



# Microbiological Parameters of Technosols Monitored for Hydrophobicity

Kostadinka Nedyalkova, Galina Petkova, Irena Atanassova<sup>\*</sup>, Martin Banov, Plamen Ivanov

"N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant Protection, 7 Shosse Bankya Str., Sofia 1331, Bulgaria

## Abstract

Soil hydrophobicity causes reduced water infiltration rate and has a negative impact on plant growth. Reports on hydrophobicity of Technosols are limited, and in Bulgaria studies have been initiated only recently. The present work aimed to monitor two Technosols (non-vegetated and afforested with *Pinus nigra*) located near Obruchishte (Maritsa-Iztok coal mines) for hydrophobicity level and to assess their microbiological status. In total, 24 soil samples from 12 sampling points and two soil depths (0-10 cm and 10-20 cm) were analyzed for hydrophobicity, moisture content, numbers of cultivable microorganisms, basal respiration and microbial biomass carbon. The hydrophobicity was measured by water-drop-penetration-time (WDPT) test. Microbial numbers were determined by plate counts technique. Sample incubation in closed vials was used to determine basal respiration and microbial biomass carbon. Among the studied samples, 42% possessed severe hydrophobicity, 37% were strongly hydrophobic and 21% were non-hydrophobic (hydrophilic). Both soils were characterized with low numbers of bacteria, actinomycetes and fungi (10<sup>2</sup> CFU/g), and low levels of basal respiration rate (0.13-6.54 mg CO<sub>2</sub>-C/100g/24h) and microbial biomass carbon (1.57-18.86 mg C/100g). Values widely differed among sampling points and layer depths because of the high heterogeneity of the soil substratum. The hydrophobic samples contained a relatively higher amount of saprotrophic fungi than hydrophilic ones.

Keywords: hydrophobicity, Technosols, microbial numbers, basal soil respiration, microbial biomass carbon

### Резюме

Почвената хидрофобност причинява намалена способност за инфилтриране на водата и оказва негативно влияние върху развитието на растенията. Публикациите във връзка с хидрофобните свойства на техногенни почви (Technosols) са ограничени, а в България изследванията по този въпрос започнаха неотдавна. Настоящата работа има за цел мониторинг на две техногенни почви (незалесена и залесена с черен бор, Pinus nigra) за хидрофобност и оценка на микробиологичния им статус. Почвите са разположени в близост до с.Обручище в района на въгледобивния басейн Марица-Изток. Анализирани са 24 почвени проби, взети от два слоя (0-10 см и 10-20 см) в 12 точки на пробовземане. Определени са нивото на хидрофобност, почвената влага, числеността на основните групи почвени микроорганизми, общата микробиологична активност (почвено дишане) и микробиалния въглерод. Почвената хидрофобност е определена чрез измерване на времето, необходимо за проникване на водната капка (WDPT, s) в почвата. Числеността на микроорганизмите е отчетена върху агарови среди. Почвеното дишане и въглерода в микробиалната биомаса са определени чрез инкубация в затворени съдове. Установено е, че 42% от почвените проби са екстремно хидрофобни, 37% са силно хидрофобни и 21% са хидрофилни. Двете почви се характеризират с ниска численост на бактериите, актиномицетите и плесенните гъби (от порядъка на 10<sup>2</sup> CFU/g), и ниски нива на микробиологична активност (0.13-6.54 mg CO<sub>2</sub>-C/100g/24h) и микробиален въглерод (1.57-18.86 mg C/100g). Стойностите на тези показатели варират в широки граници в зависимост от точките на пробовземане и почвения слой поради високата хетерогенност на почвения субстрат. В хидрофобните проби относителния дял на плесенните гъби е по-висок в сравнение с хидрофилните почвени проби.

<sup>\*</sup> Corresponding author: i.d.atanassova@abv.bg

#### Introduction

Soil hydrophobicity (soil water repellency) is a reduced water retention of soils (Doerr *et al.*, 2000). The negative impact on water infiltration leads to soil erosion, nutrient loss and decrease in plant growth and crop production. Hydrophobic properties are found in different soil types - sandy, loam, clay, peat and volcanic ash soils (Dekker *et al.*, 2005) but little information is available about hydrophobic Technosols and especially on their microbiological parameters. In Bulgaria those studies have been initiated recently.

