UDC 579.26 DOI: https://doi.org/10.33730/2077-4893.4.2019.189463

ANTIBIOTICS IN AGROECOSYSTEMS: SOIL MICROBIOME AND RESISTOME

L. Symochko[1, 3], R. Mariychuk[2], O. Demyanyuk[3], V. Symochko[1]

[1] Ужгородський національний університет
[2] Пряшівський університет, Словаччина
[3] Інститут агроекології і природокористування НААН

Відомо, що забруднення агроекосистем антибіотиками є актуальною проблемою сьогодення. Вплив забруднення антибіотиками на навколишнє природне середовище, здоров'я людини і тварин є мало вивченим. Антибіотики відіграють ключову роль у боротьбі з інфекційними захворюваннями у людей, тварин та аквакультурі в усьому світі. Надходження дедалі більшої кількості антибіотиків у води та ґрунти зумовлює потенційну загрозу для всіх мікроорганізмів у цих середовищах. Забруднення навколишнього природного середовища антибіотиками є одним із чинників, що визначають формування бактеріальної резистентності. Фторхінолони — один з найбільш розповсюджених класів антибіотиків. Енрофлоксацин належить до класу антибіотиків фторхінолону, який інтенсивно використовують для лікування бактеріальних інфекцій у ветеринарії. У навколишньому природному середовищі енрофлоксацин піддається деградації за різних умов, у т.ч. шляхом фотолізу, біодеградації та окислення мінеральними оксидами, але він не є чутливим до гідролізу. Незважаючи на ці механізми деградації, час напіврозкладу енрофлоксацину в навколишньому природному середовищі є доволі довгим. Було оцінено вплив енрофлоксацину на активність та структуру мікробіому ґрунту. У модельних екосистемах з різною концентрацією енрофлоксацину культивували: Lactuca sativa var. crispa, Anethum graveolens, Thymus serpillum, Mentha piperita, Calendula officinalis. Найактивніше енрофлоксацин із ґрунту поглинали сільськогосподарські рослини, як-от: Lactuca sativa var. crispa та Calendula officinalis. Ґрунт з високою концентрацією антибіотика характеризувався низьким умістом мікроорганізмів, що фіксують азот, і значною кількістю оліготрофної та спороутворювальної мікробіоти. Забруднення антибіотиками також є важливим чинником формування резистому ґ рунту — сукупності ґрунтових мікроорганізмів із високим рівнем стійкості до антибіотиків. З ґрунту модельних агроекосистем було виділено 37 стійких до антибіотиків бактеріальних ізолятів. Встановлено, що всі ізоляти є стійкими до антибактеріальних препаратів, з яких понад 64% були резистентними до 12 антибіотиків (майже всіх класів). В експерименті було виділено п'ять бактерій, стійких до всіх тестованих антибіотиків — анаеробні бактерії: Clostridium difficile, Clostridium perfringens та аеробні бактерії: Enterococcus faecalis, Yersinia enterocolitica, Enterobacter cloacae. Усі вони є стійкими до антибіотиків, а також збудниками інфекційних хвороб, що спричиняють загрозу для здоров'я людини. Забруднення ґрунту антибіотиками спричиняє негативні зміни у мікробних угрупованнях і є одним із важливих чинників формування резистому ґрунту.

