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MINIREVIEW

Earthworms - role in soil fertility to the use in medicine and as a food

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Abstract

Earthworms are important regulators of soil structure and dynamics of soil organic matter. They are a major component of soil fauna communities in most ecosystems and comprise a large proportion of macro fauna biomass. Their activities are beneficial because they can enhance soil nutrient cycling through the rapid incorporation of detritus into mineral soil. However, mucus production associated with water excretion in earthworm guts also enhances the activity of other beneficial soil microorganisms. Earthworms alter soil structure, water movement, nutrient dynamics and plant growth. The medical value of earthworms has been known for centuries. The extracts prepared from earthworm tissues have been used for the treatment of numerous diseases since they are valuable source of proteins, peptides, enzymes and physiologically active substances. Several studies have shown that the earthworm extracts contain different macromolecules which exhibited the variety of activities, such as antioxidative, antibacterial, antiinflammatory, anticancer etc. Some of these activities are involved in wound healing process, using the earthworm preparation. In some countries the earthworms are used as a part of healthy food. They have very high nutritive value because their bodies contain the high percentage of various proteins. Besides the human food, the earthworms are used in the feeding of animals (fish, chicken, etc.).

Key Words: earthworms; food; medicine; soil

Introduction

From evolutionary point of view the earthworms (EWs) are very old species. They survived over a million years due to their ability of adjusting to different environmental conditions. Their living place is damp soil enriched with organic substances. They are breading through the skin, and they are very sensitive on changing the temperature, the light and on the touch. During the winter they are burying in deeper layer to protect from low temperature, and during the summer and dryness to protect from dehydratation (Brusca and Brusca, 2003).

Their muscle system is built with circular (segments) and longitudinal muscles and with their shrinkage and spread the EWs are able to move. The body of EWs is covered with small fluffs, which is important in environmental adjustment and for search of the food in the soil. Waste products of EW diet enrich the soil with nutritive substances, which stimulate the growth of plants. However, the EWs are very important source of diet for numerous animals in the soil. They are hermaphrodites, meaning that the each individual has both female and male systems for reproduction. This characteristic also contributes to well environmental adjustment, because that the animals are reproduced very easily. The eggs are hutched in soil protected with capsule, which arises from the secrets from *clitellum* (front part). The capsule protects the young worm until complete maturation (Pechenik, 2009).

The EWs are the major decomposers of dead and decomposing organic matter, and acquire their nutrition from the bacteria and fungi that grow upon these materials. They decompose the organic matter and make the major contributions to recycling the containing nutrients. The EWs occur in the warmest soils and many tropical soils. They are divided into 23 families, more than 700 genera, and more than 7,000 species. Their size ranges from an inch to two yards, and are found seasonally at all depths of the soil (Pechenik, 2009).

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This review partially comprises the published information about versatile use of the earthworms.

Earthworms in soil fertility

The role of EWs in soil fertility is known since 1881, when Darwin published the book entitled "The formation of vegetable mould through the action of worms with observations on their habits". Thereafter, several studies have been published (Wardle, 2007). The soil macro invertebrates play a key role in soil organic matter (SOM) transformations and nutrient dynamics through perturbation and the production of biogenic structures, resulting in amelioration of soil fertility and land productivity (Mora *et al*., 2003; Barious, 2007). SOM is an important active carbon reservoir and fundamental component for soil fertility. It contributes to a number of soil properties, such as soil structure, porosity, water retention, cationic exchange and pH buffering capacity (Lal, 2004; Weil and Magdoff, 2004). For that purpose the soil aggregates have been proposed as the structural units within the soil that control the dynamics of SOM and nutrient cycling (Tisdall and Oades, 1982; Lavelle and Spain, 2001; Chevallier *et al*., 2004; Fonte *et al*., 2007; Hong *et al.*, 2011). The major component of soil fauna communities are the EWs. In cultivated tropical soil organic matter they are often related to fertility and productivity. In such ecosystem the invertebrate communities, especially EWs, may play an essential role in SOM dynamics by the regulation of mineralization and humification processes (Bouche, 1977; Lavelle and Martin, 1992).