In a recent study (Nedyalkova *et al.*, 2018), pioneer information on the level of hydrophobicity in the spring and microbial properties of samples of hydrophobic Technosols from the area of Maritsa-Iztok coal mines was reported. It is known that hydrophobicity, in general, is strongly dependent on the soil moisture and often is reversible (Doerr *et al.*, 2000). We intended to check the hydrophobicity level of the same soils in the hot (summer) season when shifts in soil hydrophobicity status are expected.

Among microbiological parameters, basal soil respiration and microbial biomass carbon content were widely used in ecological studies of different soil types (Nannipieri *et al.*, 1990; Alef, 1995) and were successfully applied for reclaimed mine soils (Ingram *et al.*, 2005).

The aim of the study was to monitor two Technosols for hydrophobicity level in the summer season and to assess soil microbiological status.

#### **Material and Methods**

Two Technosols located near the village of Obruchishte, Maritsa–Iztok coal mines (Bulgaria) were investigated for hydrophobicity level. They consisted of loam-textured Pliocene overburden sediments compiled more than 30 years ago during open-cast lignite mining activities and later were subjected to reclamation with coal ash.

Samples from a non-vegetated site and an afforested with pine trees (*Pinus nigra*) site, situated at about 30 m from each other, were collected in the summer (end of July) of 2017. At each site, 6 sampling points were chosen and samples from two layers - 0- (5)10 cm and 10-20 cm, were taken using a core sampler (3 cm-wide and 25 cm-long). In total, 24 soil samples (as listed in Table 1) were analyzed for hydrophobicity, moisture content, microbial amount, basal soil respiration and microbial biomass carbon content. All measurements were made in triplicates.

Soil hydrophobicity was assessed by water-drop-penetration-time (WDPT) test. Three water drops were placed onto the sample surface and the time for their complete infiltration into soil was recorded. The median value of the triplicate time was considered as hydrophobicity value of a sample. According to WDPT, samples were classified in the following classes (De Bano, 1981): non-hydrophobic or hydrophilic (WDPT<5 s), strongly hydrophobic (5<WDPT<600 s), and severely hydrophobic (WDPT>600 s). Soil moisture content was calculated after drying the samples at 105°C.

The amount of the main groups of cultivable microorganisms was determined by plate count technique. Ten-fold serial dilutions of samples were used to inoculate soil suspension on selective agar media. Soil bacteria were cultivated on Nutrient broth agar, actinomycetes – on starch-ammonium agar (Hutchinson's) medium and saprotrophic fungi – on Czapek's agar medium (Grudeva *et al.*, 2007). After incubation at 28°C, microbial colonies were counted and results were calculated as colony-forming units per gram of absolutely dry soil (CFU/g).

The respiration was measured in the laboratory after roots and macrofauna were removed from the samples, thus the CO<sub>2</sub> evolution rate (basal respiration) represented the total microbial activity in the soil (Nannipieri et al., 1990). The soil was sieved (2 mm sieve), then fine roots were taken out and the samples were adjusted to 60% (w/w) moisture content. Basal soil respiration was determined in tightly closed vials after 24 h -incubation, as described by Alef (1995). After that samples were amended with glucose and incubated for another 4 hours at 22°C to determine microbial biomass carbon  $(C_{mic})$ . The CO<sub>2</sub> evolved was determined by titration. Microbial carbon was calculated according to the equation proposed by Anderson and Domsch (1978).

Mean values were compared by the least significant differences (LSD) at  $p \le 0.05$  under ANO-VA. Correlation analyses were used to examine relations between the parameters.

#### Results

Among the studied samples, 42% possessed severe hydrophobicity, 37% were strongly hydrophobic and 21% were non-hydrophobic (hydrophilic). All but one samples from the non-vegetated soil were hydrophobic. Eight of the samples from *Pinus nigra* vegetated Technosols were severely and strongly hydrophobic and the remaining four samples were hydrophilic (Table 1).

Soil moisture ranged from 9.29 to 21.95 % in the non-vegetated soil, and from 13.64 to 31.58% in *Pinus nigra* vegetated soil. No correlation between moisture content and WDPT of samples was found.

In non-vegetated Technosol, bacteria numbers widely varied (0.07 to 6.04 CFU/g x  $10^2$ ) among soil layers of different hydrophobicity. The amount of actinomycetes (0 - 0.21 CFU/g x  $10^2$ ) was too low or absent in some sampling points. Saprotrophic fungi numbers (0.26 - 0.61 CFU/g x  $10^2$ ) did not change substantially among samples (Table 1).