Ключові слова: *ґрунт, мікробіом, резистом, антибіотик, забруднення.*

Global use of antibiotics increased steadily over the past decades, both due to an augmentation of antibiotic use in human medicine and in other sectors of commercial activity [1]. For example, antibiotic consumption in livestock reached 63.151 tons in 2010 and is predicted to increase by another 67% by 2030. Antibiotic use is also rising in aquaculture, the fastest-growing food sector worldwide due to intensive farming [2, 3]. For this reason, antibiotics of pharmaceutical origin are now found in large quantities in human-made environments such as sewage and waste water treatment plants [4]. Moreover, because antibiotic pollution is poorly regulated on a local and global scale, antibiotic molecules are increasingly found in terrestrial, freshwater, and marine environments [5] The fluoroquinolones are one of the most used classes of antibiotics. Enrofloxacin belongs to the class of fluoroquinolone antibiotics that have been intensively used for the treatment of bacterial infections in veterinary medicine. Once antibiotics enter the ecosystems, they can be treated as an ecological factor, driving the evolution of the community structure [6, 7]. Accordingly, the change of community structure influences the ecological function of soil and water ecosystems such as biomass production and nutrient transformation. Indirect effects from the antibiotic disturbance to the micro-ecosystem are largely unknown, and it is expected that such disturbance might have significant and long-term effects on the rate and stability of ecosystem functioning [8–10]. In the environment, enrofloxacin can undergo degradations by different processes including photolysis, biodegradation and oxidation by mineral oxides but it is not sensitive to hydrolysis. Despite these degradation mechanisms, environmental half life time of enrofloxacin is very long. This long environmental persistence of enrofloxacin can affect the growing of plant and the activity of the soil microbial communities. As final products of metabolism, enrofloxacin and its metabolite ciprofloxacin end up in excrement [11, 12]. Livestock manure is commonly used as organic fertilizer. One of its uses is on the fields where food plants are grown. The manure includes the residue of fluoroquinolones in addition to other drug residue. Plants can also intake fluoroquinolones along with minerals. The intake of drugs in small amounts can lead to drug resistance in pathogenic microbes and cause allergies and liver damage. Raw materials of animal origin are subject to strict state controls. There are no limits concerning raw materials of plant origin from agroecosystems. In the case of raw materials of plant origin, only pesticide residue, nitrates, heavy metals are controlled at state level, but not antibiotics. One of its uses is on the fields where food plants are grown. The manure includes the residue of fluoroquinolones in addition to other drug residue. Plants can also intake fluoroquinolones along with minerals. The intake of drugs in small amounts can lead to drug resistance in pathogenic microbes and cause allergies and liver damage. Manure is often contaminated with veterinary antibiotics which enter the soil together with antibiotic resistant bacteria. However, little information is available regarding the main responders of bacterial communities in soil affected by repeated inputs of antibiotics via manure [13–15] (Cavigelli, M. A., & Robertson, G.P., 2000; Hammesfahr U., Heuer H., Manzke B., Smalla K., Thiele-Bruhn S., 2008; Heuer H., Smalla K., 2007; Heuer H., Schmitt H., Smalla K., 2011; Torsvik 2002). Nevertheless, some investigators have commented that antibiotic resistance transfer via vegetables represents a risk to human health. The aim of this work has been to evaluate enrofloxacin effect on soil microbiome and uptake in crop plants by a multiple concentration test, controlling after fixed times (90 days) the effects of different concentrations.

