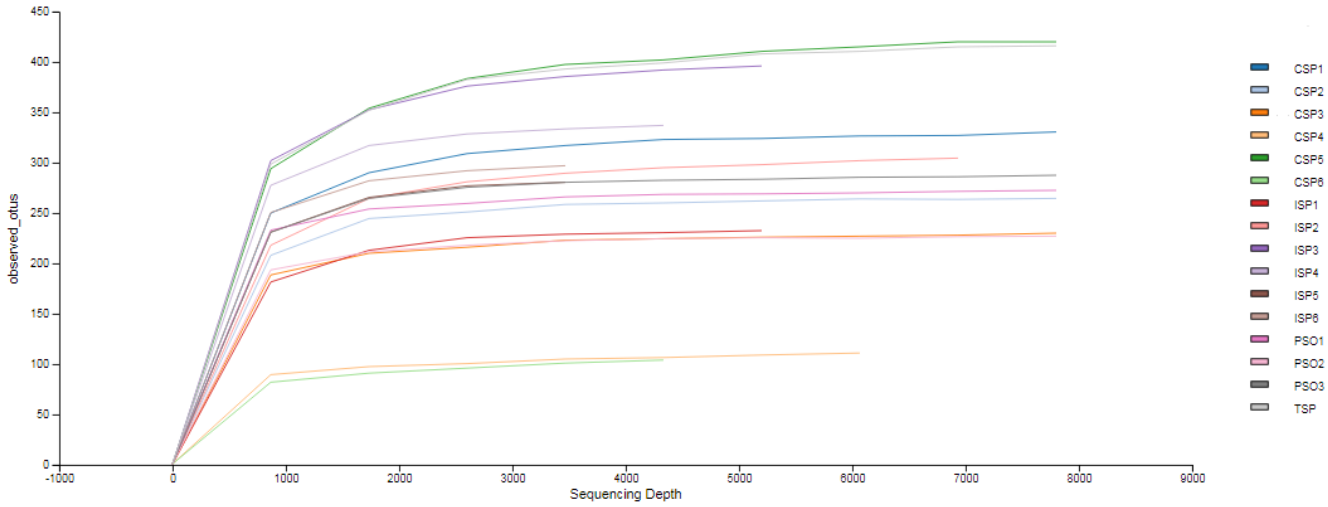

Figure 1. Rarefaction curve based on observed OTU for 16 samples show acceptable sampling with median frequency 7800 as maximum sampling depth