In the *Pinus nigra* vegetated Technosol bacteria numbered between 0.19 - 12.33 CFU/g x  $10^2$ ,

actinomycetes increased to  $0 - 3.2 \text{ CFU/g x } 10^2$ , and fungi reached  $0.48 - 3.5 \text{ CFU/g x } 10^2$  considering both soil layers (Table 1).

The relative amount of bacteria, actinomycetes and fungi in all hydrophilic and all hydrophobic samples of both soils are shown in Fig. 1.

In non-vegetated soil, CO<sub>2</sub> production ranged from 1.51 to 6.54 mg CO<sub>2</sub>-C/100g/24h in 0-10 cm layer and between 0.75-5.52 mg CO<sub>2</sub>-C/100g/24h in 10-20 cm layer. In *Pinus nigra* vegetated soil, CO<sub>2</sub> values varied between 0.14-3.67 mg CO<sub>2</sub>-C/100g/24h in top layer and between 0.13-2.21 mg CO<sub>2</sub>-C/100g/24h in the lower layer. Mean values are presented in Fig.2.

**Table 1.** Hydrophobicity level of Technosol samples according the water-drop-penetration-time (WDPT), soil moisture content and microbial counts (CFU/g) in distinct sampling points. Values in columns followed by similar letters are not significantly different at p<0.05

№	Soil layer depth	Soil moisture (%)	WDPT (s)	Hydropho- bicity level	Bacteria	Actino- mycetes (CFU/g) x 10	Sapro- trophic fungi
Non-vegetated Technosol							
1	0-10 cm	18.34	205	strong	0.69 e	0.08 c	0.32 de
	10-20 cm	16.28	4	hydrophilic	0.48 e	0.14 b	0.25 e
2	0-10 cm	14.94	8895	severe	0.49 e	0.11 bc	0.37 cde
	10-20 cm	15.07	795	severe	3.19 c	0.21 a	0.47 bcd
3	0-10 cm	11.73	20	strong	1.63 d	0 e	0.26 e
	10-20 cm	21.95	323	strong	0.40 e	0.02 de	0.25 e
4	0-10 cm	11.11	17	strong	2.13 d	0 e	0.31 de
	10-20 cm	14.29	87	strong	0.53 e	0 e	0.61 ab
5	0-10 cm	9.89	9900	severe	6.04 a	0 e	0.37 cde
	10-20 cm	9.29	16	strong	4.10 b	0 e	0.65 a
6	0-10 cm	12.36	7820	severe	0.07 e	0.07 cd	0.26 e
	10-20 cm	13.64	3880	severe	0.73 e	0.07 cd	0.50 abc
Pinus nigra vegetated Technosol							
7	0-5 cm	23.46	4520	severe	5.67 c	1.00 d	2.25 b
	10-20 cm	31.58	21	strong	1.48 d	0.15 e	0.88 ef
8	0-5 cm	13.64	11510	severe	0.80 de	0.11 e	2.01 b
	10-20 cm	28.21	11510	severe	0.57 de	0.05 e	1.51 c
9	0-5 cm	22.10	34	strong	6.93 b	3.20 a	3.50 a
	10-20 cm	29.03	1	hydrophilic	5.60 c	1.87 c	1.41 cd
10	0-5 cm	14.94	11058	severe	0.46 de	0.09 e	1.10 ef
	10-20 cm	20.48	1	hydrophilic	0.17 e	0 e	0.48 g
11	0-5 cm	19.76	8	strong	12.00 a	2.27 bc	1.13 de
	10-20 cm	24.22	7102	severe	0.19 e	0.01 e	0.70 fg
12	0-5 cm	14.94	1	hydrophilic	12.33 a	3.00 ab	2.05 b
	10-20 cm	19.62	0	hydrophilic	5.27 c	1.80 c	2.16 b



Fig. 1. Relative amounts of bacteria, actinomycetes and saprotrophic fungi in hydrophilic (A) and hydrophobic (B) soil layers



**Fig. 2**. Mean values of basal soil respiration (CO<sub>2</sub> production) and microbial biomass carbon ( $C_{mic}$ ) in different soil layers of non-vegetated and *Pinus nigra* vegetated hydrophobic Technosols

Microbial biomass carbon ( $C_{mic}$ ) content varied between 8.18-18.86 mg C/100g for 0-10 cm layer, and between 3.17-14.98 in 10-20 cm layer of non-vegetated soil. In the vegetated soil  $C_{mic}$  values of 1.67-14.38 mg C/100g in 0-10 cm layer and 1.57-14.38 mg C/100g in 10-20 cm layer were measured. Figure 2 shows the average values for both layers of each soil.

