IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

Microbial structure of nitrogen utilizers in *Populus nigra* L. compost and vermicompost

To cite this article: E Kornievskaya *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **433** 012001

IOP Conf. Series: Earth and Environmental Science **433** (2020) 012001 doi:10.1088/1755-1315/433/1/012001

Microbial structure of nitrogen utilizers in *Populus nigra* L. compost and vermicompost

E Kornievskaya, A Kurovsky, A Babenko, K Petrochenko and O Sechko

Biological Institute, *Tomsk State University*, 36 Lenin Avenue, Tomsk 634050, Russian Federation

E-mail: e_biotech@mail.tsu.ru

Abstract. *Eisenia fetida* worms affecting the amount of bacteria from four trophic groups in poplar leaf litter vermicompost was investigated. As a control, composted and vermicomposted horse manure was used. The number of ammonifiers in the vermicomposted manure was higher than in the composted; the opposite situation was observed in poplar litter substrates – the number of ammonifiers was higher in compost than in vermicompost. Nitrogen fixers of the genus *Azotobacter* were detected only in vermicomposts and were absent in composts. The activity of nitrogen fixers of the genus *Azotobacter* was higher in vermicomposted poplar leaf litter than in vermicomposted manure. The total amount of ammonifiers and utilizers of inorganic nitrogen is considered to be an integral indicator of the quality of organic fertilizers. This indicator was independent of the presence or absence of worms and was higher in the substrates based on poplar leaf litter compared to the ones based on manure. As the amount of nitrate nitrogen (NO_3-nitrogen) is larger in the leaf litter substrates than in the horse manure substrates, we recommend using composted and vermicomposted poplar leaf litter as organic fertilizer with a microbial structure, which provides enough nitrogen available to plants.

1. Introduction

Greening agriculture results in the increasing demands for organic fertilizers. Among so far insufficiently used resources for the creation of organic fertilizers, an important position is occupied by wood leaf litter, which is annually formed in large quantities and can be considered as a valuable raw material for composting. Leaf litter composts can be obtained using microorganisms on leaf surfaces with or without invertebrate animals. Composts made with earthworms are called vermicomposts. Composts and vermicomposts can have different quality. The microflora of different trophic groups can serve as one of the indicators of the quality of composts and vermicomposts.

In the majority of modern works, the quantitative composition of vermicompost microflora is taken into account by molecular genetic analysis methods [1-4], enzyme activity determination methods [5-7], and the Extraction and Analysis of Microbial Phospholipid Fatty Acids method [8-10], by direct counting using a microscope [11]. In other studies on nutrient media, bacteria, actinomycetes, and fungi were separately counted [6,12,13]. However, such methods do not allow us to reveal the role of certain microorganisms in the nitrogen cycle.

For the diagnosis of the group composition of soil micropopulation, the method of cultivation on various nutrient media has long been used. Meat-peptone agar (MPA), starch-ammonia agar (SAA) and some other nutrient media are used. The quantitative composition of different ecological trophic groups microorganisms is determined on nutrient media. These groups are: free-living nitrogen fixers, ammonifiers, inorganic nitrogen utilizers, and oligotrophs. They play an important role in the nitrogen cycle. [11,14]. The decomposition of organic nitrogen-containing compounds is carried out mainly by ammonifying bacteria, which, using the enzymes released into the substrate, convert the protein into forms available for plant nutrition. The number of groups of microorganisms growing on media with mineral nitrogen sources (for example, SAA) shows the potential ability of the microbial community of the substrate to immobilize nitrogen in the microbial biomass, which increases the content of biologically bound nitrogen and reduces its unproductive losses. The immobilization of mineral nitrogen is carried out by microorganisms capable of assimilating ammonia, nitrate nitrogen. The degree of mineralization, the degree of immobilization, the degree of oligotrophy of the substrates can be estimated by the corresponding coefficients [11,14]. The content of readily available nitrogen in the substrate depends on the degree of immobilization, since 25 to 35% of the total nitrogen of the substrate can be included in the microbial biomass [14]. Mineral nitrogen content is an important indicator of fertilizer value. The work is aimed at assessing the quality of composts and vermicomposts from poplar leaf litter as potential fertilizers based on determining the number of microorganisms of four trophic groups.

2. Materials and methods

In the experiments, vermicompost and compost samples were used before drying. Compost / vermicompost preparation was carried out according to the method described earlier [15,16].