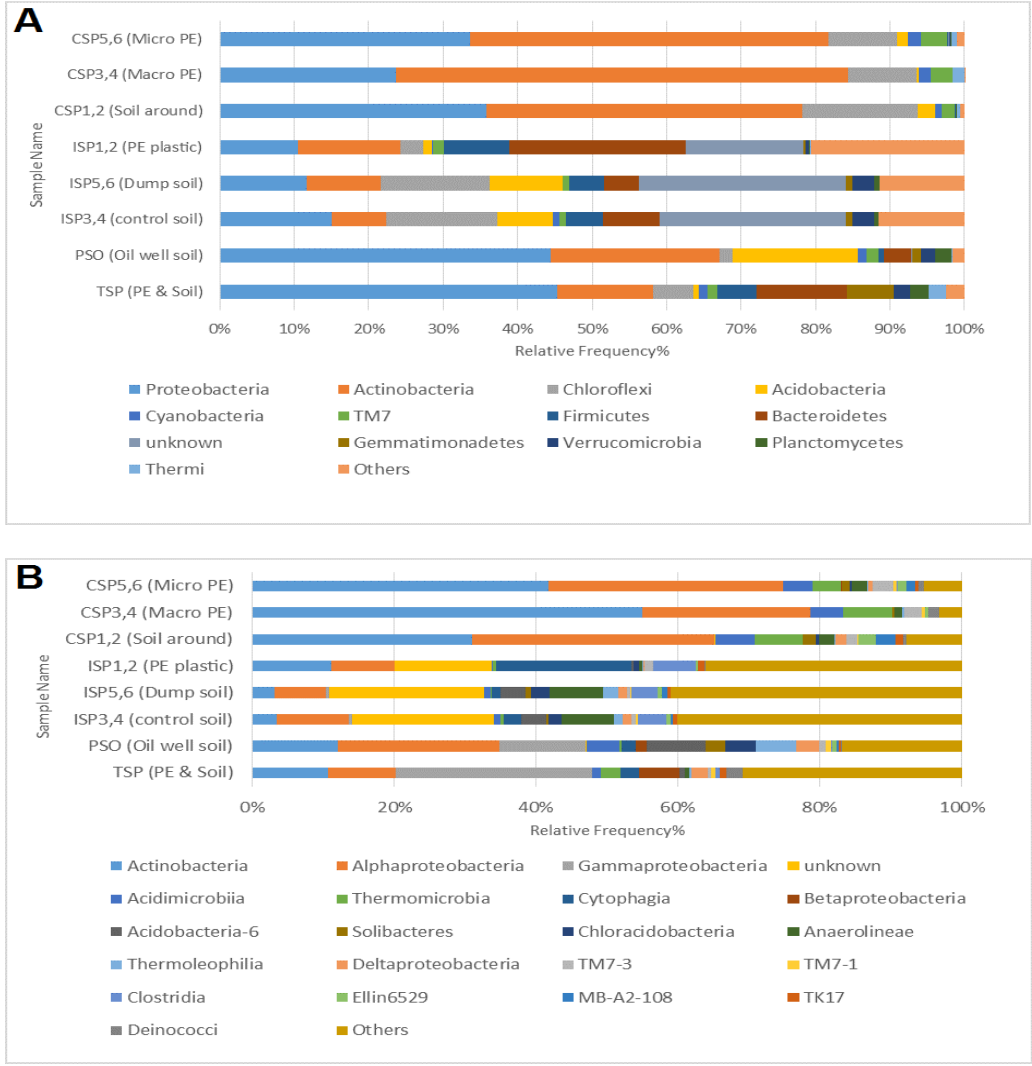

Figure 2. Taxonomic distribution of microbial communities **A** At the phylum level. **B** At the class level (the classification with less than 1% categorized in "others")

class, *Pseudomonas* sp., *Alcanivorax* sp., *Pseudidiomarina* sp., *Crenothrix* sp. and *Thermomonas* sp. in the *Gammaproteobacteria* class. Most of the mentioned genera are active in petroleum and n-alkane degradation.

Differences in abundance and diversity of taxonomies in various samples are dependent on many different factors. Bacterial profiles in various environment follow different patterns which may be related to environment temperature, latitude, nutrient availability, soil structure and the ecosystems (21, 32, 33). Beta diversity results are specified on heatmap for frequencies above 50 abundance (**Fig. 3A**) and three dimentional Principal Coordinates Analysis (PCoA) plot based on weighted and unweighted unifrac of community profiles based on different treatments in diverse samples (**Fig. 3B**).