MATERIALS AND METHODS OF RESEARCH

A feature of this work was the study of the sorption of enrofloxacin by crop plants. For this, we used the following plants as test objects: *Lactuca sativa var. crispa, Anethum graveolens, Thymus serpillum, Mentha piperita, and Calendula officinalis.* Based on the literature data, we selected three model concentrations of enrofloxacin for our studies: 1000 mg·kg^{-1}; 100 mg·kg^{-1}; 10 mg·kg^{-1} in model agroecosystems. Studies were conducted *in vivo* and *in vitro*. Spiked soil was placed into nonporous plastic plant pots to give a total of 60 pots. This gave 3 replicates per compound with different concentration for assessing uptake by crop plants plus control. Each pot received 20 seeds. The plants were grown under controlled conditions in phyto-chamber: light intensity, 10000 lx with a 16/8 h light/dark cycle; humidity, 70%; and temperature, 20°C during the light cycle and 15°C during the dark cycle. Plants were grown for 90 days. After this time, samples of plant material were removed from each pot, weighed, and placed in glass jars prior to analysis. The amount of enrofloxacin was determined in triplicate on each sample by High Performance Liquid Chromatography (HPLC). Enrofloxacin in plants was extracted according to the method of Palmada et al. [18]: 250 mg plants (dry weight) were extracted in 10 ml acetonitrile containing 1% acetic acid, then homogenized, sonicated (50), vortexed (10) and centrifuged (100) at 3000 g. The supernatant was then collected and dried by nitrogen stream. The residue was suspended in 5 ml phosphate buffer pH 7.4, defatted by a double liquid–liquid partition with 3 + 3 ml N-hexane followed by a double liquid–liquid partition with 3 + 3 ml chloroform. The organic phases were pooled and dried by nitrogen stream. The residue was suspended in mobile phase and 50 μl were injected into the HPLC. Microbiological analyses were conducted following the standard protocol [19]. Soil samples were analyzed within 24 hours. Microbiological study of soil was performed in sterile conditions. The method of serial dilution was used to obtain the suspension where microorganisms titres were 10^{-3}–10^{-5} CFU/ml. 100 μl of the soil suspension was evenly distributed on the surface of the medium with a sterile spatula. For the study we used the following media: Endos agar, Meat peptone agar, Strepto agar and Entero agar, Agar-Agar, Eshbi agar, Soil agar, Chapek agar, Starch agar in 4 repetitions. Petri dishes with studied material were incubated in the thermostat at 37°C for 48 hours in aerobic conditions. All isolated microorganisms were identified by applying of appropriate biochemical test-systems LACHEMA according to the instructions. Antibiotic resistance of the identified microorganisms was analysed by Kirby-Bauer method with the aim to find antibiotic resistant strains of pathogenic microorganisms. All isolates from the soil were examined for resistance to 12 antibiotics of the main pharmacological groups: TE30 Tetracycline; VA30 Vancomycin; L10 Lincomycin; CXM30 Cefuroxime; AMP10 Ampicillin; CIP5 Ciprofloxacin; GEN10 Gentamicin; DO30 Doxycycline; AK30 Amikacin; AMX10 Amoxicillin; E15 Erythromycin; OL15 Oleandomycin. Anaerobic microbiota was additionally tested to Metronidazole MT5; Rifampicin RIF5; Clindamycin CD2. Results were expressed as means (±) standard deviation (SD) and (SSD05) smallest significant differences of experiments conducted in quadruplicating. Data were evaluated using the software Statistica 10.0.

RESULTS AND DISCUSSION

Veterinary and human medicines are increasingly being monitored in slurry, soils, surface waters, and groundwaters. Concerns have therefore been raised over the impacts of environmental exposure routes on human and environmental health [20]. In this study the potential for medicines to enter the food chain via uptake from soil into food plants was explored. The results demonstrate that following application of enrofloxacin to plants at environmentally realistic concentrations, selected substances are taken up at detectable levels. Table 1 shows the results of the accumulation of enrofloxacin by such plants as *Lactuca sativa var. crispa, Anethum graveolens*, *Thymus serpillum*, *Mentha piperita*, *Calendula officinalis*.

The most actively enrofloxacin was absorbed from the soil by *Lactuca sativa var. crispa,* and *Calendula officinalis*. When enrofloxacin was applied to the soil at concentration of 1000 mg·kg^{-1} its concentration was 60.71 mg·kg^{-1} in lettuce's phytomass and 49.03 mg·kg^{-1} in calendula one.

