UNIVERSITY OF NOVI SAD
Technical faculty "Mihajlo Pupin"
Zrenjanin, Republic of Serbia

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Industrial Engineering and Environmental Protection

IIZS conference

PROCEEDINGS

XI International Conference – Industrial Engineering And Environmental Protection (IIZS 2021)

Zrenjanin, 7th-8th October 2021.

University of Novi Sad
Technical faculty “Mihajlo Pupin”
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XI International Conference - Industrial Engineering and Environmental Protection (IIZS 2021)

Proceedings

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INTRODUCTION

Departments of Mechanical engineering and Department of Environmental protection of Technical Faculty "Mihajlo Pupin", Zrenjanin, has organized the XI International Conference Industrial Engineering and Environmental Protection – IIZS 2021.

The topics of scientific conference «IIZS 2021», cover the fields of Industrial engineering and Environmental protection: Mechanical engineering, Energetics and process technique, Designing and maintenance, Oil and gas engineering, Health and environmental protection, Environmental management, Occupational safety and Engineering management.

The main goals of the conference are: innovation and expansion of knowledge engineers in industry and environmental protection; support to researchers in presenting the actual results of research projects, establishing new contacts with leading national and international institutions and universities; popularization of the faculty and its leading role in our society and the immediate environment, in order to attract quality young population for studying at our faculty, cooperation with other organizations, public companies and industry; initiative for collecting ideas in solving specific practical problems; interconnection and business contacts; introducing professional and business organizations with results of scientific and technical research; presentation of scientific knowledge and exchange of experiences in the field of industrial engineering.

We would like to express our gratitude to the partners of the conference – „Aurel Vlaicu" University of Arad, Faculty of engineering, Arad, Romania; University "St. Kliment Ohridski", Technical faculty, Bitola, Macedonia; University Politehnica Timisoara, Faculty of engineering, Hunedoara, Romania; University of East Sarajevo, Faculty of mechanical engineering East Sarajevo, B&H, Republic of Srpska; University of Giresun, Faculty of engineering, Giresun, Turkey for supporting the organization of the 11th International Conference «IIZS 2021». We are also grateful to all the authors who have contributed with their papers to the organization of the scientific meeting «IIZS 2021».

We would like to extend our special thanks to the Ministry of Education, Science and Technological Development, Republic of Serbia and the management of Technical Faculty "Mihajlo Pupin", University of Novi Sad, for supporting the organization of the Conference «IIZS 2021».

The IIZS Conference became a traditional meeting of researchers from all over the world, every year. We are open and thankful for all useful suggestions which could contribute that the next, XII International Conference - Industrial Engineering and Environmental Protection, become better in organizational and program sense.

Chairman of the Organizing Committee
Asst. prof. Snežana Filip

Zrenjanin, 7 - 8th October 2021.

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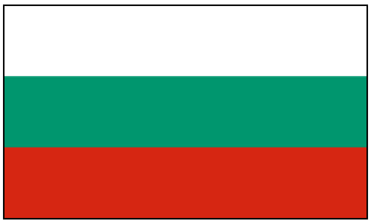

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CONTENTS

PLENARY SESSION

I - INDUSTRIAL ENGINEERING

Session 1: Mechanical Engineering

Session 3: Designing and Maintenance

Session 4: Oil and Gas Engineering

Session 5: Engineering Management

II – ENVIRONMENTAL ENGINEERING

Session 6: Health and Environmental protection

Session 7: Environmental Management

Session 8: Occupational Safety

IDENTIFICATION AND SIGNIFICANCE OF GRAM-NEGATIVE BACTERIA IN MUNICIPAL SOLID WASTE LANDFILL LEACHATE FROM VOJVODINA REGION

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Abstract: Landfill leachate is a medium affected by a considerable number of factors, both within the landfill body (landfill age, morphological composition of waste, temperature and moisture content, landfill fluid migration, waste treatment technologies before disposal, landfill body thickness, waste decomposition phases), and outside (meteorological parameters, with an emphasis on annual precipitation). Variable and almost unpredictable qualitative composition of leachate from municipal solid waste landfills, that often includes emerging substances, such as antibiotics and endocrine disruptors, as well as heavy metals, significantly affects the structure and diversity of the present microbiological communities. By implementing leachate sampling campaigns in the winter, spring and autumn in 2019 at three non-sanitary (L_1, L_2, L_3) and one sanitary (L_4) municipal solid waste landfills in the region of Vojvodina, the presence of microorganisms *Escherichia coli, Klebsiella pneumoniae* and *Citrobacter freundii*, Gram-negative bacteria from the family of *Enterobacteriaceae*, in the phylum *Proteobacteria*, were detected. Due to the frequent causing of urinary, extraintestinal, diarrheal and respiratory infections, meningitis, colitis, sepsis, as well as high resistance to a range of antibiotics, Gram-negative bacteria may represent a potential risk to human and animal health, under specific exposure circumstances. In addition to the use of Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae* and *Citrobacter freundii* as indicators of fecal contamination, the potential of the use of the mentioned bacteria as indicators of contamination of leachate with heavy metals and organic compounds, as well as the functionality and condition of municipal solid waste landfills.
Key words: municipal solid waste landfills, municipal solid waste landfill leachate, microbiological parameters, Gram-negative bacteria