Initial components of composts and vermicomposts: horse manure was used as a non-nutritional component (absorbent material), which was kindly provided by Siberian Research Institute of Agriculture and Peat Federal State Institution and collected at the Ust-Bakchar peat deposit in the Tomsk Region (57°34'47" N; 82°16'22" E).

Horse manure was used as the first (control) component in the form of clean excrement, cleaned of litter. Manure was provided by Bagira, a private farm (Tomsk, Russia (56°26'19" N; 84°59'22" E).

As the second (experimental) nutritional component for vermicomposting, fallen leaves of woody plants of the species *Populus nigra* L. (black poplar), growing on the territory of the Tomsk State University Park, were used (Tomsk, Russia, 56°28′08″ N; 84°56'55" E) [10].

Fallen leaves were collected from September 20 to October 20, 2017 at a positive average daily temperature until a stable snow cover formed. The collected litter was dried in a universal oven MEMMERT UN30 (Memmert, Germany) at a temperature of 85±0.5ºC to constant weight and stored in an air-dry state. Shortly before vermicomposting, the litter was crushed with scissors and tweezers to a size of 1 to 10 mm.

Vermiculture *Eisenia fetida* (Savigny) was provided by the head of the Department of Agricultural Biology of the Tomsk State University, Prof. A S Babenko. The initial population of worms was obtained from Dr. Yu B Morev (Institute of Biology, Academy of Sciences of the Kyrgyz SSR) in 1991 and has been supported up to now.

In total, during the experiment, the following variants of the investigated substrates were obtained:

1. Compost (without the use of vermiculture) based on a mixture of peat and horse manure;
2. Compost (without using vermiculture) based on a mixture of peat and poplar litter;
3. Vermicompost based on a mixture of peat and horse manure;
4. Vermicompost based on a mixture of peat and poplar litter.

Before composting / vermicomposting, the nutritional and non-nutritional components for each option were mixed in a weight ratio of 1:1 – 84 g and 84 g. Thus, the total weight of the dry mixture was 168 g; the volume occupied by each mixture was approximately 2 liters. After mixing, the substrates were placed in 10 l plastic buckets, mixed again and moistened with settled tap water until a moisture content of 77% by weight was reached.

After holding for 1 day, 20 individuals of worms with a total mass of 8 g were introduced into buckets with mixtures for vermicomposting; nothing was added to the control options. The duration of composting and vermicomposting was 85 days.

The amount of ammonifiers was taken into account on a standard medium meat-peptone agar [11]. To determine the amount of inorganic nitrogen utilizers, starch-ammonia agar (SAA) medium was used

[11,14]. The number of oligotrophs was counted on pure agar (PA). Ashby medium was used to determine the amount of nitrogen fixers [11].

The preparation of dilutions and crops on nutrient media MPA, SAA and PA were carried out according to standard methods used in soil microbiology [11]. 1 g of the substrate was triturated in a sterile mortar, transferred to a flask with 100 ml of sterile 0.85% NaCl, shaken for 5 minutes and dilutions were made. Crops were carried out from dilutions 10^{-4} and 10^{-5}. The repetition of the experiment was fourfold.

Actual activity of free-living nitrogen fixers was determined by the method of fouling lumps of substrate on Ashby medium [11]. The lumps of the substrate were laid out on a nutrient medium in a checkerboard pattern at a distance of about 1 cm from each other. The repetition of the experiment was twofold.

Also, simultaneously with the crops, 4 g of each compost and vermicompost sample were taken for moisture determination. The samples were dried to absolutely dry weight at 105ºC.

The statistical processing of the results was carried out using Google spreadsheets and MS Excel.

Confidence intervals were calculated by the formulas [11]:

$$I_{95} = 2\sqrt{\frac{\overline{N}}{n}} = \frac{2\sqrt{T}}{n} \quad (1)$$

where I_{95} is the confidence interval, $\overline{N}$ is the average number of counted colonies, T is the total number of counted colonies, n – is the number of replicates.

Subsequently, the average content of colony forming units (CFU) in one gram of wet substrate was calculated according to the formula [11]:

$$CFU_W = \left(\overline{N} \pm I_{95}\right) \times K \times \frac{1}{V} \quad (2)$$

where $CFUw$ is the number of colony forming units of microorganisms in 1 g of wet substrate, I_{95} is the confidence interval, $\overline{N}$ is the average number of counted colonies, K is the dilution from which the sowing is made with a degree times – 1, V is the volume of seed.