The effects of EWs on soil biological process and fertility level differ in ecological categories. Anecic species are active in deep mineral layers of the soil, endogeic species live in the upper mineral layer of soil, and epigeic species live on the soil surface (Jones *et al*., 1994). Mostly, the combinations of these ecological categories are responsible for maintaining the fertility of the soil (Sinha *et al*., 2003; Bhadauria and Saxena, 2009).

EWs have important role in supplying the nutrients (N, P, K and Ca) through production of aggregates and pores (*i.e.*, biostructures) in the soil and/or on the soil surface, by affecting its physical properties, nutrient cycling and plant growth (Scheu, 2003; Mora *et al*., 2005). The effect of EWs on the dynamics of organic matter varies depending on the time and space scale considered (Mora *et al*., 2005). In the humid tropical environment endogeic EWs accelerate initial SOM turnover through indirect influence on soil C as entry of microbial activity (Haynes and Fraser, 1998; Parmelee *et al*., 1998). Also it has been reported that EWs increase the incorporation of cover crop-derived C into macro aggregates, as well as into micro aggregates formed within macro aggregates (Fonte *et al*., 2007). Thus, the increased transfer of organic C and N into soil aggregates implies the potential for EWs to provide SOM stabilization and accumulation in agricultural systems.

In addition, EWs also increase nitrogen mineralization through direct and indirect effects on the microbial community. The studies have shown that the amount of soil nitrogen available for plants was produced more by activity of EWs than the total input through the addition of slashed vegetation, inorganic and organic manure, recycled crop residues, and weeds (Bhadauria and Ramakrishan, 1996). An important role of EWs is the huge increase in soil pH. The influence of EWs on N cycling seems to be defined by the type of cropping system and the fertilizer applied (mineral versus organic) (Postma-Blaauw, 1996). Furthermore, the EWs can also enhance the nutrient availability in system with reduced human influence, with respect to tillage, less using mineral fertilizer, and low organic matter content (Brown *et al*., 1998; Cortez and Hameed, 2001). The role of EWs in the enhancing soil fertility is ancient knowledge, but now is better explain by scientific results. More details about this subject have been reviewed by Bhaduaria and Saxena (2010).

The most important family of EWs in enhancing agricultural soil is *Lumbricidae*, which includes the genus *Lumbricus*, *Aporrectodea*, and several others. *Lumbricides* originate from Europe and by human activities they have been transported to many parts of the world.

Earthworms in medicine

The use of EWs in a medicine was documented at very early date, in 1340 AD. (Stevenson, 1930; Reynolds *et al*., 1972). Moreover, in the folk medicine (North American Indians, doctors in East Asia) the EWs have been used for the treatment of various diseases (Cooper *et al*., 2004). Traditional Chinese medicine has also widely used the EWs for a long time. The research on the pharmaceutical effects of EWs has been initiated along with the development of biochemical technologies. Many bioactive molecules which can be consider as potential drug have been detected in the EWs. These molecules exhibited different activities, such as fibrinolytic, anticoagulative, anticancer, antimicrobial and thus may be exploited for the treatment of variety diseases (Cooper *et al*. 2012).