INTRODUCTION

Municipal Solid Waste Landfill Leachate

Landfill leachate is a medium affected by a considerable number of factors, both within the landfill body (landfill age, morphological composition of waste, temperature and moisture content, landfill fluid migration, waste treatment technologies before disposal, landfill body thickness, waste decomposition phases), and outside (meteorological parameters, with an emphasis on annual precipitation). The process of landfill filtrate formation includes dissolution and dispersion of solid substances in water that is percolated through the landfill body and dissolved or suspended substances formed through biological and chemical processes taking place inside the landfill body [1].
The process of generating leachate is an integral part of the water movement cycle at a landfill. The beginning of the cycle is characterized by percolating of the received atmospheric precipitation through active layers of the deposited waste. In the process, organic and inorganic substituents, heavy metals and potentially hazardous substances present in the active layers of deposited waste are dissolved, and the process of combining percolated precipitation with moisture percolated from active layers of deposited waste is realized, thus forming an optimal medium for pollutant transport. A certain part of the formed filtrate flows away from the landfill, part returns to the atmosphere through evaporation from the upper surface of the landfill body or vegetation (transpiration), while the remaining amount of formed filtrate remains in the upper layer of the landfill, whereby moisture in waste increases and conditions for the continuous production of new volumes of leachate are ensured.
It is very difficult to predict the composition of the landfill leachate due to the complex dynamics of the processes taking place in the landfill body and the impact of a large number of factors. Qualitative, as well as quantitative composition of leachate can vary over time, and the load of pollutants reaches its peak several years after waste disposal, with a subsequent decline [2,3]. The qualitative

composition of leachate is characterized by pollutants that fall into four basic groups [4,5]: *soluble organic components* (volatile fatty acids, varieties of humic and fulvic acids), *inorganic macrocomponents* (ions of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ammonium (NH_4^{+}), iron (Fe^{2+}), manganese (Mn^{2+}), chloride (Cl^{-}), sulphate (SO_4^{2-})), *heavy metals* (ions of cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+})), *xenobiotic organic components* (hydrocarbons, phenols, chlorinated aliphatic compounds, pesticides, dioctyl phthalate).

Microbial Structures present in Landfill Leachates

The general division of living organisms is made into three domains - *bacteria, archaea and eukaryotes* [6,7]. The basic mechanisms and processes that take place within the municipal solid waste landfill are characterized by the presence and activity of representatives of all three previously mentioned categories.
Variations in the composition of microbiological communities due to the action of a substantial number of factors within the landfill body, such as landfill age, morphological composition of waste, temperature and moisture content, landfill fluid migration, waste treatment technologies before disposal, landfill body thickness, waste decomposition phases, are known from the literature and presented in Table 1.
Municipal solid waste landfills more than 10 years old are characterized by lower acetate concentration, lower microbiological activity, as well as a smaller number of cellulolytic bacteria [8,9]. Conditions at the mentioned landfills, namely low content of hydrocarbon (6.4 ± 1.6% of dry mass) and high pH values (7.9 ± 0.35) are in favor of the survival of gram-positive bacteria from the *Bacillaceae* family, especially the genus *Bacillus*. However, the mentioned conditions are not in favor of the survival of bacteria from *Proteobacteria* and *Methanosaeta* families. Different depths of sampling disposed waste indicate significant variations in the microbiological communities present. The deeper layers of the disposed waste are characterized by lower concentrations of acetate, due to which they represent the optimal environment for the development of *Methanoculleus*. Contrary to the above, higher layers of disposed waste are characterized by substantial concentrations of acetate that are in favor of the survival of *Methanosarcin*, resulting in a greater activity related to methane production [10]. High intake of organic matter, as well as the accumulation of acetate characterizes landfills with implemented leachate recirculation treatment. These conditions are in favor of *Methanosarcin*, but not of bacteria from *Proteobacteria* and *Methanosaeta* families [10]. Low temperatures in general lead to the formation of more diverse microbiological communities [10]. *Proteobacteria* dominate landfill sites contaminated primarily with hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and heavy metals [11].