The activity of the *Azotobacter* was calculated as the ratio of the number of lumps of soil with the growth of *Azotobacter* to the total number of lumps in percent [11].

A day after sowing, the moisture content of each substrate was determined by the formula [11]:

$$W = \frac{m_w - m_d}{m_d} \times 100 \quad (3)$$

where W is the moisture content of the substrate in percent, m_w is the mass of the wet substrate, m_d is the mass of the dry substrate.

For each substrate, a moisture coefficient was also calculated by the formula for peat-containing soil [11]:

$$K_w = \frac{100}{100 - W} \quad (4)$$

where K_W is the moisture coefficient, W is the moisture content of the substrate in percent.

The conversion of the number of CFU in 1 g of wet substrate to CFU in 1 g of dry substrate is carried out according to the formula:

$$CFU = CFU_w \times K_w \quad (5)$$

where CFU is the number of colony forming units in 1 g of dry substrate, CFU_w is the number of colony forming units in 1 g of wet substrate, K_W – is the moisture coefficient.

The coefficients of mineralization, immobilization, oligotrophy were also calculated [14].

The mineralization coefficient was calculated by the formula:

$$K_{min} = \frac{CFU_{MPA}}{CFU_{SAA}} \quad (6)$$

where K_{min} is the mineralization coefficient, CFU_{MPA} is the number of ammonifiers, CFU_{SAA} is the number of inorganic nitrogen utilizers.

The immobilization coefficient was calculated by the formula:

$$K_{imm} = \frac{CFU_{SAA}}{CFU_{MPA}} \tag{7}$$

where K_{imm} is the immobilization coefficient, CFU_{SAA} is the number of inorganic nitrogen utilizers, CFU_{MPA} is the number of ammonifiers.

The oligotrophic coefficient was calculated by the formula:

$$K_{olig} = \frac{CFU_{PA}}{CFU_{MPA}} \tag{8}$$

where K_{olig} is the oligotrophic coefficient, CFU_{PA} is the number of oligotrophs, CFU_{MPA} is the number of ammonifiers.

The extracts of experimental substrates were made of dry weighed samples of compost and vermicompost (1 g). Distilled water was added to the weighed samples at a ratio of 1:99 and quantitatively transferred to dark glass bottles, capped, stirred for 3 min in a shaker, and left at a room temperature (+21±3°C) for 24 h. The extracts were then filtered and assayed to measure pH (by potentiometry), Ca^{2+} content [17] (by complexometry), and K^+ and NO_3^- concentrations [18] (by ionometry). Total nitrogen, total phosphorus and total potassium in dry matter were determined in the test laboratory of the Tomsk agrochemical service, on the atomic absorption spectrophotometer QANT-2AT and spectrophotometer UNICO 2100.

3. Results and discussion

The quality of new fertilizers can be assessed by their chemical composition and microflora diversity. Data on chemical parameters of the obtained substrates are shown in Table 1.

Table 1. The physicochemical parameters of composts and vermicomposts.

Variants	Parameter						
	pH of water extract	K^+, [mEq/kg DW]	NO_3^-. [mEq/kg DW]	Ca^{2+}, [mEq/kg DW]	Total N in DM, %	Total P in DM, %	Total K in DM, %
Manure compost	5.75± 0.08	77.41± 9.09	Below detection threshold	32.31± 9.02	1.4± 0.2	0.98± 0.05	1.3± 0.1
Litter compost	6.44± 0.11	138.11± 17.02	225.99± 30.99	54.17± 13.4	1.9± 0.2	0.51± 0.05	1.3± 0.1
Manure vermi-compost	5.64± 0.11	84.27± 4.22	66.06± 8.35	26.00± 4.06	1.5± 0.2	0.72± 0.05	0.51± 0.1
Litter vermi-compost	6.76± 0.25	128.31± 8.66	221.32± 22.78	58.11± 8.03	1.8± 0.2	0.42± 0.05	1.5± 0.1

Notes: mEq, milliequivalent; DW, dry weight; DM, dry matter; the data are presented as means and 95% confidence intervals.