The lowest content of enrofloxacin was found in *Mentha piperita* with all three experimental concentrations of antibiotic in the soil. Experimental studies have shown that *Anethum graveolens* and *Thymus serpillum* absorb antibiotic at the same level. At an experimental concentration of 10 mg·kg^{-1} of antibiotic, its content after cultivation of 90 days was 0.34 mg·kg^{-1} in *Anethum graveo-*

Table 1

Accumulation antibiotic by plants

	Concentration of antibiotic, mg•kg^{-1}	Contain of antibiotics in plants after 90 days of experiment				
		Anethum graveolens	Thymus serpillum	Lactuca sativa	Mentha piperita	Calendula officinalis
1	10	0.34±0.06	0.33±0.02	0.50±0.07	0.22±0.08	0.48±0.07
2	100	2.53±0.43	3.02±0.33	6.42±0.54	1.56±0.27	5.08±0.51
3	1000	24.80±1.20	24.23±0.91	60.71±1.03	18.41±1.14	49.03±1.09

lens, and 0.33 mg•kg^{-1} in *Thymus serpillum*, at a concentration of enrofloxacin 1000 mg•kg^{-1} its content in plants was significantly greater and was 24.80 and 24.23 mg•kg^{-1}.

The results of the studies showed that there is a species differentiation of cultivated plants according to activity of absorbing the antibiotic from the soil. The most actively the antibiotic is absorbed by lettuce and calendula, and less actively by mint. Antibiotic effects on ecosystems are related to its concentration, bioavailability, exposure time and the addition of substrates. When antibiotics get into the arable land, they could possibly impact vegetation growth and development as well as soil microbial activity. The class of fluoroquinolone antibiotics have been intensively used for treatment of bacterial infections in veterinary medicine.

The effects of enrofloxacin on the function and structure of soil microbial communities were evaluated (Table 2).

It should be noted, the soil with a high concentration of antibiotic 1000 mg•kg^{-1} was characterized by a low content of nitrogen-fixing microorganisms and a high number of oligotrophic and sporeforming microbiota.

Among AR microorganisms there were such anaerobic bacteria as *Clostridium difficile, Clostridium perfringens* and such aerobic bacteria as *Enterococcus faecalis, Yersinia enterocolitica, Enterobacter cloacae*. Other dominant bacteria were characterized by a high or moderate level of antibiotic resistance. From the soil bacteria resistant to all tested antibiotics were isolated. They were such representatives of aerobic microbiota as *Bacillus licheniformis*, *Serratia fonticola*, *Hafnia alvei*, *Bacillus cereus*, *Pantoea agglomerans*, *Bacillus megaterium* and one of anaerobic bacteria — *Clostridium difficile*.

In natural conditions, from the soil of model ecosystems mostly bacteria of the genus *Bacillus* were isolated. All of them are antibiotic resistant and are the causative agents of foodborne infections and pose a threat not only to environment but also to human health.

The presence of enrofloxacin in the soil, especially in high concentrations, causes negative changes in the microbial community, significant increasing number of antibiotic-resistant bacteria loses stability and integrity of soil microbiome.

Contamination by antibiotics is one of the important factors in the formation of soil resistome. One of the important indicators of the ecological and sanitary state of the soil and the whole ecosystem is the presence of conditionally pathogenic and pathogenic microorganisms.

Particularly dangerous are the antibiotic-resistant microorganisms, which, together with the bioproduction, can enter to the human and animal organisms from the terrestrial ecosystems. The structure of microbial communities of the soil is interrelated with the presence of antibiotic-resistant pathogenic microorganisms.

In the soil of agroecosystems where the number of pedotrophes and oligotrophes was higher, a greater number of antibiotic-resistant microorganisms were isolated. *Clostridium perfringens* (resistant to tetracycline, rifampicin, amoxicillin, moderately sensitive to vancomycin), *Clostridium oedematiens*

Table 2

Microbial community composition (CFU/gr.d.s.) in soils of agroecosystems contaminated by antibiotic