Table 1. Complex connections of microbial structures with specific landfill features [12]

Feature		Note	Characteristic
SAMPLE AGE	Old refuse samples	Lower concentration of acetate; Lower microbial activity; Lower number of cellulolytic bacteria.	Domination of the family *Bacillaceae*.
			Domination of the genus *Bacillus*.
			Identification of extremophiles (alkaliphiles and halophiles).
			High abundance of *Bacillus*-like DNA sequences.
			Proteobacteria are favoured.
			Favorable conditions for *Methanosaeta*.
			Favorable conditions for hydrogenotrophic and formate-using methanogens.

	Young refuse samples	Higher concentration of acetate; Higher microbial activity; Higher number of cellulolytic bacteria.	Unfavorable conditions for gram- bacteria like *Proteobacteria*.
			Favorable conditions for *Methanosarcina*.
			Favorable conditions for acetolactic, hydrogenotrophic and formate-using methanogens.
	--	--	Significant structure changes of the methanogenic community happen shortly after waste burial.
DEPTH	Deeper waste layers	Lower concentration of acetate.	Favorable conditions for *Methanoculleus*.
	Upper waste layers	Higher concentration of acetate.	Favorable conditions for *Methanosarcina*.
			Higher methane production activity.
HIGH pH	Incineration ash layer in landfill	--	Dominated by *Bacillus*.
ORGANIC MATTER CONCENTRATIONS	High organic matter concentrations	--	High diversity of microbial communities.
LEACHATE	Full-scale recirculation of leachate	High input of organic matter associated with leachate recirculation; Accumulation of acetate.	Favorable conditions for *Methanosarcina*; No detection of *Methanosaeta*.
			Very low abundance of *Proteobacteria*.
	Closed landfill	Stable conditions.	Favorable conditions for *Methanosaeta*; Low abundance of *Methanosarcina*.
			High abundance of *Proteobacteria*, mainly *Gammaproteobacteria*.
TEMPERATURE	Lower Temperature	--	Higher microbial diversity.
	High Temperature	40-50 °C	Low archaeal diversity.
CONTAMINATED SITE	Pollutants: Hydrocarbons; PAH; PCB; Heavy metals.	--	Domination of *Proteobacteria*.
	Contamination intensity		Lowest microbial diversity and lowest enzymatic activity in areas with highest pollutant concentrations

Optimal conditions for the development and survival of Gram-negative bacteria from the family *Enterobacteriaceae*, phylum *Proteobacteria*, are the pH value of the environment between 6.6 and 7.5, as well as the temperature of 35 °C [13]. An overview of the identification of Gram-negative bacteria in municipal solid waste landfill leachate done by some other authors are shown in Table 2.

Table 2. Identification of Gram-negative bacteria in the MSW landfill leachate

SPECIES	LANDFILL SITE	REFERENCE
Proteus mirabilis; *Klebsiella pneumoniae*; *Escherichia coli*; *Acinetobacter calcoaceticus*; *Yersinia enterocolitica*; *Stenotrophomonas maltophilia*; *Citrobacter freundii*.	Noble Hill Sanitary Landfill at Springfield, USA	[14]
Actinobacillus pleuropneumoniae; *Bordetella* sp.; *Escherichia coli* var II; *Brucella* sp.; *Acinetobacter baumannii*; *Klebsiella pneumoniae*; *Ochrobactrum anthropi*; *Salmonella* spp.	San Nicolás landfill at Aguascalientes, Mexico	[15]
Klebsiella pneumoniae; *Escherichia coli*; *Citrobacter freundii*; *Proteus mirabilis*; *Vibro* spp.; *Salmonella* spp.	Five active landfill sites in the Ashanti Region, Ghana	[16]

MATERIAL AND METHODS

Leachate sampling campaigns were carried out in the winter, spring and autumn of 2019, in cycles of 2 hours at each location. Sampling was carried out in accordance with instruction Q3.BP. 853 (in accordance with SRPS EN ISO 19458:2009) at three non-sanitary (L_1, L_2, L_3) and one sanitary (L_4) municipal solid waste landfill in the region of Vojvodina. At each site, 0.5 L of leachate was collected for the purpose of microbiological analyses. All samples were delivered to the laboratory and stored at a temperature of 4 °C until the sample was prepared for analysis.