There was no significant correlation between hydrophobicity (WDPT) and  $CO_2$  production, microbial biomass carbon or microbial numbers for all samples tested.

#### Discussion

The majority of soil samples studied possessed hydrophobic properties. Most of the samples from non-vegetated soil possessed strong and severe level of hydrophobicity. The highest WDPT values were registered in the *Pinus nigra* vegetated soil. This is in agreement with other studies in which hydrophobicity is associated with evergreen tree types (Doerr *et al.*, 2000). Differences in WDPT values are attributed to the heterogenic composition of the samples and confirm the spatial variability of water repellency of lignitic mine soils reported by Gerke et al. (2001).

Comparing hydrophobicity levels of the spring (Nedyalkova *et al.*, 2018) and summer (this study) soil samples, it could be pointed out that hydrophobicity decreased in 13 out of 24 samples at both plots.

Microbial number in the Technosols studied was, in general, very low. Values differed among sampling points and layer depths because of the high heterogeneity of the substrata. A decreasing trend of bacteria and actinomycetes numbers with increasing the WDPT values was noticed. Microbial counts in the *Pinus nigra* vegetated Technosol were higher than those in the non-vegetated soil which was, obviously, due to the influence of vegetation. It is well known that root exudates provide available nutrients for microorganisms and support microbial growth. In addition, the higher soil moisture content of the vegetated soil stimulated microbial growth.

The increase in the relative amount of fungi in all hydrophobic samples compared to the hydrophilic samples pointed to some relation of fungi with hydrophobicity but the relationship was not significant.

Values of basal respiration and microbial biomass carbon were low and due to sample heterogeneity both parameters varied widely. Vanhala *et al.* (2005) also pointed out the impact of heterogeneous soil environment in variations of basal soil respiration rate. In this study, the average soil respiration and  $C_{mic}$  values were higher in the non-vegetated soil and in the summer samples as compared to the spring samples (Nedyalkova *et al.*, 2018). This could be explained by the higher temperature and hygroscopic moisture, both stimulating organic carbon mineralization in the July samples taken shortly after drizzle in the area.

#### Conclusion

The Technosols located near Obruchishte (Maritsa-Iztok coal mines) possessed hydrophobic properties. Most of the samples collected from non-vegetated and *Pinus nigra* vegetated soils studied were classified as strongly and severely hydrophobic. The highest level of hydrophobicity was measured in the soil under *Pinus nigra* trees. A trend of lowering of hydrophobicity level in the summer samples from both Technosols compared to the spring samples was noticed.

In general, both soils were characterized with low microbial counts, low basal respiration rate and microbial biomass carbon. Values widely differed among sampling points and layer depths because of the high heterogeneity of the substrata. The relative amount of saprotrophic fungi was higher in the hydrophobic samples than in the hydrophilic ones.

#### Acknowledgments

The study was supported by the National Science Fund, Bulgarian Ministry of Education and Science, project DN 06/1 (2016-2019).