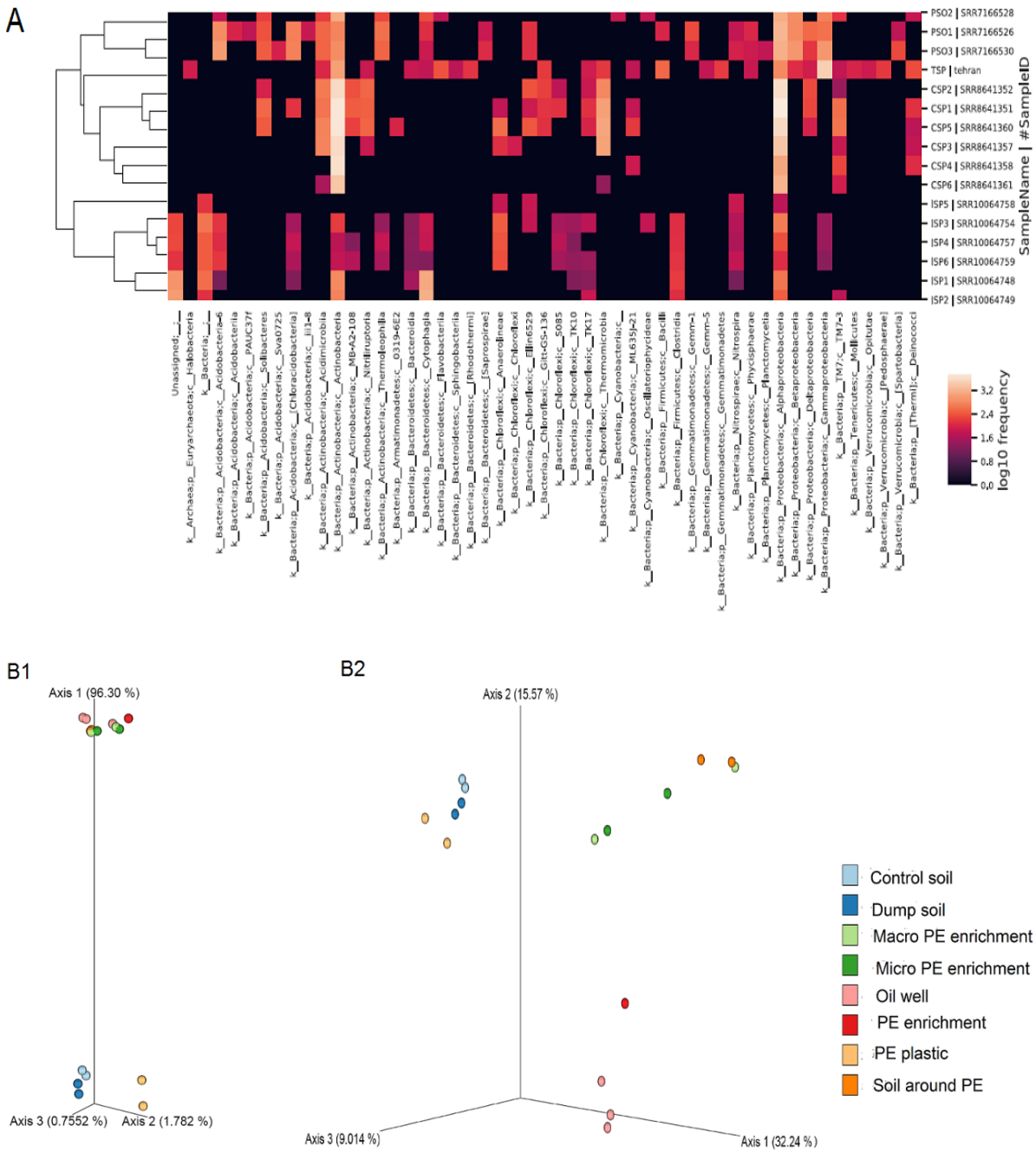

Figure 3. Beta diversity. **A** The heatmap of the relative abundance of bacteria with frequency above 50 in diverse samples (Sample name are in rows and taxonomies at class level are on columns). **B** PCoA plot of different treatment in diverse samples, **B1** weighted-unifrac and, **B2** unweighted-unifrac.

4.2. Prediction of Functional Profile

To evaluate the functional composition and potential of the microbial community, sequence data from 16S rRNA genes of all samples was used in PICRUSt software to predict metabolic structures. Predicted enzymes and proteins were classified by KEGG Orthology (KO) (34). 7610 KO and 428 metabolic pathways reconstructed by HUMAnN2, were implemented in PICRUSt. 428 pathways were identified in MetaCyc analysis across all samples, among which 48 pathways are involved in degradation of aromatic, aliphatic and xenobiotic compounds.

These 428 pathways were categorized in 14 pathway classes: 1) cofactor, vitamin, electron carrier, prosthetic group biosynthesis, 2) secondary metabolite metabolism, 3) generation of precursor metabolite and energy, 4) amino acid metabolism (biosynthesis and degradation), 5) C1 compound utilization and assimilation, 6) carbohydrate metabolism, 7) nucleoside and nucleotide metabolism (biosynthesis and degradation), 8) fatty acid and lipid metabolism (biosynthesis and degradation), 9) amine and polyamine metabolism, 10) cell structure

