Agraarteadus
1 • XXXIII • 2022 95–100

Journal of Agricultural Science
1 • XXXIII • 2022 95–100

EARTHWORMS (*Oligochaeta: Lumbricidae*) AND HEAVY METALS: CONTENT AND BIOACCUMULATION IN THE BODY

Mzia Kokhia, Manana Lortkipanidze, Oleg Gorgadze, Madona Kuchava, David Nebieridze

Institute of Zoology, Ilia State University, 3/5 K. Cholokashvili Ave, 0162, Tbilisi, Georgia

Saabunud: Received: 04.02.2022

Aktsepteeritud: Accepted: 01.05.2022

Avaldatudveebis: Published online: 01.06.2022

Vastutavautor: Corresponding author: Mzia Kokhia

E-mail: mzia.kokhia@iliauni.edu.ge

ORCID:
0000-0002-9654-7670 (MKo)
0000-0002-7075-5340 (ML)
0000-0002-5963-1777 (OG)
0000-0003-1295-1957 (MKu)
0000-0001-7768-9414 (DN)

Keywords: earthworms, heavy metals, copper, zinc, lead, *Aporrectodea rosea*, *Eisenia veneta*, *Allolobophora chlorotica*.

DOI: 10.15159/jas.22.20

ABSTRACT. Nowadays, when one of the most significant problems for mankind is saving the soil from pollution. It is well-known that one of the most important means of soil rehabilitation and remediation are soil inhabitants, their biodiversity and products of their life activity. Given the significant role of soil inhabitants in soil formation processes, it is important to consider their role in the processes of reprocessing and bioaccumulation of heavy metals. That especially concerns the earthworms, whose role in soil formation and maintenance of natural fertility is well-known and causes the interest of soil scientists and ecologists. The paper shows the degree of bioaccumulation of heavy metals (copper, zinc and lead) in the body of earthworms. Study involved three species of earthworms, which were collected in the vicinity of Tbilisi – *Aporrectodea rosea* (Savigny, 1826), *Eisenia veneta* (Rosa, 1886) and *Allolobophora chlorotica* (Savigny, 1826) – showed that earthworms of different species accumulate different amounts of heavy metals – copper, zinc and lead and, depending on the species, after being placed in heavy metals solutions, they demonstrate the different intensity of movement. The amount of heavy metals in the body of an earthworm depends on the structure of the body tissues and maybe on the structure of the skin-muscular sac.

Introduction

Soil is a layer of terrestrial biogeocenoses, where the transformation, decomposition, mineralization and humification of organic matter take place. In terrestrial ecosystems, there is no layer comparable to the soil, which would correspond to such a large number of ecological requirements of various groups of animals. Soil is one of the most valuable natural resources used by man for food production. The role of earthworms in soil formation and maintenance of natural soil fertility is well-known and interesting for soil scientists and ecologists. The knowledge gained to study earthworms is actively used in carrying out activities for environmental protection, in the development of programs for the restoration of natural ecosystems. The paper deals with the problem of soil and heavy metals, which are toxic, even at low concentrations and unlike organic compounds cannot be biodegraded into less harmful materials (Mudhoo, Mohee, 2011).

Nowadays in the presence of climate change, land degradation and biodiversity loss, soils have become one of the most vulnerable resources in the world. Notwithstanding the enormous scientific progress made to date, protection and monitoring of soil resources at national and global levels still face complicated challenges impeding effective on-the-ground policy design and implementation that varies widely from region to region. There is still insufficient global support for the protection and sustainable management of the world's soil resources.

Our society still is not sufficiently aware of the excessive danger of agriculture chemicalization. Currently, the dearth of healthy soil is a major problem resulting from enormous waste generation. Uncontaminated soil is vital for the survival of living things.

Caring for high productivity is primarily associated with the improvement of the biosphere. The continuous use of chemical fertilizers leads to a decrease in soil organic matter (SOM) combined with a deterioration in the quality of agricultural soils reducing their fertility (increases soil erosion and reduces humus formation). (Pahalvi *et al*., 2021).

Soil loss problem is one major issue, as soil formation is very long (thousands of years are needed to form only a few centimetres of soil depth) and 24 billion tonnes of topsoil are lost every year (Guterres, 2019). Soil erosion is a major global soil degradation threat to land, freshwater, and oceans (FAO, 2015; Borrelli *et al*., 2020). The consequences for farmers and communities are too important: loss of fertility, landslide, sediments accumulation in rivers, depletion of deepwater reservoirs and water pollution.