No.	Agroecosystems	Ammonifiers $\cdot 10^6$	Spore forming bacteria $\cdot 10^6$	Micromycetes $\cdot 10^3$	Actinomyces $\cdot 10^3$	Bacteria wich are using mineral forms of nitrogen $\cdot 10^4$	Anaerobic bacteria $\cdot 10^3$	Aerobic nitrogen fixing bacteria, %	Anaerobic nitrogen fixing bacteria $\cdot 10^3$	Oligotrophic bacteria $\cdot 10^6$	Oligonitrophic bacteria $\cdot 10^4$	Pedotrophic bacteria $\cdot 10^6$
1	*Mentha piperita*	4.07	3.88	11	7.21	4.32	35.20	19.22	3.77	2.63	4.56	3.68
2	*Calendula officinalis*	8.30	1.88	20	10.33	3.64	41.22	28.56	5.96	1.61	2.17	1.88
3	*Thymus Serpillum*	5.46	4.45	15	14.11	3.22	50.22	22.34	8.22	2.87	3.27	3.52
4	*Anethum graveolens*	7.93	1.74	28	21.22	3.14	94.68	38.23	11.35	1.24	1.68	2.26
5	*Lactuca sativa var. crispa*	8.66	2.23	25	12.38	2.18	73.82	29.67	9.23	1.70	2.95	2.96
SSD_{05}		0.48	0.23	0.36	0.37	0.32	0.21	1.12	1.34	0.41	0.55	0.18

(moderately susceptible to amoxicillin and vancomycin), *Clostridium difficile* (sensitive to metronidazole). Nevertheless, the enrichments of *Clostridium* in soil which was continually treated with manure containing can be dangerous for public health [21–23]. The enrichment of these bacteria, which are phylogenetically closely related to human pathogens, may improve the chance of transferring antibiotic resistance genes to human pathogens, since horizontal gene transfer is more prevalent between closely related organisms than between distantly related ones [24–26]. Selective pressures associated with antibiotic pollution can act on the overall microbial community composition by reducing taxa diversity or by shifting microbial composition. Generally speaking, antibiotic exposure tends to favour an increase in Gram-negative bacteria as opposed to Gram-positive bacteria. Presence of antibiotics in soil was found to alter microbial community structure, leading to a loss of biomass and a reduction in microbial activity including nitrification, denitrification, and respiration [27, 28]. Moreover, antibiotics can also affect bacterial enzyme activity, including dehydrogenases, phosphatases, and ureases, which are considered important indicators of soil activity [29]. The different terrestrial toxicological effects of enrofloxacin were observed through using a series of bioassays and including sorption of fluoroquinolone antibiotic by five crop plants.

Enrofloxacin is highly resistant, its biodegradation process is longer than other antibiotics, it is actively absorbed by the plants, and therefore it is necessary to control its content in phytoproducts.

CONCLUSION

The use of fluoroquinoline antibiotic in the farm leads to its accumulation in the soil, which is due of its long biodegradation. Experimental studies *In vivo* and *In vitro* have shown that the absorption activity of an antibiotic depends on its concentration in the soil and the species of agricultural plants. The most actively enrofloxacin is absorbed by *Lactuca sativa* and *Calendula officinalis*.

The smallest content of enrofloxacin was in *Mentha piperita* among five crop plants. The presence of enrofloxacin in the soil, especially in high concentrations, causes negative changes in the microbial community, significantly increasing number of antibiotic-resistant bacteria loses stability and integrity of soil microbiome. Thirty seven antibiotic resistant bacterial isolates were cultured from soil. All isolates were multidrug resistant, of which greater than 64% were resistant to 12 antibiotics, comprising almost all classes of antibiotic. Contamination of agroecosystems by antibiotics plays a key role in formation of soil resistome.

Acknowledgment. This work was supported by the Slovak Academic Information Agency (SAIA), grant number 18032.