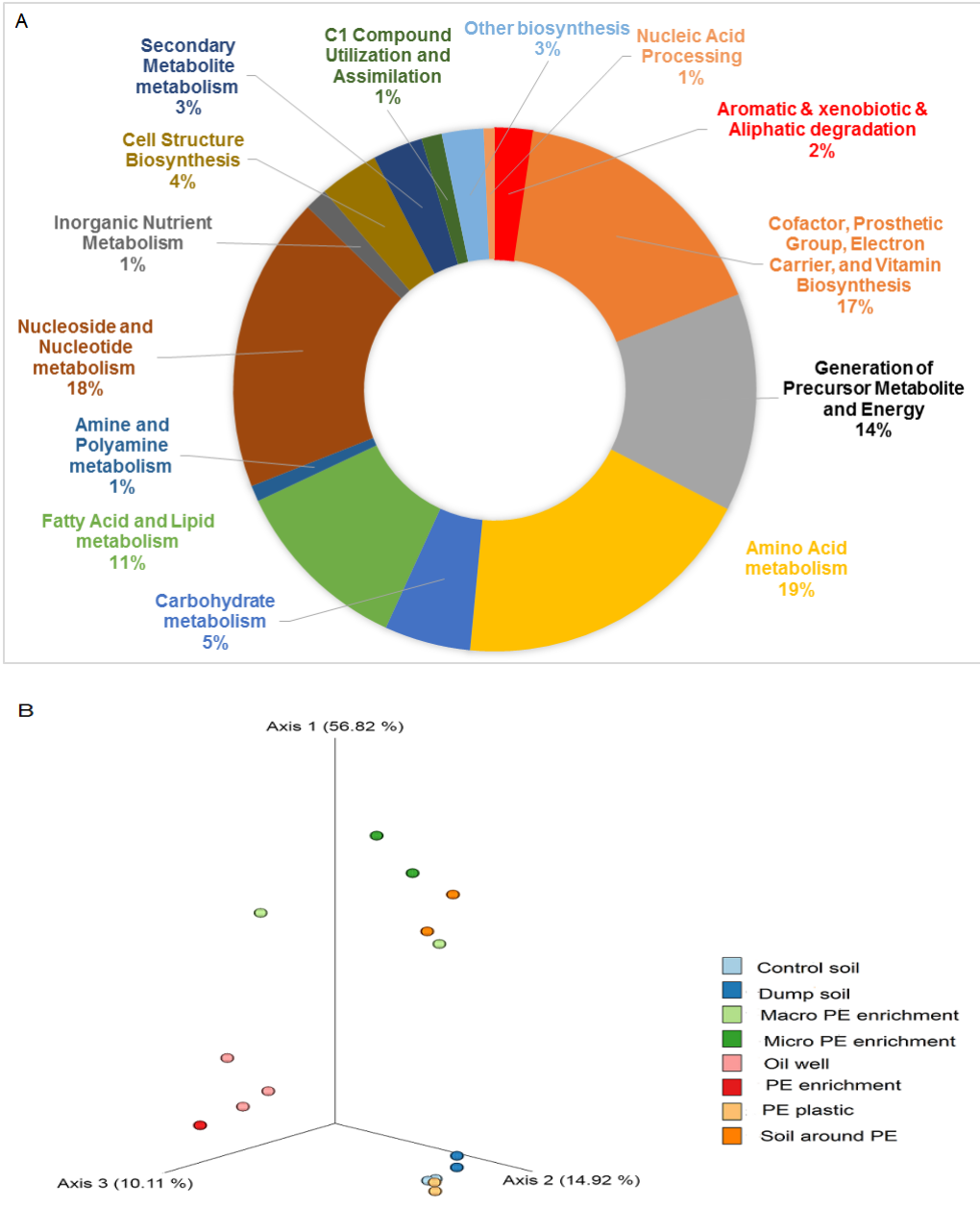

Figure 4. Predicted functional profile. **A** Pie chart showing mean percentage of metabolic pathway classes of microbial communities. **B** Similarity between predicted function of different treatment sample based on Bray-Curtis dissimilarity distance.

biosynthesis, 11) inorganic nutrient metabolism and 12) aromatic, aliphatic and xenobiotic degradation, 13) nucleic acid processing, 14) other biosynthesis. Most pathways are involved in vital activities of bacteria and a low percentage of predicted pathways are involved in bioremediation if necessary (**Fig. 4A**).

Predicted metabolic profiles of samples show most pathways and gene families are involved in vital cell activities (33) which is not surprising since microorganisms try to adapt to the fluctuations and stresses in the environment. Therefore, they must tune their metabolic pathways to allow for the consumption and degradation of these pollutants to survive in these environments. Bray-Curtis dissimilarity distance based on predicted KOs and MetaCyc pathway between different samples (treatments) are shown in PCoA plot (**Fig. 4B**).