Soil-forming rocks are one of the main sources of chemical elements, which, due to the vital activity of earthworms, turn into vermicomposts – peat-like, dark and homogeneous mixtures (Gupta *et al*., 2014; Babita, Thakur, 2015). At the same time, earthworms processing manure in both liquid and solid form produce vermicompost, which is an excellent fertilizer. Plant cells contain about 40 chemical elements – almost all found in the soil, but for normal growth and fruiting plants need only 16 of them (Semhi *et al*., 2009). These are the elements absorbed by plants from air and water – oxygen, carbon and hydrogen, and elements absorbed from the soil, among which macro elements are distinguished – nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and heavy metals – molybdenum, copper, zinc, manganese, iron, boron and cobalt.

Based on the foregoing, each research conducted to study the problems of soil improvement and rehabilitation is very important and not only for Georgia. The importance of this problem has forced us to provide the study with the role of earthworms and vermitechnologies generally concerning soil remediation. In this case, in the process of vermicomposting, there were included the earthworms of the following species: *Eisenia andrei* (Bouche, 1972), *E. lagodechiensis* (Michaelsen, 1910) and *E.veneta* (Rosa, 1886).

Previous research (Striganova, 1980; Kokhia, 2011; 2021) has shown the importance of soil dwellers and their livelihoods for improving and maintaining soil quality and fertility. It is well known that in this case, earthworms play an important role in the soil formation process. Due to the high rate of food intake and motor activity, worms modify their habitat by changing the kinetics of various soil processes that directly or indirectly affect the growth of fertility (Bityutskiy, Kaidun, 2008). At the same time, the earthworms are an excellent indicator of soil conditions given their many important roles and sensitivity to problems such as low pH, compaction, waterlogging and intensive cultivation.

An important group of indicators that are the result of human influence on ecosystems and determine the ecological condition of the soil include soil erosion, fertilizer doses, soil profile, ploughed up areas, quality and quantity of organic and mineral compounds earthworms.

The results of numerous scientific studies (Edwards *et al*., 2010; Dai *et al*., 2004) showed a close correlation between the content of humus in various types of soil and the magnitude of the yields obtained, all other conditions being equal, and the crop technology (Maksimova, 2011). An indicator of the humus level reduction in the soils is one of the most important diagnostic features for the classification of soils with varying degrees of erosion. In this case, we tried to show that the soil invertebrates with their life activity have a significant role in the soil making process. The main objective of the research was to study the role of earthworms, as one of the main representatives of soil fauna, in soil enrichment and bioremediation. Taking into account the importance of their life activity and their role in the processing of all constituent elements of the soil, we tried to determine and evaluate their role in the processing of heavy metals.

Materials and Methods

Given the importance of the earthworms' vital activity in soil formation processes, we conducted a series of experiments to determine the rate of accumulation of some micronutrients.

Previous studies (Edwards *et al.*, 2010) have shown that the composition of vermicompost is represented by the following constituent elements (Maksimova, 2011) (Table 1).

Table 1. Vermicompost chemical composition

Elements	Amount, %
Humidity	65–70
Ash content	10–15
Humus	22–26
Total nitrogen	2.3–2.6
Total phosphorus	2.0–2.2
Total potassium	1.9–2.1
Ca	0.6–0.7
Mg	0.10–0.12
Cu	0.0011–0.0013
Zn	0.016–0.017
C/N	10–12

Analyzes to determine the chemical composition of vermicompost were carried out at the Laboratory of Vermitechnology of Scientific and Practical Center of the National Academy of Science of Belarus for Bioresources.

Using three species of earthworms – *Aporrectodea rosea* (Savigny, 1826), *Eisenia veneta* (Rosa, 1886) and *Allolobophora chlorotica* (Savigny, 1826), with the atomic-absorption spectrometry method, the content of heavy metals (Cu, Zn, Pb) were determined and compared in different species of earthworms. The material was collected near Tbilisi (41°43´00.07´´N; 44°49´30.07´´E) and 30 samples were analyzed.