#### References

- Alef, K. (1995). Soil respiration, in: Alef, K., P. Nannipieri (Eds), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 214-218.
- Anderson, J. P. E., K. H. Domsch (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **10**: 215-221.
- De Bano, L. F. (1981). Water repellent soils: a state-of-the-art. USDA Forest Service General Technical Report PS W-46. Berkeley, CA, pp. 21. https://www.fs.fed.us/psw/publications/documents/psw\_gtr046/psw\_gtr046.pdf
- Dekker, L. W., K. Oostindie, C. J. Ritsema (2005). Exponential increase of publications related to soil water repellency. *Austr. J. Soil Res.* 43: 403-442.
- Doerr, S. H., R. A. Shakesby, R. P. D. Walsh (2000). Soil water repellency: its causes, characteristics and hydrogeomorphological significance. *Earth-Sci. Rev.* 51: 33-65.
- Gerke, H. H., E. Hangen, W. Schaaf, R. F. Hüttl (2001). Spatial variability of potential water repellency in a lignitic mine soil afforested with *Pinus nigra*. *Geoderma* **102**: 255-274.
- Grudeva, V., P. Moncheva, S. Nedeva, B. Gocheva, S. Antonova-Nedeva, S. Naumova (2007). Handbook of microbiology. University edition SU "St. Kl. Ohridski", p. 356 (in Bulgarian).
- Ingram, L. J., G. E. Schuman, P. D. Stahl, L. K. Spackman (2005). Microbial respiration and organic carbon indicate nutrient cycling recovery in reclaimed soils. *Soil Sci. Soc. Am. J.* 69: 1737-1745.
- Nannipieri, P., S. Grego, B. Ceccanti (1990). Ecological significance of the biological activity in soil, in: Bollag, J. M., G. Stotzky (Eds.), Soil Biochemistry. Marcel Dekker, New York, pp. 293-355.
- Nedyalkova, K., G. Petkova, I. Atanassova, M. Banov, P. Ivanov (2018). Microbiological properties of hydrophobic and hydrophilic Technosols from the region of Maritsa-Iztok coal mines. C. R. Acad. Bulg. Sci. 71: 577-585.
- Vanhala, P., P. Tamminen, H. Fritze (2005). Relationship between basal soil respiration rate, tree stand and soil characteristics in boreal forests. *Environ. Monit. Assess.* 101: 85-92.





## **Short Communication**

## Meningitiis Due to Sphingomonas paucimobilis in a Pediatric Patient: A Case Report

Nurullah Ciftci<sup>1\*</sup>, Hatice Turk Dagi<sup>1</sup>, Gulsum Alkan<sup>2</sup>, Fatih Ates<sup>1</sup>, Inci Tuncer<sup>1</sup>

<sup>1</sup>Department of Microbiology, Faculty of Medicine, Selcuk University, Konya, Turkey <sup>2</sup>Department of Pediatric Infectious Diseases, Faculty of Medicine, Selcuk University, Konya, Turkey

## Abstract

Sphingomonas paucimobilis is a gram-negative, nonfermentative, aerobic, oxidase and catalase positive, non-spore producing bacterium characterizied with yellow pigment production and motile with polar flagella. In this study, we describe an unusual case of S. paucimobilis meningitis in a patient with ventriculoperitoneal shunt. A 13-years-old girl was brought to the emergency room with complaints of fever and headache for two days. She had ventriculoperitoneal shunt surgery following posterior fossa tumor surgery 10 years before. Upon physical examination, the patient was uncomfortable and had 39°C body temperature. Laboratory results were as follows: hemoglobin, 12.6 g/dL; leukocyte count, 18,800/mm<sup>3</sup> (76% neutrophil); platelet count, 179,000/mm<sup>3</sup>; and C-reactive protein, 34 mg/dL. The patient was prediagnosed with meningitis and cerebrospinal fluid sample (CSF) was taken before the empirical vancomycin and meropenem treatment. Shunt was removed, CSF was drained externally. The sample was sent to the Microbiology laboratory. CSF sample was inoculated on 5% sheep blood agar and Eosin-Methylene Blue agar. After 24 hours of incubation at 37°C, Gram-negative bacilli were grown on media. The isolate was identified as S. paucimobilis using the VITEK 2 automated system. The bacterium was susceptible to imipenem, colistin, levofloxacin, meropenem and cefepime. The patient was treated successfully with appropriate antibiotic treatment. We describe this unusual case of ventriculoperitoneal shunt infection with S. paucimobilis. In conclusion, S. paucimobilis is an infectious agent that is prevalent in nature but may also be isolated in the hospital setting. It can lead to nosocomial or community acquired infections. Although it can be eliminated with prophylactic therapy, sensitivity pattern should be definitely studied to determine the optimal treatment.