In this study, relative abundance percentage of mentioned predicted KEGG Orthology in various samples were calculated (**Fig. 5**).

5. Discussion

Microbial degradation of xenobiotic waste from the environment is a promising approach in bioremediation. However, identification and characterization of the microbiota profile in the polluted environment is a priority and a necessity (33). As the bioinformatic analysis of the microbial community profiles from different soil samples and PE biofilm specimen elucidate, *Proteobacteria, Actinobacteria, Chloroflexi* and *Acidobacteria* are the dominant phyla in the compared samples; these most abundant bacteria have also been reported in studies on long term diesel contaminated soil (35, 36).

Proteobacteria was the most dominant phylum in control (ISP 3-4) and enriched soil samples (TSP, CSP 1-2, PSOs) but in PE biofilm specimens, *Actinobacteria* were predominant. According to research of Janssen and coworkers (2006), as expected, the most abundant phylum in soil are *Proteobacteria* (37). Militon and colleagues (2010) showed that before and after a 12 month period soil treatment with hydrocarbon, the most abundant phylum in soil were still *Proteobacteria*, but after a 24 month period, *Actinobacteria* became dominant (38). These results confirm that with changes in available nutrients over time, soil bacterial community structure changes and *Proteobacteria* is typically the dominant phylum in soil. Moreover, it seems that *Actinobacteria* play a key role in biofilm formation and bioremediation of xenobiotic compounds.

In addition to the mentioned dominant taxa, bacterial communities are diverse in all samples and according to Bell and coworkers (2005), in order to complete metabolic cycles, diverse communities prepare and perform better in ecosystems (39). A study by Delgado-Baquerizo and colleagues has, for the first time, identified the most abundant bacteria with a collection of soil samples from around the world and suggested these dominant taxa account only for 2% of soil diversity and this small portion of the total diversity forms a major fraction of the soil sample sequences worldwide (40). Therefore, some taxa are present and abundant in soil samples ; however, a large fraction of diverse taxa present in soil are discounted for even though they may play pivotal and important roles in environment (41).

PE is an n-alkane polymer with petroleum origin and

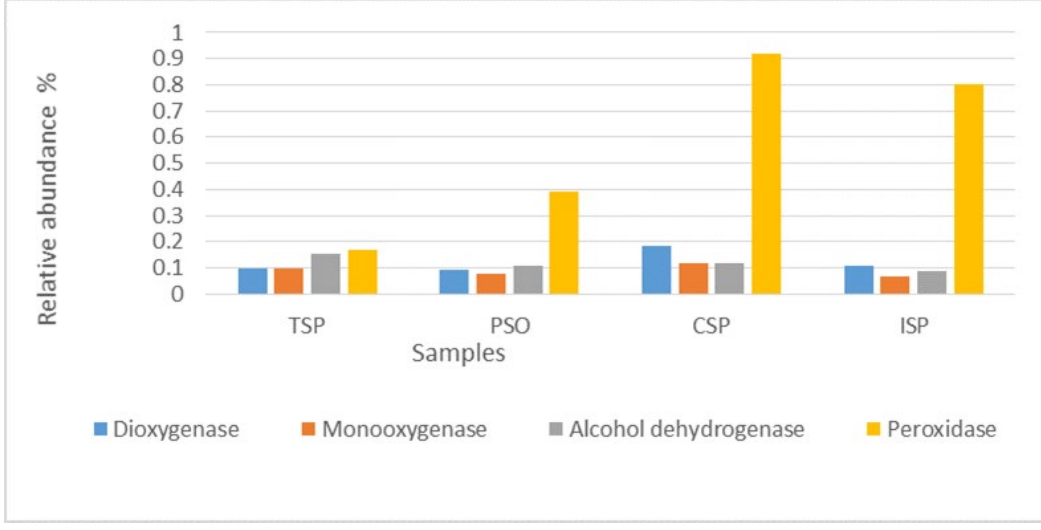

Figure 5. Relative aboundance percentage of notable KO in alkane and aliphatic degradation in various samples.