The pH, NO_3^-, K^+ and Ca^{2+} values in aqueous extracts of leaf litter-based substrates were significantly higher compared to similar indicators of manure-based substrates extracts (Table 1). In manure-based compost, the nitrate ion content was below the detection limit (10^{-4} Mol/l). The total nitrogen content was also higher in leaf litter substrates, and the total phosphorus content of manure-based substrates was superior to leaf litter substrates. The total potassium content was the same in leaf litter and manure-based compost, but very different in the different vermicompost. In leaf litter-based vermicompost, the total potassium was almost three times greater than in manure-based vermicompost.

The larger the total number of microorganisms growing on MPA and on SAA, the more mineral forms of nitrogen are in the soil [19]. Therefore, in order to determine which of the composts obtained in the experiment is of great value as a fertilizer, data were obtained on the sum of the number of microorganisms ammonifying and utilizing inorganic nitrogen, the coefficients of mineralization of organic nitrogen, the coefficients of immobilization of inorganic nitrogen, the coefficients of oligotrophy of the actual activity of the *Azotobacter*; the results are presented in Table 2.

Table 2. Microbiological characteristics of composts and vermicomposts.

Variants	The number of CFU per 1 g of compost on the medium				Coefficients			Actual activity of *Azotobacter* %
	Ammonifiers on MPA	Inorganic nitrogen utilizers on SAA	Oligotrophs on the PA	Sum CFU on MPA and SAA	mineralization	immobilization	oligotrophy	
Manure compost	$2.17\times10^9\pm$ 1.04×10^8	$3.78\times10^9\pm$ 1.04×10^8	$3.08\times10^9\pm$ 1.04×10^8	5.95×10^9	0.57	1.74	1.42	0
Litter compost	$9.88\times10^9\pm$ 1.04×10^8	$3.68\times10^9\pm$ 1.37×10^8	$4.65\times10^9\pm$ 1.24×10^8	1.36×10^{10}	2.69	0.37	0.47	0
Manure vermicompost	$2.88\times10^9\pm$ 2.22×10^8	$1.99\times10^9\pm$ 1.36×10^8	$3.30\times10^9\pm$ 1.52×10^8	4.87×10^9	1.44	0.69	1.15	76.67
Litter vermicompost	$4.67\times10^9\pm$ 1.20×10^8	$5.94\times10^9\pm$ 9.99×10^7	$1.99\times10^9\pm$ 1.22×10^8	1.06×10^{10}	0.79	1.27	0.43	88.04

Two variants of composts and two variants of vermicomposts were investigated – on the basis of horse manure and on the basis of poplar litter. The number of ammonifiers was greater in compost and vermicompost based on poplar litter than in compost and vermicompost based on horse manure, which seems somewhat unusual and may indicate low concentration of organic nitrogen compounds in horse manure, which, in turn, presumably can be due to malnutrition of animals. The data of other authors [7,20-22] are somewhat different from the results of the experiment, which can be explained by the use of different base and feed substrates, as well as different types of worms.

Worms influenced the number of microorganisms of all the studied ecological and trophic groups, but in different ways and without definite patterns. So, the number of ammonifiers for the variants with horse manure was increased by worms, but for the variants with poplar litter, they were reduced. Possibly, the lower amount of ammonifying microorganisms in vermicompost with poplar litter compared to compost was due to intensification of the ammonification process, in which, apart from epiphytic microorganisms of peat and leaf litter, the native microflora of earthworms participated.

The amount of inorganic nitrogen utilizers in composts was approximately equal. In vermicompost based on poplar litter, there were more inorganic nitrogen utilizers, in manure based vermicompost less than in compost. The worms did not affect the number of oligotrophs in substrates with manure and approximately halved the number of CFU of oligotrophs in substrates with poplar litter. The worms did not affect the oligotrophicity coefficient; oligotrophicity coefficients were approximately equal in compost and vermicompost based on poplar litter and did not differ much in compost and vermicompost based on manure. The mineralization coefficient of organic nitrogen for substrates with horse manure increased sharply, and the immobilization coefficient of inorganic nitrogen reduced. The worms affected the substrates with poplar litter in the opposite way – the mineralization coefficient of organic nitrogen reduced while the immobilization coefficient of inorganic nitrogen increased.