Keywords: Shunt infection, Sphingomonas paucimobilis, Cerebrospinal fluid, Meningitis

### Резюме

Sphingomonas paucimobilis е Грам-отрицателна, неферментативна, аеробна, оксидаза- и каталаза-положителна, не-спорообразуваща бактерия, характеризираща се с продуцирането на жълт пигмент, подвижна, с полярни флагели. В настоящото изследване се съобщава за необичаен случай на менингит, причинен от *S. paucimobilis*, в пациент с вентрикулоперитонеален шунт. Момиче на 13-години постъпва в спешното отделение с оплаквания за треска и главоболие от два дни. Тя е с вентрикулоперитонеален шунт след операция на мозъчен тумор преди 10 години. След първоначалния преглед пациентката е в дискомфорт, с телесна температура 39°С. Лабораторните резултати са: хемоглобин 12.6 g/dL; левкоцити 18,800/mm<sup>3</sup> (76% неутрофили); тромбоцити 179,000/ mm<sup>3</sup> и С-реактивен протеин 34 mg/dL. Предварителната диагноза е менингит и е взета проба от цереброспинална течност (ЦСТ) преди емпиричното третиране с ванкомицин и меропенем. Шунтът е отстранен, а ЦСТ се дренира външно. Изпратена е проба в микробиологичната лаборатория. ЦСТ е инокулирана върху 5% овчи кръвен агар и еозин-метилен блу агар. След 24 ч. инкубация при 37 °C, върху средите се развиват Грам-отрицателни бацили. Чрез автоматизирана система VITEK 2

<sup>\*</sup> Corresponding author: ciftcinurullah72@gmail.com

изолатът е идентифициран като *S. paucimobilis*. Бактерията е чувствителна към имипенем, колистин, левофлоксацин, меропенем и цефепим. Пациентката е третирана успешно с подходящи антибиотици. В статията описваме този необичаен случай на инфекция на вентрикулоперитонеален шунт с *S. paucimobilis*. В заключение, *S. paucimobilis* е инфекциозен агент, широко разпространен в природата, но освен това може да бъде изолиран и в болнична среда. Може да причини инфекции, свързани с медицинското обслужване или придобити в общността. Въпреки че може да бъде елиминиран с профилактична терапия, за оптималното третиране е важно да се проучва лекарствената му чувствителност.

#### Introduction

Sphingomonas paucimobilis is a non-fermentative, obligately aerobic, gram negative, non-spore producing bacillus and motile by a single polar flagellum. It is oxidase, catalase and esculine hydrolysis positive, urease and indole negative and characterized with a yellow pigment on sheep blood agar. The bacterial growth requires minimum 24-48 hours of incubation, at 30-37°C (not at 42°C) and 5% CO<sub>2</sub> or ambient atmosphere. Bacterial growth occurs nearly 48 hours on sheep blood agar (Smalley *et al.*, 1983; Yabuuchi *et al.*, 1990; Ryan and Adley, 2010; Tai and Velayuthan, 2014; Tille, 2014).

S. paucimobilis is rarely isolated from human materials and has a limited role as a causative agent. Because it is rarely encountered in clinical settings there is limited information about its epidemiology and ability to cause human infection. According to recent literature, although S. paucimobilis has minor clinical importance immune-compromised patients, diabetes mellitus and alcoholism are considerable risk factors for primary bacteremia. Infection, especially in immune compromised patients, can lead to septic shock (Tille, 2014) . S. paucimobilis has been isolated from many clinical specimens such as wound infections (brain abscess, splenic abscess, leg ulcer), urine, vaginal, cervical samples, blood cultures and cerebrospinal fluid (CSF). In the literature, there are a few reported cases of meningitis caused by S. paucimobilis (Ryan and Adley, 2010; Tai and Velayuthan, 2014; Tille, 2014). In this study, we describe an unusual case of S. paucimobilis meningitis in a patient with ventriculoperitoneal shunt. This case is reported to emphasize that S. paucimobilis should be kept in mind as a nosocomial infectious agent and the infections should be treated according to the susceptibility test results.

#### **Case Presentation**

A 13-year-old girl was brought to the emergency room with complaints of fever and headache for two days. She had had ventriculoperitoneal (VP) shunt following posterior fossa tumor surgery