consists of high molecular weight hydrocarbon with C-C and C-H bonds (4). Many microorganisms can degrade alkanes as energy and carbon source. The most important enzymes involved in the degradation of environment pollutants, encoded by major gene families, are methane monooxygenases, soluble cytochrome P450s, non-heme iron monooxygenases (alkane hydroxylase, alkB), alcohol and aldehyde dehydrogenases and dioxygenases (42, 43). Ligninolytic enzymes such manganese peroxidase and laccase can cause oxidation and molecular weight reduction of PE (4, 44-48). All of mentioned enzymes belong to the family of oxidoreductases; these enzyme families, with different electron acceptors, have notable potential for degradation of the xenobiotic compounds (49). The most abundant KO relative with PE degradation in all samples belong to peroxidases, alcohol dehydrogenases, dioxygenases and monooxygenases.

6. Conclusion

The accumulation of plastic pollutants in terrestrial and marine ecosystems is a major environmental problem. Many microorganisms with ability to degrade and eliminate these contaminants have been identified by available culture-dependent methods. Metagenomic approaches provide extensive information on the microorganism populations, their relationship with the environment and can consequently, identify their roles in the process of polyethylene degradation. In this study, we re-identified some of active bacterial genera in n-alkanes bioremediation such as *Mycobacterium* sp., *Rhodococcus* sp., *Streptomyces* sp., *Nocardia* sp., *Sphingomonas* sp., *Agrobacterium* sp., *Alcanivorax* sp. and *Pseudomonas* sp. The most dominant KO in these samples are related to peroxidase, alcohol dehydrogenase and dioxygenase pathways that exist in the mentioned phyla. A deeper understanding of the complex interactions between different bacterial communities and environmental milieu can expedite and improve extant bioremediation processes.

Acknowledgments

The support of VC Research, Alzahra University is hereby acknowledged. This study was partially financed by grant No: 97/6239 from the Biotechnology Development Council of the Islamic Republic of Iran.

Reference

1. Barnes DK, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. *Philos Trans R Soc Lond B Biol Sci*. 2009;**364**(1526):1985-1998. doi: 10.1098/rstb.2008.0205
2. Ahmed T, Shahid M, Azeem F, Rasul I, Shah AA, Noman M, *et al*. Biodegradation of plastics: current scenario and future prospects for environmental safety. *Environ Sci Pollut Res Int*. 2018;**25**(8):7287-7298. doi: 10.1007/s11356-018-1234-9
3. Ghosh SK, Pal S, Ray S. Study of microbes having potentiality for biodegradation of plastics. *Environ Sci Pollut Res Int.* 2013;**20**(7):4339-4355. doi: 10.1007/s11356-013-1706-x
4. Krueger MC, Harms H, Schlosser D. Prospects for microbiological solutions to environmental pollution with plastics. *Appl Microbiol Biotechnol*. 2015;**99**(21):8857-8874. doi: 10.1007/s00253-015-6879-4
5. Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim CS, Sakharkar KR. Biodegradation of Low Density Polythene (LDPE) by Pseudomonas Species. *Indian J Microbiol*. 2012;**52**(3):411-419. doi: 10.1007/s12088-012-0250-6
6. Sivan A. New perspectives in plastic biodegradation. *Curr Opin Biotechnol*. 2011;**22**(3):422-426. doi: 10.1016/j.copbio.2011.01.013
7. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. *Sci Adv*. 2017;**3**(7):e1700782. doi: 10.1126/sciadv.1700782
8. Prakash T, Taylor TD. Functional assignment of metagenomic data: challenges and applications. *Brief Bioinform*. 2012;**13**(6):711-727. doi: 10.1093/bib/bbs033
9. Shah AA, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: a comprehensive review. *Biotechnol Adv*. 2008;**26**(3):246-265. doi: 10.1016/j.biotechadv.2007.12.005
10. Su C, Lei L, Duan Y, Zhang KQ, Yang J. Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. *Appl Microbiol Biotechnol*. 2012;**93**(3):993-1003. doi: 10.1007/s00253-011-3800-7
11. Streit WR, Schmitz RA. Metagenomics--the key to the uncultured microbes. *Curr Opin Microbiol.* 2004;**7**(5):492-498. doi: 10.1016/j.mib.2004.08.002
12. Pavloudi C, Kristoffersen JB, Oulas A, De Troch M, Arvanitidis C. Sediment microbial taxonomic and functional diversity in a natural salinity gradient challenge Remane's "species minimum" concept. *PeerJ*. 2017;**5**:e3687. doi: 10.7717/peerj.3687
13. Tringe SG, Hugenholtz P. A renaissance for the pioneering 16S rRNA gene. *Curr Opin Microbiol.* 2008;**11**(5):442-446. doi: 10.1016/j.mib.2008.09.011
14. Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G, *et al*. Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. *Bioinform Biol Insights*. 2015;**9**:75-88. doi: 10.4137/bbi.s12462
15. Carter MR, Gregorich EG. Soil sampling and methods of analysis: *CRC press*; 2007. doi: 10.1201/97814200052
16. Soleimani Z, Gharavi S, Soudi M, Moosavi-Nejad Z. A survey of intact low-density polyethylene film biodegradation by terrestrial Actinobacterial species. *Int Microbiol*. 2021;**24**(1):65-73**.**
17. Gyung Yoon M, Jeong Jeon H, Nam Kim M. Biodegradation of Polyethylene by a Soil Bacterium and AlkB Cloned Recombinant Cell *J Bioremediation Biodegrad*. 2012;**03**(04). doi: 10.4172/2155-6199.1000145
18. Harshvardhan K, Jha B. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, *India. Mar Pollut Bull*. 2013;**77**(1-2):100-106. doi: 10.1016/j.marpolbul.2013.10.025
19. Puglisi E, Romaniello F, Galletti S, Boccaleri E, Frache A, Cocconcelli PS. Selective bacterial colonization processes