ЛІТЕРАТУРА

1. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015 / E.Y. Klein, T.P. Van Boeckel, E.M. Martinez et al. // Proc. Natl. Acad. Sci. USA. — 2018. — Vol. 115. — P. 3463–3470.
2. Global trends in antimicrobial use in food animals / T.P. Van Boeckel, C. Brower, M. Gilbert et al. // Proc. Natl. Acad. Sci. USA. — 2015. — Vol. 112. — P. 5649–5654.
3. Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management. A review from a systems perspective / P.J.G. Henriksson, A. Rico, M. Troell et al. // Sustain. Sci. — 2018. — Vol. 13. — P. 1105–1120.
4. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review / L. Rizzo, C. Manaia, C. Merlin et al. // Sci. Total Environ. — 2013. — Vol. 447. — P. 345–360.
5. Soil microbial diversity and antibiotic resistance in natural and transformed ecosystems / L. Symochko, Hosam E.A.F. Bayoumi Hamuda, O. Demyanyuk et al. // International Journal of Ecosystems and Ecology Sciences (IJEES). — 2019. — Vol. 9(3). — P. 581–590.
6. *Aminov R.* Evolution and ecology of antibiotic resistance genes / R. Aminov, R. Mackie // FEMS Microbiol Lett. — 2007. — Vol. 271. — P. 147–161.
7. *Migliore L.* Phytotoxicity and uptake of enrofloxacin in crop plants / L. Migliore, S. Cozzolino, M. Fiori // Chemosphere. — 2003. — No. 52. — P. 1233–1244.
8. *Thiele-Bruhn S.* Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass / S. Thiele-Bruhn, I. Beck // Chemosphere. — 2005. — Vol. 59. — P. 457–465.
9. Laboratory models to evaluate phytotoxicity of sulphadimethoxine on terrestrial plants / L. Migliore, C. Civitraele, S. Cozzolino et al. // Chemosphere. — 1998. — Vol. 37. — P. 2957–2961.
10. Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure / A. Kotzerke, S. Sharma, K. Schauss et al. // Environ Pollut. — 2008. — Vol. 153. — P. 315–322.
11. Uptake of Veterinary Medicines from Soils into Plants / A. Boxall, P. Johnson, E. Smith et al. // J. Agric. Food Chem. — 2006. — No. 54. — P. 2288–2297.
12. *El-Mahmood A.* Phytochemical screening and antibacterial evaluation of the leaf and root extracts of Cassia alata Linn / A. El-Mahmood, J. Doughari // Afr J Pharm Pharacol. — 2008. — No. 2. — P. 124–129.
13. *Cavigelli M.A.* The functional significance of denitrifier community composition in a terrestrial ecosystem / M.A. Cavigelli, G.P. Robertson // Ecology. — 2000. — Vol. 81(5). — P. 1402–1414.
14. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities / J.C. Chee-Sanford, R.I. Aminov, I.J. Krapac et al. // Applied and environmental microbiology. — 2001. — Vol. 67(4). — P. 1494–1502.
15. Determination of antibiotic residues in manure, soil, and surface waters / T. Christian, R.J. Schneider, H.A. Färber et al. // CLEAN–Soil, Air, Water. — 2003. Vol. 31(1). — P. 36–44.
16. Effects of Apramycin on microbial activity in different types of soil / X. Diao, Y. Sun, Z. Sun et al. // Ecology and Environment. — 2004. — Vol. 13(4). — P. 565–568.
17. *Dıaz-Cruz M.S.* Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge / M.S. Dıaz-Cruz, M.J.L. de Alda, D. Barcelo // TrAC Trends in Analytical Chemistry. — 2003. — Vol. 22(6). — P. 340–351.
18. Determination of enrofloxacin and its active metabolite (ciprofloxacin) at the residue level in broiler muscle using HPLC with fluorescence detector / J. Palmada, R. March, E. Torroella et al. // Proceedings of Euroresidue IV. Residues of veterinary drugs in food. ADDIX, Wijk bij Duurstede, Netherlands, 2000. — P. 822–826.
19. Practical handbook of microbiology / E. Goldman, L.H. Green (Eds.). — Third Edition, 2015.
20. *Symochko L.* Assessment of sorption and toxicity of fluoroquinolone antibiotic in agroecosystems / L. Symochko // Агроекологічний журнал. — 2017. — № 3. — С. 147–151.
21. *Symochko L.* Microbial monitoring of soil as additional tools for conservation biology / L. Symochko, H.B. Hamuda // Obuda University e-Bulletin. — 2015. — No. 5(1). — P. 177–185.
22. Microbiological control of soil-borne antibiotic resistance human pathogens in agroecosystems /