10 years before. Upon physical examination, the patient was uncomfortable and had 39°C body temperature. Laboratory results were as follows: hemoglobin, 12.6 g/dL; leukocyte count, 18 800/mm<sup>3</sup> (%76 neutrophil); platelet count, 179 000/mm<sup>3</sup>; and C-reactive protein, 34 mg/dL. The patient was prediagnosed with meningitis and CSF sample was taken before the empirical vancomycin and meropenem treatment. Shunt was removed, CSF was drained externally. On different days, triplicated CSF samples were sent to the Microbiology laboratory and inoculated on to 5% sheep blood agar and Eosin-Methylene Blue agar (bioMérieux, France). After 24-48 hours of incubation at 37°C, yellow pigmented, slow growing, catalase and oxidase positive, urease and indol negative Gram-negative bacilli grew on media. The same bacteria were isolated in all three samples. The isolates were identified as S. paucimobilis using the VITEK 2 (Biomerieux, France) automated system. Antimicrobial susceptibility test was carried out with VITEK 2 automated system and the results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) break point for Pseudomonas sp (EUCAST, 2016). The bacteria were susceptible to imipenem (MIC 2 µg/mL), colistin (MIC  $< 0.5 \,\mu\text{g/mL}$ ), levofloxacin (MIC  $0.5 \,\mu\text{g/mL}$ ), meropenem (MIC 0.5 µg/mL) and cefepime (MIC  $8 \,\mu g/mL$ ). Although the patient was being treated in the clinic, in the third week CSF culture reproduction continued with S. paucimobilis. Levofloxacin was added to the treatment, two weeks after CSF sterilization. Shunt revision was performed again. The patient was treated successfully with appropriate antibiotic treatment. Therefore, we present this unusual case of VP shunt infection with S. paucimobilis.

#### Discussion

*S. paucimobilis* was first described in 1977 as *Pseudomonas paucimobilis* and was changed in the 1990s to its recent name (Yabuuchi *et al.*, 1990). Bacteria can be found in soil and water but can proliferate in distilled water, hemodialysis fluids and sterile drug solutions. So it can cause both community-acquired and nosocomial infections. *S. paucimobilis* has minor clinical significance and its virulance is low so the bacterium is usually not checked in routine hospital analysis, but it may be clinically important for immuno-compromised patients, diabetes mellitus, post operative and nosocomial infection (Tille, 2014). Therefore, it should be considered as a causative agent alternative to the *Pseudomonas, Stenotrophomonas* and *Burkholderia* species for these patients (Ryan and Adley, 2010; Tille, 2014).

Recently, there have been many studies about this bacterium, which show that the importance of S. paucimobilis has increased over the years (Maragakis et al., 2009; Ryan and Adley, 2010; Deveci et al., 2017). Bacteraemia, ventilator-associated pneumonia, myositis, peritonitis, postoperative postoperative endophthalmitis and catheter-related infections have been reported in literature (Ryan and Adley, 2010; Tille, 2014). But meningitis caused by S. paucimobilis have been reported only in a few case (Hajirousou et al., 1979; Tai and Velayuthan, 2014; Bolen et al., 2015; Deveci et al., 2017). Ryan et al. (2010) have evaluated 240 case reports published about S. paucimobilis and have discovered 52 different cases of infection relating to the presence of S. paucimobilis. In the study, they reported 20 cases of bacteraemia/sepsis, five cases of peritonitis, three cases of pneumonia, three cases of urinary tract infection and the others in only one case. In our country, Erdem et al. (2010) have reported a case of surgical-site surgical-site infection owing to S. paucimobilis. Basoglu et al. (2013) isolated 11 S. paucimobilis strains from tracheal secretion samples (7), urine (2), wound (1) and blood culture (1). Bulut et al. (2008) reported a case of hospital acquired bloodstream infection caused by S. paucimobilis.

In the literature, Hajiroussou *et al.* (1979) first reported a case of meningitis in a 39-year-old patient, caused by *P. paucimobilis* (recently *S. paucimobilis*), and since that time there have been a few cases of meningitis caused by *S. paucimobilis* (Tai and Velayuthan, 2014; Bolen *et al.*, 2015; Deveci *et al.*, 2017). Deveci *et al.* (2017) have reported a case of community-acquired *S. paucimobilis* meningitis in an adolescent patient. The study was the first case of adolescent meningitis caused by *S. paucimobilis*. Tai and Velayuthan (2014) have reported *S. paucimobilis* meningitis in a 31-year-old farmer who was working in soil and had a wound in the leg. They reported that the bacteria entered his body through the wound resulting in bacteraemia and later meningitis. In another study, Bolen *et al.* (2015) presented the case of *S. paucimobilis* meningitis in an immuno-compromised patient, which was also the first reported case of ventriculitis caused by this bacterium. In this study, we describe an unusual case of *S. paucimobilis* meningitis in a 13-years old patient with ventriculoperitoneal shunt.