on polyethylene waste samples in an abandoned landfill site. *Scientific reports.* 2019;**9**(1):1-13. doi: 10.1038/s41598-019-50740-w

20. Zhang M, Zhao Y, Qin X, Jia W, Chai L, Huang M, *et al*. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. *Sci Total Environ*. 2019;**688**:470-478. doi: 10.1016/j.scitotenv.2019.06.108
21. Galazka A, Grzadziel J, Galazka R, Ukalska-Jaruga A, Strzelecka J, Smreczak B. Genetic and Functional Diversity of Bacterial Microbiome in Soils With Long Term Impacts of Petroleum Hydrocarbons. *Front Microbiol*. 2018;**9**:1923. doi: 10.3389/fmicb.2018.01923
22. Fatima F, Pathak N, Rastogi Verma S. An Improved Method for Soil DNA Extraction to Study the Microbial Assortment within Rhizospheric *Region. Mol Biol Int*. 2014;**2014**:518960. doi: 10.1155/2014/518960
23. Devi SG, Fathima AA, Radha S, Arunraj R, Curtis WR, Ramya M. A Rapid and Economical Method for Efficient DNA Extraction from Diverse Soils Suitable for Metagenomic Applications. *PLoS One*. 2015;**10**(7):e0132441. doi: 10.1371/journal.pone.0132441
24. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019;**37**(8):852-857. doi: 10.1038/s41587-019-0209-9
25. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;**13**(7):581-583. doi: 10.1101/024034
26. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, *et al*. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol*. 2006;**72**(7):5069-5072. doi: 10.1128/aem.03006-05
27. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, *et al*. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol.* 2013;**31**(9):814-281. doi: 10.1038/nbt.2676
28. Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, *et al*. EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. *Syst Biol*. 2019;**68**(2):365-369. doi: 10.1101/291658
29. Louca S, Doebeli M. Efficient comparative phylogenetics on large trees. *Bioinformatics*. 2018;**34**(6):1053-1055. doi: 10.1093/bioinformatics/btx701
30. Ye Y, Doak TG. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. *PLoS Comput Biol*. 2009;**5**(8):e1000465. doi: 10.1371/journal.pcbi.1000465
31. Franzosa EA, McIver LJ, Rahnavard G, Thompson LR, Schirmer M, Weingart G, *et al*. Species-level functional profiling of metagenomes and metatranscriptomes. *Nat Methods*. 2018;**15**(11):962-968. doi: 10.1038/s41592-018-0176-y
32. Delgado-Baquerizo M, Maestre FT, Reich PB, Trivedi P, Osanai Y, Liu YR, *et al.* Carbon content and climate variability drive global soil bacterial diversity patterns. *Ecol Monogr*. 2016;**86**(3):373-390. doi: 10.1002/ecm.1216
33. Mukherjee A, Chettri B, Langpoklakpam JS, Basak P, Prasad A, Mukherjee AK, *et al*. Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments. *Sci Rep*. 2017;**7**(1):1108. doi: 10.1038/s41598-017-01126-3
34. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;**28**(1):27-30. doi: 10.1038/s41598-017-01126-3
35. Sutton NB, Maphosa F, Morillo JA, Abu Al-Soud W, Langenhoff AA, Grotenhuis T, *et al*. Impact of long-term diesel contamination on soil microbial community structure. *Appl Environ Microbiol.* 2013;**79**(2):619-630. doi: 10.1128/aem.02747-12