The worms also influenced the nitrogen fixation process carried out by free-living aerobic nitrogen fixers. Free-living aerobic nitrogen fixers were detected in vermicomposts and were not found in composts, which is consistent with published data [21]. According to previous studies, free-living aerobic nitrogen fixers are one of the groups of microorganisms that are closely associated with earthworms. Actual activity of bacteria of the genus *Azotobacter* was significantly higher in vermicompost based on poplar litter compared to manure-based vermicompost – 88 and 77%, respectively. It can be assumed that the composts obtained in the experiment are less valuable as fertilizers compared to vermicomposts due to the absence of nitrogen-fixing bacteria of the genus *Azotobacter* in composts. However, composts obtained without the participation of cultivated earthworms are widely used by many farms due to the lack of the ability to conduct their own vermiculture on an industrial scale and are used as fertilizer.

There were two options for composting – based on manure and poplar leaf litter. The number of ammonifiers and oligotrophs in the compost based on poplar litter was less, and there were more inorganic nitrogen utilizers than in compost based on manure; however, other characteristics are more important for fertilizer. The oligotrophic coefficient of compost based on manure is more than unity – 1.47, which indicates the predominance of the conversion of organic substances into carbon dioxide over the mineralization of organic nitrogen in this substrate. Despite the fact that the number of oligotrophs in compost based on poplar litter was greater than in compost based on manure, the oligotrophic coefficient of compost based on poplar litter is less than unity – 0.47, which indicates the high preservation of nutrients in compost based on poplar litter. The mineralization coefficient of organic nitrogen compounds is less than unity for compost based on manure and much more than unity for compost based on poplar litter – 0.57 and 2.69, respectively, which indicates that compost based on poplar litter contains significant amounts of ammonia nitrogen, which can be used by plants. The total amount of ammonifiers and utilizers of inorganic nitrogen is also significantly higher in compost based on poplar litter than in compost based on manure – 1.36×10^{10} and 5.95×10^{9} CFU per 1 g of substrate, respectively, which according to published data [19] indicates to a higher content of mineral nitrogen compounds in compost based on poplar litter. Thus, compost and vermicompost based on peat and poplar litter are more suitable for use as fertilizers than compost and vermicompost based on peat and horse manure.

4. Conclusion

Nitrogen fixers of the genus *Azotobacter* were found only in vermicomposts. The actual activity of free-living aerobic nitrogen fixers was higher in vermicompost from poplar leaf litter than in vermicompost from manure. Ammonifiers, inorganic nitrogen utilizers and oligotrophs were identified in all composts and vermicomposts. The total amount of ammonifiers and utilizers of inorganic nitrogen was higher in composts and vermicomposts from poplar litter. This indicates a higher content of valuable mineral nitrogen for plants in compost and vermicompost from poplar leaf litter than in manure compost and vermicompost. According to the totality of the results of quantitative studies of microorganisms of the four trophic groups, the best nitrogen fertilizers are vermicompost and compost based on poplar leaf litter.

References

[1] Domínguez J, Aira M, Kolbe A R, Gómez-Brandón M and Pérez-Losada M 2019 Changes in the composition and function of bacterial communities during vermicomposting may explain beneficial properties of vermicompost. *Sci. Reports* **9** 11 https://doi.org/10.1038/s41598-019-46018-w

[2] Chen Y, Chang S K C, Chen J, Zhang Q and Yu H 2018 Characterization of microbial community succession during vermicomposting of medicinal herbal residues. *Bioresource Technology* **249** 542 doi: 10.1016/j.biortech.2017.10.021

[3] Lv B, Xing M, Yang J and Zhang L 2015 Pyrosequencing reveals bacterial community differences in composting and vermicomposting on the stabilization of mixed sewage sludge and cattle dung. *Appl. Microbiol. Biotechnol.* **99** 10703 DOI: 10.1007/s00253-015-6884-7

[4] Huang K, Li F, Wei Y, Chen X and Fu X 2013 Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia foetida*. *Bioresource Technology* **150** 235 DOI: 10.1016/j.biortech.2013.10.006

[5] García-Sánchez M, Taušnerová H, Hanč A, and Tlustoš P 2017 Stabilization of different starting

materials through vermicomposting in a continuous-feeding system: Changes in chemical and biological parameters. *Waste Management* **62** 33 DOI: 10.1016/j.wasman.2017.02.008

[6] Emperor G N and Kumar K 2015 Microbial Population and Activity on Vermicompost of *Eudrilus eugeniae* and *Eisenia fetida* in Different Concentrations of Tea Waste with Cow Dung and Kitchen Waste Mixture. *Int. J. Curr. Microbiol. App. Sci.* **4**(10) 496