Immunological recognition

The EW (phylum *Annelida*, family *Lumbricidae*) is one of the first organisms in the evolution that possess immunological recognition and memory. The EWs like the other complex invertebrates produce several types of leukocytes and synthesize and secrete the variety of immunoprotective molecules. They possess innate immunity, as well as some functions associated with the adaptive immunity (allogenic tissue rejection) (Cooper *et al*., 1995, 1999). The celomocytes involved in innate immunity, play a central role in the EW immune system (phagocytosis, releasing of lytic factors) (Stein *et al*., 1977, 1981; Cossarizza *et al*., 1996; Beshin *et al*., 2002). The EW celomocyte cells also provide immune functions and possess several CD markers (CD11, CD24, CD45RA, CD45RO, CD49b, CD54 and CD90) associated with innate immunity (Engelmann *et al*., 2011). Immuno-protective molecules synthesized and secreted from celomocytes induce agglutination, opsonisation and lysis of foreign material. In addition, they are employed in clotting reactions and phenoloxidase cascade (Cooper *et al*., 2002; Mohrig *et al*., 1996). The carbohydrates, so called lectins are the target recognition fragments for the EW agglutinins (Kauschke *et al*., 2000). The proteases are also very important factors in the immune system with their contribution to the destruction of foreign materials (clot formation, complement activation) (Söderhäll and Cerenius 1998). The patterns of celomic fluid protease can be considered as species specific in EW (Mohrig *et al*., 1989; Kauschke *et al*., 1997, 2000). The level of protease pattern and activity in celomic fluid might be considered as promising biomarker in environmental monitoring studies (Kauschke *et al*., 2007). More details about EWs proteases can be find in review paper of Pan *et al*. (2010).

Fibrinolytic activity

The fibrinolytic system is responsible for the proteolytic degradation of fibrin and therefore plays a role in hemostasis and thrombosis (Cesarman-Maus and Hajjar, 2005). Intravascular thrombosis, as a result of aggregation of fibrin in the arteries, is one of the main causes of cardiovascular disease. The main component of blood clots is fibrin. The clots arise from fibrinogen after thrombin action. Formation of fibrin clot and fibrinolysis are normally well balanced in biological systems. However, the thrombosis can occur if fibrin is not hydrolyzed as a consequence of any disorder. The usual outcome of such thrombosis is myocardial infarction. The fibrin clots are dissolved by fibrinolytic enzymes. For that purpose plasminogen activator (t-PA), urokinase and streptokinase are mostly used. These enzymes exhibit low specificity for fibrin and have undesired side effect and are also relatively expensive. Therefore, the search for other fibrinolytic agent from various sources continues. The EWs are an attractive source of the fibrinolytic enzymes and various physiologically active compounds.

Fibrinolytic enzymes, which are potent and safe, have been purified and studied from several species of earthworm, including *Lumbricus rubellas* and *Eisenia fetida*. Its therapeutic and preventive effects on thrombosis-related disease have been clinically confirmed.

The presence of fibrinolytic activity in coelomic fluid or tissue homogenate from EWs has been reported previously. First isolation of the EWs fibrinolytic enzymes (EFE) were published in 1980's. In crude extract of EW *L. rubellus* Mihara *et al*. (1983) found the lumbrokinase that exerted the fibrinolytic and thrombolytic activities. Many authors have isolated and characterized similar enzymes from different species (*E. foetida, Lumbricus bimastus*) (Lu *et al*., 1988; Hrženjak *et al*., 1991, 1998; Cheng *et al*., 1996; Lin *et al*., 2000; Xu *et al*., 2002; Wang *et al*., 2003; Li *et al*., 2003; 2012), and since that time, the medical value of EWs has been investigated in more details. Most earthworm fibrinolytic enzymes showed distinctive high stability and strong tolerance to organic solvents and high temperature. It was found that EW extracts could significantly diminish the coagulation of platelets and promote the dissolving of thrombi in the blood. Because of that, they should be used for the treatment of cardiac and cerebro-vascular diseases. After oral administration to the patients, EW fibrinolytic enzyme reduced coagulation of fibrin and blood platelets (Ryu *et al*., 1994; Lijnen *et al*., 1995; Gao *et al*., 1999: Zheng *et al*., 2000), and had no adverse effects on the functions of the nervous system, respiratory system, cardiovascular vessels, liver and kidneys (Stein *et al*., 1982; Valenbois *et al*., 1982; Hirigoyenberry *et al*., 1990; Cho *et al*., 1998). The fibrinolytic enzymes have very specific way of absorption. They can exert the biological function in circulation after the transport into blood through intestinal epithelium (Fan *et al*., 2001). On the other hand the fibrinolytic enzymes like urokinase and tissue plasminogen activator could be administrated by intaraperitoneal injection rather than orally. The potential use of fibrinolytic enzymes in the prevention and treatment of serious cardiac and cerebro-vascular diseases has been very attractive in medicine and pharmacology. Besides their use as therapeutics, fibrinolytic enzymes could be also used in degradation of organic waste products from the food and livestock industry (Nakajima *et al*., 2000). In addition the EWs are very cheap source of biologically active molecules. However, many details about fibrinolytic enzymes from EWs have been reviewed by Grdiša *et al*. (2009).