36. Yu Y, Zhang Y, Zhao N, Guo J, Xu W, Ma M, *et al*. Remediation of Crude Oil-Polluted Soil by the Bacterial Rhizosphere Community of Suaeda salsa Revealed by 16S rRNA Genes. *Int J Environ Res Public Health*. 2020;**17**(5). doi: 10.3390/ijerph17051471
37. Janssen PH. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ. Microbiol*. 2006;**72**(3):1719-1728. doi: 10.1128/aem.72.3.1719-1728.2006
38. Militon C, Boucher D, Vachelard C, Perchet G, Barra V, Troquet J, *et al*. Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil. *FEMS Microbiol Ecol*. 2010;**74**(3):669-681. doi: 10.1111/j.1574-6941.2010.00982.x
39. Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK. The contribution of species richness and composition to bacterial services. *Nature*. 2005;**436**(7054):1157-1160. doi: 10.1038/nature03891
40. Delgado-Baquerizo M, Oliverio AM, Brewer TE, Benavent-González A, Eldridge DJ, Bardgett RD, *et al*. A global atlas of the dominant bacteria found in soil. *Science*. 2018;**359**(6373):320-325. doi: 10.1126/science.aap9516
41. Martiny JBH, Walters KE. Towards a Natural History of Soil Bacterial Communities. *Trends Microbiol.* 2018;**26**(4):250-252. doi: 10.1016/j.tim.2018.02.010
42. Garrido-Sanz D, Redondo-Nieto M, Guirado M, Pindado Jiménez O, Millán R, Martin M, *et al*. Metagenomic insights into the bacterial functions of a diesel-degrading consortium for the rhizoremediation of diesel-polluted soil. *Genes*. 2019;**10**(6):456. doi: 10.3390/genes10060456
43. Wang W, Shao Z. Enzymes and genes involved in aerobic alkane degradation. *Front Microbiol*. 2013;**4**:116. doi: 10.3389/fmicb.2013.00116

44 Iiyoshi Y, Tsutsumi Y, Nishida T. Polyethylene degradation by lignin-degrading fungi and manganese peroxidase. *J Wood Sci*. 1998;**44**(3):222-229. doi: 10.1007/bf00521967

45. Orr IG, Hadar Y, Sivan A. Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcus ruber. *Appl Microbiol Biotechnol*. 2004;**65**(1):97-104. doi: 10.1007/s00253-004-1584-8
46. Bhardwaj H, Gupta R, Tiwari A. Communities of microbial enzymes associated with biodegradation of plastics. *J Polym Environ.* 2013;**21**(2):575-579. doi: 10.1007/s10924-012-0456-z
47. Restrepo-Flórez J-M, Bassi A, Thompson MR. Microbial degradation and deterioration of polyethylene – A review. *Int Biodeterior Biodegrad*. 2014;**88**:83-90. doi: 10.1016/j.ibiod.2013.12.014
48. Zhurina M, Gannesen A, Martyanov S, Plakunov V. Express method for determining the relation between polyethylene biocorrosion by Chromobacterium violaceum biofilms and their ability to form extracellular matrix. *Microbiology*. 2020;**89**(1):44-49. doi: 10.1134/s0026261720010178
49. Khatoon N, Jamal A, Ali MI. Polymeric pollutant biodegradation through microbial oxidoreductase: A better strategy to safe environment. *Int J Biol Macromol*. 2017;**105**(Pt 1):9-16. doi: 10.1016/j.ijbiomac.2017.06.047