A series of experiments were carried out. Acids of H_2SO_4, HNO_3, and HCl (Chemapol, Prague, Czech)

were used in the experiments. Microelements were determined on an atomic-absorption spectrophotometer Opton FMD3 (Germany). The earthworms of the same species were placed in a solution with different concentrations of lead, zinc and copper (2000, 1500 and 50 μg). Distilled water was used as a control. Samples were processed in the following way: a mixture of 1.5 ml of concentrated HNO_3 and 6 ml of concentrated HCl was filled with worms dried to constant weight at a temperature of 105 °C and placed in Kjeldahl flasks (50 ml). After an hour, the flasks were carefully heated on closed electric stoves, avoiding the charring of the mixture, in the case of charring a few drops of HNO_3 were added to it (Reznichenko, 2016; Baibotaeva *et al*., 2019).

It should be noted that during the experiment we also observed the movement of worms, depending on their sizes and concentration of solutions.

By dry ashing, the samples were brought to full discolouration and then cooled. After cooling, several ml of water was added. All contents were mixed and filtered through an ashless filter Whatman paper (Germany) into volumetric flasks, and then the concentration of heavy metals was measured using a calibration curve (Korostelev, 1988).

Statistical analysis carried out by the method of linear regression and analysis of variance in R showed that there are no statistically significant differences between earthworms' species in terms of the content of trace elements in them, however, it should be noted that the intensity of movement of earthworm species differed depending on the concentration of heavy metals in solution. R version 4.2.0 (R Core Team, 2022) and the *multicompView* package (Graves *et al.*, 2019) were used to conduct the statistical analyses.

Results and Discussion

Earthworms of different species accumulate different amounts of copper, lead and zinc from solutions with different concentrations of these elements. The analysis of the data shows that the loss amount of lead and copper during the experiment depends on the species of earthworms and the duration of their storage in solutions with heavy metals. At the same time, the zinc loss for all three species of earthworms being placed in water is 40%, despite the solution concentration. The heavy metals distribution in the soil is important for the bioaccumulation by earthworms, as the main pathways for chemical absorption are the skin for soluble elements, gut transit and digestion (Weltje, 1998). There is a factor in the connection of heavy metals with tissues and the density of the skin-muscular sac.

The choice of these heavy metals for the experiments was due to their great importance for soil and soil fauna. The soil contains various copper compounds with specific chemical composition, solubility and accessibility to organisms. Copper is actively involved in the processes of tissue respiration and is a part of the enzyme ceruloplasmin (copper oxidase), which catalyzes the oxidation of vitamin C (Reznichenko, 2016; Baibotaeva *et al*., 2019).

No less important is zinc, an essential heavy metal for all living organisms. The content of gross zinc in the soil depends on the conditions of soil formation (Reznichenko, 2016; Baibotaeva *et al*., 2019).

Lead was not chosen as a vital element, unlike zinc, which is part of 45 enzymes and plays a huge role in changing their properties (Bityutsky, Kaidun, 2008). It was chosen as a toxic heavy metal, the amount of which has increased significantly in soils as a result of the development of industry, transport and chemicalization of agriculture. This factor is obvious and caused the unequal content, absorption and subsequent retention of heavy metals – copper, zinc and lead. There are various compounds of copper in the soil, specific for their chemical composition, solubility and accessibility to organisms. Have to be noted that some heavy metal ions are very important micronutrients for plant and human metabolism and are natural substances found usually at low levels, in soils (Gamalero, *et al*., 2009; Lokhande, *et al.*, 2010).

The conducted determinations showed that heavy metals in the bodies of three species of earthworms' *A. chlorotica*, *E. veneta* and *A. rosea* are contained in the following amounts (Fig. 1). As above mentioned the statistical analysis no statistically significant differences between earthworms' species.