Hemostatic activity of tissue extract of *E. foetida* (G-90) was also proved in an *in vivo* system (Mataušić-Pišl *et al*., 2011). Because of remarkable degree of fibrinolytic and anticoagulation activity this extract exhibited the significant effect on bleeding and coagulation times, after administration in Wistar rats. The effect was very similar to that of heparin. Therefore, G-90 could represent a new source of fibrinolytic and anticoagulation enzymes suitable for future application in human and veterinary medicine. A similar effect has already been seen with a crude extract of earthworms *L. rubellus* (Nakajima *et al*., 1993; Cho *et al*., 2004; Popović *et al.,* 2005). The authors have shown that mixing of blood with that extract prolonged the clotting time.

The studies of different authors have also indicated that the celomic fluid of EWs exhibits other biological functions, including bacteriostatic (Cooper *et al*., 2004; Popović *et al*., 2005), proteolytic (Nakajima *et al*., 1993; Wang *et al*., 2003), cytolytic (hemolytic) (Popović *et a*l., 2001; Procházková *et al*., *2006*; Mataušić-Pišl *et al*., 2011), and mitogen activity (Hrženjak *et al*., 1993).

Antitumor activity

Antitumor effect of the macromolecules from EWs has been determined in *in vitro* and *in vivo* studies. However, the interest in EFE has also been increased. It has been shown that EFE isolated from *E. foetida* exhibits antitumor activity against the human hepatoma cells *in vitro* and *in vivo* (Chen *et al*., 2007). Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer related mortality worldwide (Sherman and Takayama, 2004). It seems that EFE induced apoptosis in these cells. The results indicated that EFE could be used in treatment of hepatoma. Moreover macromolecular mixture (G-90) from the tissue homogenate of *E. foetida* inhibited the growth of melanoma cells *in vitro* and *in vivo* (Hrženjak *et al*., 1993). Such effects have also been seen with coelomic fluid of *E. foetida*. Isolated coelomic cytolytic factor 1 (CCF-1) was capable of lysing different mammalian tumor cell lines (Bilej *et al*., 1995). Similar antitumor effect of the earthworm extracts has been detected by different authors (Chen *et al*., 2001; Hu *et al*., 2002; Xie *et al*., 2003; Yuan *et al*., 2004).

Antipyretic and antioxidative activities

Antipyretic activity has also been detected in the EWs *Lumbricus* spp. and *Perichaeta* spp. (Hori *et al*., 1974), as well as in the paste obtained from EW *Lampito mauritii* (Balamurugan *et al*., 2007). This activity was similar to that obtained with aspirin (Ismail *et al*., 1992). The paste from *L. mauritii* has also shown remarkable antipyretic and antioxidative actions in the treatment of peptic ulcer in rats (Prakash *et al*., 2007). After paracetamol-induced liver injury in Wistar rats, the hepatoprotective potential of extract from *L. mauritii* has been observed (Balamurugan *et al*., 2008).

The protection of human body from free radicals is very important since it is connected with the retention of progress for many chronic diseases. Non-enzymatic antioxidants such as glutathione, vitamins C and E, Tocopherol and Ceruloplasmin protect the cells from oxidative damage (Aldrige, 1981). The enzymatic antioxidants, such as superoxide dismutase, catalase and cyclooxigenase protect the cells from lipid peroxidation and they are very important scavengers of superoxide ion and hydrogen peroxide (Scott *et al*., 1991). Discovery of antioxidative activity in different EW preparations has been promising (Grdiša *et al*., 2001; Balamurugan *et al*., 2007).