L. Symochko, T. Meleshko, V. Symochko et al. // International Journal of Ecosystems and Ecology Sciences (IJEES). — 2019. — Vol. 8(3). — P. 591–598.
23. Toxicity of the fluoroquinolone antibiotics enrofloxacin and ciprofloxacin to photoautotrophic aquatic organisms / I. Ebert, J. Bachmann, U. Kühnen et al. // Environmental toxicology and chemistry. — 2011. — Vol. 30(12). — P. 2786–2792.
24. *Heuer H.* Antibiotic resistance gene spread due to manure application on agricultural fields / H. Heuer, H. Schmitt, K. Smalla // Curr opin microbiol. — 2011. — Vol. 14. — P. 236–243.
25. *Martínez J.L.* Antibiotics and antibiotic resistance genes in natural environments / J.L. Martínez // Science. — 2008. — Vol. 321(5887). — P. 365–367.
26. The shared antibiotic resistome of soil bacteria and human pathogens / K.J. Forsberg, A. Reyes, B. Wang et al. // Science. — 2012. — Vol. 337. — P. 1107–1111.
27. Effects of tylosin as a disturbance on the soil microbial community / K. Westergaard, A.K. Müller, S. Christensen et al. // Soil Biol. Biochem. — 2001. — Vol. 33. — P. 2061–2071.
28. *Thiele-Bruhn S.* Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass / S. Thiele-Bruhn, I.C. Beck // Chemosphere. — 2005. — Vol. 59. — P. 457–465.
29. *Cyco´n M.* Antibiotics in the Soil Environment — Degradation and their impact on microbial activity and diversity / M. Cyco´n, A. Mrozik, Z. Piotrowska-Seget // Front. Microbiol. — 2019. — Vol. 10. — P. 338.

REFERENCES

1. Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. USA, 115*, 3463–3470 [in English].
2. Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA, 112*, 5649–5654 [in English].
3. Henriksson, P.J.G., Rico, A., Troell, M., Klinger, D.H., Buschmann, A.H., Saksida, S., Chadag, M.V., Zhang, W. (2018). Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management. A review from a systems perspective. *Sustain. Sci. 13*, 1105–1120 [in English].
4. Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I., Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Sci. Total Environ, 447*, 345–360 [in English].
5. Symochko, L., Hosam E.A.F. Bayoumi Hamuda, Demyanyuk, O., Symochko, V., Patyka, V. (2019). Soil microbial diversity and antibiotic resistance in natural and transformed ecosystems. I*nternational Journal of Ecosystems and Ecology Sciences (IJEES), 9(3)*, 581–590 [in English].
6. Aminov, R., Mackie, R. (2007). Evolution and ecology of antibiotic resistance genes. *FEMS Microbiol Lett., 271*, 147–161 [in English].
7. Migliore, L., Cozzolino, S., Fiori, M. (2003). Phytotoxicity and uptake of enrofloxacin in crop plants. *Chemosphere, 52*, 1233–1244 [in English].
8. Thiele-Bruhn, S., Beck, I. (2005). Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere, 59*, 457–465 [in English].
9. Migliore, L., Civitraele, C., Cozzolino, S., Casoria, P., Brambilla, G., Gaudio, L. (1998). Laboratory models to evaluate phytotoxicity of sulphadimethoxine on terrestrial plants. *Chemosphere, 52*, 2957–2961 [in English].
10. Kotzerke, A., Sharma, S., Schauss, K., Heuer, H., Thiele-Bruhn, S., Smalla, K., Wilke, B.M., Schloter, M.A. (2008). Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. *Environ Pollut., 153*, 315–322 [in English].
11. Boxall, A., Johnson, P., Smith, E., Sinclair, C., Stutt, E., Levy, L. (2006). Uptake of veterinary medicines from soils into plants.*J. Agric. Food Chem., 54*, 2288–2297 [in English].
12. El-Mahmood, A., Doughari, J. (2008). Phytochemical screening and antibacterial evaluation of the leaf and root extracts of Cassia alata Linn. *Afr J Pharm Pharacol, 2*, 124–129 [in English].
13. Cavigelli, M.A., Robertson, G.P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology, 81(5)*, 1402–1414 [in English].
14. Chee-Sanford, J.C., Aminov, R.I., Krapac, I.J., Garrigues-Jeanjean, N., Mackie, R.I. (2001). Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Applied and environmental microbiology, 67(4)*, 1494–1502 [in English].
15. Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T., Goldbach, H.E. (2003). Determination of antibiotic residues in manure, soil, and surface waters. *CLEAN–Soil, Air, Water, 31(1)*, 36–44 [in English].
16. Diao, X., Sun, Y., Sun, Z., Shen, J. (2004). Effects of Apramycin on microbial activity in different types of soil. *Ecology and Environment, 13(4)*, 565–568 [in English].
17. Dıaz-Cruz, M.S., de Alda, M.J.L., Barcelo, D. (2003). Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *TrAC Trends in Analytical Chemistry, 22(6)*, 340–351 [in English].
18. Palmada, J., March, R., Torroella, E., Espigol, C., Baleri, T. (2000). Determination of enrofloxacin and its active metabolite (ciprofloxacin) at the residue