[7] Srivastava P K, Singh P C, Gupta M, Sinha A, Vaish A, Shukla A, Singh N and Tewari S K 2011 Influence of earthworm culture on fertilization potential and biological activities of vermicomposts prepared from different plant wastes. *J. Plant Nutr. Soil Sci.* **174**. 420 https://doi.org/10.1002/jpln.201000174

[8] Neher D A, Cutler A J, Weicht T R, Sharma M and Millner P D 2019 Composts of poultry litter or dairy manure differentially affect survival of enteric bacteria in fields with spinach. *J. of Applied Microbiology* **126** 1910 doi:10.1111/jam.14268

[9] Zhao C, Wang Y, Wang Y, Wu F, Zhang J, Cui R, Wang cnd Mu H 2018 Insights into the role of earthworms on the optimization of microbial community structure during vermicomposting of sewage sludge by PLFA analysis. *Waste Management* **79** 700 DOI: 10.1016/j.wasman.2018.08.041

[10] Gómez-Brandón M, Lores M, Domínguez J 2013 Changes in chemical and microbiological properties of rabbit manure in a continuous-feeding vermicomposting system. *Bioresource Technology* **128** 310 doi: 10.1016/j.biortech.2012.10.112

[11] Tereshchenko N N, Akimova E E, Minaeva O M 2011 Microbiology workshop to assess soil fertility and soil quality (Tomsk: TSU) p 96 [in Russian]

[12] Karmegam N, Vijayan P, Prakash M, Paul J A J 2019 Vermicomposting of paper industry sludge with cowdung and green manure plants using *Eisenia fetida*: A viable option for cleaner and enriched vermicompost production. *J. of Cleaner Production* **228** 718 doi: 10.1016/j.jclepro.2019.04.313

[13] Ganguly R K and Chakraborty S K 2018 Assessment of microbial roles in the bioconversion of paper mill sludge through vermicomposting. *J. of Environmental Health Sci. and Eng.* **16** 205 doi: 10.1007/s40201-018-0308-4

[14] Stakhurlova L D, Shcheglov D I and Svistova I D 2007 Biological Activity as an Indicator of Chernozem Fertility in Different Biocenoses. *Eurasian Soil Science* **40**(6) 694 doi: 10.1134/S1064229307060117

[15] Kurovsky A V, Petrochenko K A, Babenko A S and Yakimov Yu E 2016 The Peculiar Physicochemical and Agrochemical Properties of Vermiculture-processed Poplar Leaf Litter. *Key Eng. Mater.* **683** 519 doi:10.4028/www.scientific.net/KEM.683.519

[16] Petrochenko K A, Kurovsky A V, Babenko A S, Yakimov Yu E 2015 Leaf Litter-Based Vermicompost As Promising Calcium Fertilizer. *Tomsk State University Report. Biology* **2 (30)** 20 doi: 10.17223/19988591/30/2 [in Russian]

[17] Panumati S, Chudecha K, Vankhaew P, Choolert V, Chuenchom L, Innajitara W and Sirichote O 2008 Adsorption efficiencies of calcium (II) ion and iron (II) ion on activated carbon obtained from pericarp of rubber fruit Songklanakarin. *J. Sci. Technol*. **30** 179

[18] Moore E V 1969 *Studies with ion-exchange calcium electrodes in biological fluids: some applications in biomedical research and clinical medicine*. In: R.A. Durst (Ed.), Ion-selective electrodes National Bureau of Standards (U.S., Washington) pp 215–286

[19] Dymova L V, Nesterova L B 2006 Interrelation of the number of microorganisms and mineral forms of nitrogen in the soil. *Agrochemical Bulletin* **4** 8 [in Russian]

[20] Tereshechenko N N 2011 Influence of the psychophilic conditions of cultivation of earthworms on the quality of vermicompost and the level of its biological activity. *Achievements of Science and Technology of Agro-Industrial Complex* **7** 21 [in Russian]

[21] Tereshchenko N N, Yunusova T V, Pisarchuk A D 2012 Microorganisms - Unique Indicators of Vermicompost Quality. *Achievements of Sci. and Technol. in Agroindustrial Complex* **5** 58 [in Russian]

[22] Yakushev A V, Byzov B A, Blagodatsky S A 2009 The Effect of Eathworms on the Physiological State of the Microbial Community at Vermicomposting. *Microbiology* **78**(4) 510 DOI: 10.1134/S002626170904016X