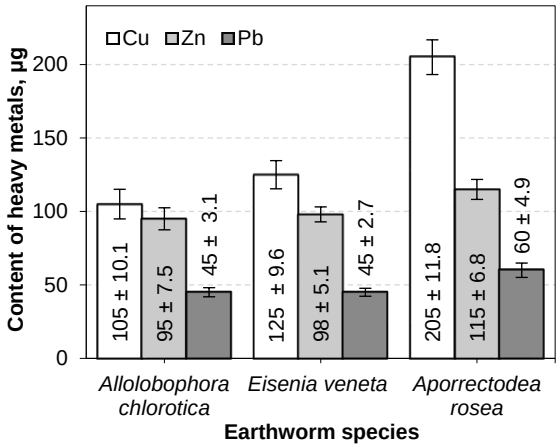

Figure 1. The content of heavy metals in the sample

Accumulation of heavy metals in the body of the earthworm and their retention

It should be mentioned, that earthworms are one of the most important indicators of soil health, and there have been many studies performed on the response to heavy metals of the earthworms and their role during their processing (Dai *et al*., 2004; Nahmani *et al*., 2007; Azizi *et al*., 2013; Panasin *et al*., 2015). The experiments have shown that in solutions containing lead ions, the mobility of worms initially increases, but after an hour the worms of the species *A. chlorotica* stop moving. *E. veneta* worms continue to move vigorously for 5–6 hours, and then stop moving from the very beginning in a solution with a concentration of 2000 μg,

and then in more dilute solutions. The lead solution with a concentration of 2000 μg A. *rosea* worms continued intensive movement within 12 hours, and then stop moving.

As above mentioned, the statistical analysis did not show statistically significant differences between earthworms' species.

In solutions with zinc, all three species of earthworms' mobility continued for two days, while its intensity decreased with increasing concentration. Similarly, worms behave in solutions containing copper.

The comparisons showed that the mobility of the representatives of the species *A.rosea* is significantly higher than the mobility of *E.veneta*, or in particular, the species of the worms *A. chlorotica*, which can be explained by the different levels of skin-muscular sac development in different species of worms.

Interestingly, the absorption of zinc depends obviously on the size of the worm. We used worms of *A. rosea* species of different lengths. It turned out that a 4 cm worm absorbs 250 μg of zinc from a solution concentration of 2000 μg and a 9 cm worm size absorbs an amount of 800 μg.

Retention of heavy metals by the body of earthworms

The worms that absorbed ions of heavy metals were kept in clear water for 15 minutes and then checked the number of heavy metals that had passed into the water. It turned out that different species of earthworms accumulate and contain different amounts of heavy metals (Fig. 1). This effect is of great importance for soils since the water rinses heavy metals in different quant–ities, and, as a result, their migration occurs in the soil.

In addition, when taking earthworms or the soil itself for analysis, it is necessary to consider the factor of rain and watering. To achieve the effectiveness of the soil rehabilitation adding extra vermiculture should be added and seed new plants possessing special biological properties. The improvement of the grass grounds productivity is due to active organic fertilizers concentrated on the soil's surface (humus) and collecting the chemical energy caused by this process. Acceleration of humus amount addition will be possible by introducing vermiculture. We should emphasize that vermiculture should be specially chosen as "Regional vermiculture", a fluctuation of worms' number depending on the characteristics of the soil quality.

The development of vermiculture allows the growing of different types of invertebrates and soil microorganisms. It is well known that biodegradation of organic microorganisms promotes soil deactivation *in-* and *ex-situ*. The number of invertebrates is limited by soil qualities.

Ploughing-up, moistening, cultivation, vegetable cover and the presence of organic fertilizers – the earthworms, will help to secure the effect of soil fertility. Some factors require specific studies so that researchers can better understand the metabolism involved in the process (Singh, Singh, 2018). Vermicomposting protects the environment and augments crop productivity, which is why it is very interesting to study the vermicomposting process. The humus helps the soil to keep feeding elements in an available plant form. The existence of appropriate vegetation cover is necessary for the growth of vermiculture.

It is not excluded that several items from the proposed recommendations might be the theme of our further research.

All this indicates the importance of soil humus content. Thus, the enrichment of soil with organic matter is one of the important means of saving the soil from erosion.

Composting is defined as a bio-oxidative process leading to organic matter mineralization and transformation (Zucconi, de Bertoldi, 1987).

Conclusion

At last, we conclude that one of the most important ways to counteract soil erosion is the protection of soil macrofauna and its biodiversity.

The experimental results showed that the earthworms of different species – *Aporrectodea rosea*, *Eisenia veneta* and *Allolobophora chlorotica* – contain different amounts of heavy metals and, when placed in solutions, exhibit the different intensity of movement depending on the type of worms and the concentration of the solution.

The amount of heavy metals (lead, zinc, copper) in the body of worms depends on the structure of their tissue and maybe on the skin-muscular sac structure too.

Earthworms of various species from solutions with different concentrations absorb different amounts of heavy metals and this absorption is dependent on its size. Earthworms of various species, when placed in water, lose the different amounts of accumulated heavy metals, which is of great importance in the migration of heavy metals in the soil.