Microbiological analyses were carried out at the Institute of Public Health of Vojvodina in Novi Sad, Republic of Serbia.

Fig. 1. Three non-sanitary, L_1 (upper left), L_2 (upper right), L_3 (lower left) and one sanitary, L_4 (lower right) MSW landfills from the region of Vojvodina on which landfill leachate sampling was conducted

RESULTS AND DISCUSSION

The results of microbiological analyses of leachate sampled at three non-sanitary (L_1, L_2, L_3) and one sanitary (L_4) municipal solid waste landfill in the region of Vojvodina in the winter, spring and summer period are shown in Table 3.

Table 3. Species of identified microorganisms at four MSW landfill sites by seasons

	L_1	L_2	L_3	L_4
Winter	*Escherichia coli* (37°C, 44°C); *Klebsiella pneumoniae* (37°C, 44°C).	/	*Escherichia coli* (37°C, 44°C); *Klebsiella pneumoniae* (37°C, 44°C).	*Escherichia coli* (37°C, 44°C).
Spring	*Klebsiella pneumoniae* (37°C, 44°C).	*Citrobacter* spp. (37°C, 44°C); *Escherichia coli* (37°C, 44°C).	*Escherichia coli* (37°C, 44°C); *Klebsiella pneumoniae* (37°C, 44°C).	*Citrobacter freundii* (37°C, 44°C); *Klebsiella pneumoniae* (37°C, 44°C).
Autumn	*Escherichia coli* (37°C, 44°C); *Klebsiella oxytoca* (37°C, 44°C).	*Escherichia coli* (37°C, 44°C).	*Escherichia coli* (37°C, 44°C); *Klebsiella oxytoca* (37°C, 44°C).	*Citrobacter freundii* (37°C, 44°C); *Escherichia coli* (37°C, 44°C).

Frequently identified microorganisms at all four municipal solid waste landfill sites are *Escherichia coli* and *Klebsiella pneumoniae*. The presence of *Citrobacter freundii* microorganisms was identified at the L_2 landfill site in the spring season, as well as at the L_4 landfill site during the spring and autumn seasons.

The detected microorganisms *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* are Gram-negative bacteria from the family of *Enterobacteriaceae*, phylum *Proteobacteria* [17].

Escherichia coli is an aerobic and facultatively anaerobic bacteria that ferments lactose. It grows well in laboratory conditions, and for most serotypes the optimal temperature for reproduction is around 37°C. It is a rather resistant bacteria and it can survive in water and soil for months, with an extended period of staying on various objects. It reproduces easily and quickly in different types of food. A temperature of 60°C can kill them after 15 min [17]. *Escherichia coli* falls into the group of coliform bacteria, as it belongs to the normal human gut flora. In respect of the above, in sanitary inspections of drinking water and food they are used as indicators of fecal contamination. The mentioned bacteria can cause extraintestinal and diarrheal diseases in humans. This bacteria can be transmitted through intake of contaminated water and food and through contact with infected humans and animals [17].

Klebsiella pneumoniae is a facultative anaerobic bacterium that ferments lactose. The mentioned bacteria are characterized by rounded ends, they have no flagella and they are immobile, and so they are distributed individually or in pairs. Fimbriae with adherent properties and a capsule are significant virulence factors [17]. *Klebsiella pneumoniae* is a common cause of a hospital-acquired pneumonia and infections in immunocompromised patients. The nasopharynx and intestines are the centers of concentration and routes of transmission of the mentioned bacterium in humans. However, fecal contamination is the most common cause of infections caused by *Klebsiella pneumoniae* [17].

Citrobacter freundii is a facultatively anaerobic bacterium that does not ferment lactose. In general, most of the mentioned bacteria are characterized by the presence of several flagella for the purpose of movement, but there are also exceptions to the mentioned case. The above bacteria can be found in soil, water, sewage, food, but also in the digestive tract of animals and humans [17]. *Citrobacter freundii* is mentioned as the cause of infections of the urinary tract and respiratory organs, meningitis, sepsis, while their presence is also detected in decubitus ulcers. They are also a cause of healthcare associated infections, especially in pediatric and immunocompromised patients [17].

Based on the above, as causes of urinary, extraintestinal, diarrheal and respiratory infections, meningitis, colitis, and sepsis, *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* may pose a significant risk to human and animal health, under certain exposure circumstances.