Antibacterial activity

During the 700 million years of their existence, EWs have evolved in the environment replete with microorganisms, some of which threaten their existence, therefore they have developed efficient defense mechanisms against invading microorganisms. There is a variety of relationships between EW and microbes: (1) microbe as food for earthworm, (2) microbes as nutritive material for growth and reproduction, (3) microbes-mostly Gram positive, pathogenic are digested by EW and thereby facilitate multiplication of useful microbes in the gut and (5) microbes are distributed to new places in soil (Ranganathan, 2006; Parthasarathi *et al*., 2007).

The molecules which defend the EWs from microbes have been detected in the celomic fluid of *Lumbricus* and *Eisenia* (Stein *et al*., 1982; Valembois *et al*., 1982). This activity is attributed to some proteins, such as lysozyme and fetidins (Hirigoyenberry *et al*., 1990; Milochau *et al*., 1997). Few reports are also available regarding antimicrobial agents from EWs tissue (Cho *et al*., 1998; Popović *et al*., 2005).

Wound-healing activity

Many scientists and medical communities have been searching for way to improve wound care and promote wound-healing. Skin wound healing is a complex process characterized by re-epithelization and restoration of the underlying connective tissue. A number of overlapping phases are involved. During this process, keratinocytes, endothelial cells, fibroblasts and inflammatory cells proliferate and/or migrate to the site of injury, interacting both with each other and with extracellular matrix (Gailit and Clark, 1994). Cell migration and tissue remodeling which take place during the course of the wound-healing process require controlled degradation of extracellular matrix and activation or release of growth factors (Vassilli and Saurat, 1996). Along the line of neo-vascularisation, matrix-generating cells move into the granulation tissue. These fibroblasts degrade the provisional matrix and respond to cytokine/growth factors by proliferating and synthesizing new extracellular matrix.

The agents with biomedical potential, prepared or isolated from the EWs, have been used in wound care. Characteristics of EWs homogenate/paste, which could contribute to better healing of wounds, have been reported (Hrženjak *et al*., 1993, 1998; Popović *et al*., 2001; Grdiša *et al*., 2001, 2004; Cooper *et al*., 2004; Popović *et al*., 2005; Balamurugan *et al*., 2007; Prakash *et al*., 2007). Mitogen, antibacterial, hemostatic and antioxidative activities have an important influence on the healing and epithelization of wound. Macromolecules from EWs also stimulate the synthesis of EGF and FGF, the factors involved in epithelization process (Grdiša *et al*., 2004). The earthworm preparations from *L. rubellus* and *E. foetida* promoted wound healing (Li *et al*., 2000; Mataušić-Pišl *et al*., 2010). Both preparations shortened the healing time by increasing epithelization, granulation and synthesis of collagen.

Pasta, obtained from EW *L. mauritii*, *Kinberg*, exhibited variety of activities, such as antiinflammatory, antioxidative and hepatoprotective activities (Balamurugan *et al*., 2007; Prakash *et al*., 2007). Due to the properties of animal extracts, they should be considered in the treatment of wounds as well as different human diseases. Thus, the EWs could be handy and low cost source of bioactive molecules that are involved in wound healing process.

Earthworms as a food

Despite unusual perception, the EWs have been used as food for humans. Due to high nutritive value and abundance of the proteins they are basic of healthy diet. According to the literature data the EWs contain about 60-70 % of proteins (Zhenjun *et al*., 1997; Medina *et al*., 2003; Zhenjun, 2005). These authors have also reported that the presence of essential amino acids, especially a tyrosine, is much higher in the body of EWs than it is recommendation of FAO. Besides the human diet, the EWs have been used in the feeding of fish (Vassilli and Saurat, 2010), as well as the chicken (Taboga, 1980).