Acknowledgements

The paper was executed in the framework of the Project "Fauna of Georgia – Current Trends and Conservations". The authors have appreciated their colleagues at the Institute of Zoology for their support in providing this study.

The authors would like to say thanks to Dr Svetlana Maksimova from the Scientific and Practical Center of the National Academy of Sciences of Belarus for Bioresources for her support in providing this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement

The authors confirm that all experiments were performed in accordance with relevant guidelines and regulations.

Author contributions

DN, MKo – study conception and design;
OG – acquisition of data;
ML, MKo – analysis and interpretation of data;
MKo, MKu– drafting of the manuscript;
MKo – critical revision and approval of the final manuscript.

References

Azizi, A.B., Lim, M.P.M., Noor, Z.M., Noorlidah, A. 2013. Vermiremoval of heavy metal in sewage sludge by utilizing *Lumbricus rubellus*. – Ecotoxicology and Environmental Safety, 90:13–20. DOI: 10.1016/j.ecoenv.2012.12.006

Babita, A.N., Thakur, M. 2015. Organic farming: A holistic approach towards sustainable fruit production. European Journal of Pharmaceutical and Medical Research. 2(6):108–115.

Baibotaeva, A., Kenzhaliyeva, G., Bosak, V. 2019. Tyazhelye metally v pochvakh urbanizirovannykh territorij. Heavy metals in soils of urbanized territories]. – Vestnik BGSHA: Nauchno-Metodicheskij Zhurnal Belorusskoj Gosudarstvennoj sel'skohozjajstvennoj Akademii [Bulletin BGSHA: Scientific and Methodological Journal of the Belarusian State Agricultural Academy], 4:126–130. [In Russian].

Bityutskiy, N.P., Kaidun, P.I. 2008. Vliyanie dozhdevykh chervej na podvizhnost` mikroelementov v pochve i ikh dostupnost` rasteniyam [Influence of earthworms on the mobility of heavy metals in the soil and their availability to plants]. – Pochvovedenie [Soil Science], 12:1479–1486. [In Russian].

Borrelli, P., Robinson, D.A., Panagos, P., Lugato, E., Yang, J.E., Alewell, C., Wuepper, D., Montanarella, L., Ballabio, C.2020. Land use and climate change impacts on global soil erosion by water (2015-2070). – Proceedings of the National Academy of Sciences. (PNAS), 117(36): 21994–22001. DOI: 10.1073/pnas. 2001403117

Dai, J., Becquerb, T., Rouillerc, J.H., Reversata, G., Bernhard-Reversata, F., Nahmania, J., Lavelle, P. 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. – Soil Biology & Biochemistry 36:91–98. DOI: 10.1016/j.soilbio.2003.09.001

Edwards, C.A., Arancon, N.Q., Sherman, R.L. (Eds.). 2010. Vermiculture technology: Earthworms, organic wastes, and environmental management (1st ed.). – CRC Press, 624 p. DOI: 10.1201/b10453

FAO. 2015. Healthy soils are the basis for healthy food production. – Food and Agriculture Organization of the United Nations, Rome, Italy, 4 p. – https://www.fao.org/documents/card/en/c/645883cd-ba28-4b16-a7b8-34babbb3c505/ Accessed on 30/02/2022

Gamalero, E., Lingua, G., Berta, G., Glick, B.R. 2009. Beneficial role of plant growth promoting bacteria and arbuscularmycorrhizal fungi on plant responses to heavy metal stress. – Canadian Journal of Microbiology, 55:501–514.

Graves, S, Piepho, H, Dorai-Raj, LSwhfS. 2019. _multcompView: Visualizations of Paired Comparisons_. R package version 0.1-8, https://CRAN.R-project.org/package=multcompView

Guterres, A. 2019. 24 billion tons of fertile land lost every year, warns UN chief on World Day to Combat Desertification. – UN News, United Nations https://news.un.org/en/story/2019/06/1040561 Accessed on 30/02/2022

Gupta, R., Yadav, A., Garg, V.K., 2014. Influence of vermicompost application in potting media on growth and flowering of marigold crop. –International Journal of Recycling of Organic Waste in Agriculture, 3(1):47. DOI: 10.1007/s40093-014-0047-1