level in broiler muscle using HPLC with fluorescence detector. *Proceedings of Euroresidue IV. Residues of Veterinary Drugs in Food. ADDIX, Wijk bij Duurstede, Netherlands* [in English].
19. Goldman, E., Green, L.H. (Eds.). (2015). *Practical handbook of microbiology. Third Edition* [in English].
20. Symochko, L. (2017). Assessment of sorption and toxicity of fluoroquinolone antibiotic in agroecosystems. *Ahroekolohichnyi zhurnal — Agroecological journal, 3*, 147–151 [in English].
21. Symochko, L., Hamuda, H.B. (2015). Microbial monitoring of soil as additional tools for conservation biology. *Obuda University e-Bulletin, 5(1)*, 177–185 [in English].
22. Symochko, L., Meleshko, T., Symochko, V., Boyko, N. (2019). Microbiological control of soil-borne antibiotic resistance human pathogens in agroecosystems. *International Journal of Ecosystems and Ecology Sciences (IJEES), 8(3)*, 591–598 [in English].
23. Ebert, I., Bachmann, J., Kühnen, U., Küster, A., Kussatz, C., Maletzki, D., Schlüter, C. (2011). Toxicity of the fluoroquinolone antibiotics enrofloxacin and ciprofloxacin to photoautotrophic aquatic organisms. *Environmental toxicology and chemistry, 30(12)*, 2786–2792 [in English].
24. Heuer, H., Schmitt, H., Smalla, K. (2011). Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr opin microbiol, 14*, 236–243 [in English].
25. Martínez, J.L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science, 321(5887)*, 365–367 [in English].
26. Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A., Dantas, G. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. *Science, 337*, 1107–1111 [in English].
27. Westergaard, K., Müller, A.K., Christensen, S., Bloem, J., Sürensen, S.J. (2001). Effects of tylosin as a disturbance on the soil microbial community. *Soil Biol. Biochem, 33*, 2061–2071 [in English].
28. Thiele-Bruhn, S., Beck, I.C. (2005). Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere, 59*, 457–465 [in English].
29. Cyco'n, M., Mrozik, A., Piotrowska-Seget, Z. (2019). Antibiotics in the Soil Environment — Degradation and their impact on microbial activity and diversity. *Front. Microbiol, 10*, 338 [in English].

Стаття надійшла до редакції журналу 28.10.2019