As yet, there is no standard procedure for antibiotic susceptibility of *S. paucimobilis*. Based on *in vitro* susceptibility studies, case specific therapy indication was required, because antibiotic resistance is reported differently in many articles (Fink, 2009). In some studies antibiotic resistance of *S. paucimobilis* is reported according to EUCAST breakpoint breakpoint for *Pseudomonas* (Deveci *et al.*, 2017). In our study, we evaluated antibiotic resistance according to EUCAST *Pseudomonas* break-point breakpoint (EUCAST, 2016). This case is reported to emphasize that *S.paucimobilis* should be kept in mind as a nosocomial infectious agent and the infections should be treated according to the sensitivity test results.

#### References

- Başoğlu, T. M., G. Ece, T. Adanır (2013). Hastanemizde üreyen Sphingomonas paucimobilis İzolatlarının Klinik Ve Mikrobiyolojik Açıdan Değerlendirilmesi. Turk. Hij. Deney. Biyol. Derg. 70: 181-184.
- Bolen, R. D., E. Palavecino, A. Gomadam, N. Balakrishman, S. Datar (2015). *Sphingomonas paucimobilis* meningitis and ventriculitis in an immunocompromised host. J. N. Sci. 359: 18-20.
- Bulut, C., M. A. Yetkin, S. Tekin Koruk, FŞ. Erdinç, E.A. Karakoç (2008). *Sphingomonas paucimobilis*: Nadir Bir Hastane Kaynaklı Bakteriyemi Etkeni. *Mikrobiyol. Bul.* 42: 685-688.
- Deveci, N., N. Gürkan, N. Belet, S. Uğur Baysal (2017). Sphingomonas paucimobilis: Az Rastlanan Bir Menenjit Etkeni. J. Pediatr. Inf. 11: Doi: 10.5578/ced.57342.
- Erdem, K. E., T. M. Işıkgöz, A. M. Öztürk, O. R. Sipahi, A. Tünger, H. Pullukçu (2010). Nadir bir cerrahi alan infeksiyonu etkeni: *Sphingomonas paucimobilis. Antimikrob. Kemoter. Derg.* 24: 234-236.
- European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 6.0, valid from 2016-01-01 http://www. eucast.org/fileadmin/src/media/PDFs/EUCAST\_files/ Breakpoint\_tables/v\_6.0\_Breakpoint\_table.xls
- Fink, B. (2009). Revision of late periprosthetic infections of total hip endoprostheses: pros and cons of different concepts. *Int. J. Med. Sci.* 6: 287-295.
- Hajirousou, V., B. Holmes, J. Bullas, C. A. Pinning (1979). Meningitis caused by *Pseudomonas paucimobilis*. J. Clin. Path. 32: 953-955.
- Maragakis, L. L., R. Chaiwarith, A. Srinivasan, F. J. Torriani, E. Avdic, A. Lee, T. R. Ross, K. C. Carroll, T. M. Perl

(2009). *Sphingomonas paucimobilis* bloodstream infections associated with contaminated intravenous fentanyl. *Emerg. Inf. Dis.* **15**: 12-18.

- Ryan, M. P., C. C. Adley (2010). Sphingomonas paucimobilis: a persistent Gram negative nosocomial infectious organism. J. Hosp. Infect. 75:153-157.
- Smalley, D. L., V. R. Hansen, V. S. Baselski (1983). Susceptibility of *Pseudomonas paucimobilis* to 24 antimicrobial agents. *Antimicrob. Agents Chemother*. 23: 161-162.
- Tai, M. L., R. D. Velayuthan (2014). *Sphingomonas paucimobilis*: an unusual cause of meningitis-case report. *Neurol.*

Med. Chir. 54: 337-340.

- Tille, P. M. (2014). Bailey and Scott's Diagnostic Microbiology. 13nd ed. Elsevier, London, pp. 376-383.
- Yabuuchi, E., I. Yano, H. Oyaizu, Y. Hashimoto, T. Ezaki, H. Yamamoto (1990). Proposals of Sphingomonas paucimobilis gen. nov. and comb. nov., Sphingomonas parapaucimobilis sp. nov., Sphingomonas yanoikuyae sp. nov., Sphingomonas adhaesiva sp. nov., Sphingomonas capsulata comb. nov., and two genospecies of the genus Sphingomonas. Microbiol. Immunol. 34: 99-119.