Kokhia, M. 2011. Sakartvelos maghalmtiani sadzovrebi da mati reabilitatsia: sakartvelos maghalmtiani sadzovrebis makroedaphoni, struktura, kveba, monitoringi (aghmosavlet sakartvelo). [Georgian high mountain pastures biodiversity: Macroedaphone, structure, feeding activity and monitoring]. – Contour, Tbilisi, Georgia, 110 p. [In Georgian]

Kokhia, M., Lortkipanidze, M., Gorgadze, O., Kuchava, M., Nebieridze, D. 2021. Soils of Georgia highlands and their biodiversity – In Proceedings of Global Symposium on Soil Biodiversity "Keep soil alive, protect soil biodiversity". Rome, Italy. 19–22 April, 2021. Food and Agriculture Organization of the United Nations, pp. 35–40. DOI: 10.4060/cb7374en

Korostelev, P.P. 1988. Khimicheskiy analiz v metallurgii. [Chemical analysis in metallurgy]. – Uchebnoe posobiye dlya SPTU. - 2-ye izdaniye pererab i dop. [Textbook for SPTU. - 2nd edition revised and add.], 384 p. [In Russian]

Lokhande, R.S., Singare, P.U., Andhale, M.L., Acharya, A., Nair, A.G.C., Reddy, A.V.R. 2010. Determination of macro, micro nutrient and trace element concentrations in Indian medicinal plants using instrumental neutron activation analysis and atomic absorption spectroscopy techniques. – International Journal of Food Safety, Nutrition and Public Health, 3:33–44.

Maksimova, S. 2011. Progress in vermicomposting in Belarus, Russia and Ukraine. – Vermiculture Technology. CRC Press. pp. 565–579. DOI: 10.1201/b10453

Mudhoo, A., Mohee, R. 2011. Fate of heavy metals exposed to composting environments and composts. – Dynamic Soil, Dynamic Plant; 5 (Special Issue 2). – In book: Global Science Books, pp. 25–35.

Nahmani, J., Hodson, M.E., Black, S. 2007. A review of studies performed to assess metal uptake by earthworms. – Environmental Pollution, 145:402–424. DOI: 10.1016/j.envpol.2006.04.009

Pahalvi, H.N., Majeed, L.R., Rashid, S., Bisma, N., Kamili, A.N. 2021. Chemical fertilizers and their impact on soil health. – Microbiota and Biofertilizers, 2:1–20. DOI: 10.1007/978-3-030-61010-4_1

Panasin, V.I., Shatokhin, A.Yu., Rymarenko, D.A. 2015. Tsink v pochvah selskohozyaystvennyh ugodiy kaliningradskoy oblasti [Zinc in agricultural soils of Kaliningrad region]. – Agrohimicheskij vestnik [Agrochemical Bulletin], 5:45–48. [In Russian].

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Reznichenko, I.S. 2016. Akkumulyacziya tyazhelykh metallov pochvenno-podstilochnym tipom dozhdevykh chervej na primere Perelia diplotetratheca (Perel, 1967) v usloviyakh zagryazneniya tochechnym istochnikom emissii [Accumulation of heavy metals soil-litter type of earthworms on example Perelia diplotetratheca (Perel, 1967) in conditions a polluted point source emission]. – Sovremennye problemy nauki i obrazovanija [Modern Problems of Science and Education], 6:1–8. [In Russian].

Semhi, K., Abdalla, O. A. E., Khirbash, S. Al, Khan, T., Asaidi, S., Farooq, S. 2009. Mobility of rare earth elements in the system soils–plants–groundwaters: a case study of an arid area (Oman). – Arabian Journal of Geosciences, 2(2(sad):143–150. DOI: 10.1007/s12517-008-0024-y

Singh, A., Singh, G.Sh. 2018. Vermicomposting: A sustainable tool for environmental equilibria. – Environmental Quality Management. 27:23–40; DOI: 10.1002/tqem.21509

Striganova, B.R. 1980. Pitanie pochvennykh saprofagov [Feeding of soil saprophages] – Nauka, Moscow, Russia, 248 p. [In Russian]

Zucconi, F., de Bertoldi, M.D. 1987. Organic waste stabilization throughout composting and its compatibility with agricultural uses. – In Global bioconversion.Wise D.L. (ed). **–** CRC, Boca Raton, USA, pp. 109–137.

Weltje, L., 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. – Chemosphere 36:2643–2660.