The presence of the mentioned Gram-negative bacteria from the phylum *Proteobacteria* can also be used as an indicator of the performance of landfill sites at which sampling was done, as well as the qualitative composition of leachate. The lower diversity of microbiological communities at landfills L_1, L_2, L_3 and L_4 indicates the prevailing conditions, which is - lower concentrations of acetate and hydrocarbons, as well as high pH value that characterize landfill sites that are more than 10 years old. Recirculation of municipal solid waste landfill leachate, as a primary treatment, is characterized by a high content of organic matter and accumulation of acetate, which are not favorable conditions for the development and survival of a range of Gram-negative bacteria. Based on the research conducted by other authors, it has been determined that due to the high concentrations of total hydrocarbons, significant microbiological enzyme activity was detected [11]. According to the above conclusions, identification of Gram-negative bacteria from the phylum *Proteobacteria* may also indicate contamination of leachate with organic pollutants, primarily hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and heavy metals [11].

Municipal solid waste landfills are very complex environments with variable and almost unpredictable qualitative composition of leachate, which often includes emergent substances, such as antibiotics and endocrine disruptors, as well as heavy metals, which significantly affect the structure and diversity of present microbiological communities. Therefore, the risk of developing antibiotic resistance in microorganisms is constantly increasing. The literature data according to which we are led to this doubt refers that the 50 *Escherichia coli* isolates tested were resistant to the antibiotics penicillin and erythromycin (100%), nalidixic acid (98%), cephalexin (94%), amoxicillin (86%), ampicillin (84%), ciprofloxacin (74%), tetracycline (64%), cefixime (54%) and gentamicin (36%) [18]. Most *Klebsiella pneumoniae* isolates showed an outstanding antibiotic resistance. A more favorable situation was only in the case of meropenem (1.20%), amikacin (4.79%) and piperacillin/tazobactam (10.53%) [19]. Gram-negative bacteria *Citrobacter freundii* showed antibiotic resistance to aminoglycoside

antibiotics (gentamicin, netilmicin and amikacin), fluoroquinolone antibiotics (levofloxacin, ciprofloxacin) and carbapenems (ertapenem, imipenem) and meropenem [20].
Due to the high prevalence of antibiotic resistance in Gram-negative bacteria from the family of *Enterobacteriaceae*, phylum *Proteobacteria*, displayed in numerous studies, there is an obvious need for careful monitoring of landfill leachate microbiological and physic-chemical properties. In addition, there is a necessity for the properly done exposure assessment of humans and animals to this leachate, in order to minimize and avoid possible health risk.

CONCLUSION

The presence of microorganisms *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii*, Gram-negative bacteria from the family of *Enterobacteriaceae*, phylum *Proteobacteria* was detected after analyzing microbiological parameters of leachate sampled from three non-sanitary (L_1, L_2, L_3) and one sanitary (L_4) municipal solid waste landfill in the region of Vojvodina in the winter, spring and autumn periods. Frequently detected Gram-negative bacteria at all four municipal solid waste landfills are *Escherichia coli* and *Klebsiella pneumoniae*. The presence of *Citrobacter freundii* bacteria was detected at the L_2 landfill site during the spring season and at the L_4 landfill site during the spring and autumn seasons.
The presence of the mentioned Gram-negative bacteria of the *Proteobacteria* phylum can also be used as an indicator of the performance of landfill sites at which sampling was done, such as landfill age, morphological composition of waste, landfill body thickness, waste decomposition phases, as well as the qualitative composition of leachate. The lower diversity of microbiological communities at landfills L_1, L_2, L_3 and L_4 indicates the prevailing conditions, i.e. lower concentrations of acetate and hydrocarbons, as well as high pH values, characteristic for landfill sites that are more than 10 years old. Recirculation of municipal solid waste landfill leachate, as a primary treatment, is characterized by a high content of organic matter and accumulation of acetate, which are not favorable conditions for the development and survival of a range of Gram-negative bacteria. Identification of Gram-negative bacteria of the *Proteobacteria* phylum also indicates contamination of leachate with organic pollutants, primarily hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and heavy metals.
Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* have been identified as common causes of urinary, extraintestinal, diarrheal and respiratory infections, meningitis, hemorrhagic and entero-hemorrhagic colitis, sepsis. Due to the data presented in the literature related to the antibiotics resistance of Gram-negative bacteria, it is necessary to pay attention to this phenomenon in the case of the analyzed leachates from the Vojvodina region.
By optimizing and enhancing the analyses of microbiological parameters of leachate, besides the use in the form of fecal contamination indicators, Gram-negative bacteria could also become potentially reliable indicators of leachate contamination with heavy metals and organic compounds, as well as indicators of functionality and condition of municipal solid waste landfills.

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