The group of scientist of Royal Society (London, UK) have published (2003) the study about nutritive properties of the earthworms and their usage in the diet of Yekuana people from Venezuela (Paoletti *et al*., 2003). In their diet they are using two species: *Andiorrhinus (Amazonindrilus) motto* (Righi *et al*., 1999), which live in drift of sludge, it is white and known under the name “motto”, and *Andiorrhinus (Andiorrhinus) kuru* (Moreno and Paoletti, 2004), which lives in forest soil, and it is known under the name “kuru”. The Yekuna people eat them raw (uncooked) or after treatment in water at the temperature around 60 - 70 °C or after drying in the smoke. These smoked EWs are considered as speciality and the price is very high. Beside using the EWs as food the folk doctors also advise their usage against malaria and anemia. Investigation on these two species of EWs, Paoletti *et al*. (2003) have found 18 amino acids incorporated in the proteins, which represents 64.4 - 72.9 % of total mass of the EWs. Also they have found 20 minerals, such as calcium and iron (the content of iron in these species is 10 times higher than in soya bean), and some elements in trace, as well as omega-3 and omega-6 fatty acids. According to this data, the EWs “motto” and “kuru” are assumed as the best food for fulfilling all requirements of the human body.

Some fatty acids (palmitate, oleic, octadecanoic acids) have been detected in *E. foetida* (Roubath-Sadiqui and Marcel, 1995) with the highest content before complete maturation. The same authors declared that *Eisenia foetida* also contain the high percentage of proteins (58 %).

Many restaurants in Mexico have on their menu variety of EWs, and in Japan the EWs are very appreciate as a food in combination with soya been or supplementation in juices. On the other hand in some part of Asia, Africa and South America the EWs have been introduce in everyday diet.

Conclusions

Earthworms improve soil fertility, for which they need certain conditions and organic matter for food. It has been shown that the presence of earthworms in soil remarkable increase the yield of crops. In addition, the earthworms are valuable and low cost source of many bioactive molecules, which could find place in human and veterinary medicine. Thus, it is obvious that earthworms have a whole variety of application, from environmental protection, medical use and nutrition production.

References

Aldrige WN. Mechanism of toxicity. New concepts are required in toxicology. Trends Pharmacol. Sci. 2: 228-231, 1981.

Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Earthworm paste (*Lampito mauritii, Kinberg*) alters inflammatory, oxidative, hematological and serum biochemical indices of inflamed rat. Eur. Rev. Med. Pharmacol. Sci. 11: 77-90, 2007.

Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Hypothetical mode of action of earthworm extract with hepatoprotective and antioxidant properties. J. Zhejiang Univ. Sci. B 9: 141-147, 2008.

Barrios E. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64: 269-285, 2007.

Beschin A, De Baetselier P, Bilej M. CCF, an invertebrate analogue of TNF is not related to the other lytic components from *Eisenia foetida* earthworm. BioEssays 24: 974-976, 2002.

Bhadauria T, Ramakrishan PS. Role of earthworms in nitrogen cycling during the cropping phase of shifting agriculture (Jhum) in north-east India. Biol. Fertil. Soil 22: 350-354, 1996.

Bhadauria T, Saxena KG. Influence of landscape modification on earthworm biodiversity in the Garhwali region of Central Himalaya. In: Edwards CA, Jeyaraaj R, Jeyaraaj I (eds), Proceedings of Indo US Workshop on Vermitechnology in Human Welfare (Indo-US Science and Technology Forum), Coimbatore, Tamil Nadu, India, pp 80-95, 2009.

Bhadauria T, Saxena KG. Role of earthworms in soil fertility maintenance through the production of biogenic structures. Appl. Environ. Soil Sci. 2010, Article ID 816073, doi:10.1155/2010/816073

Bilej M, Brys L, Beschin A, Lucas R, Vercauteren E, Hanušová R, *et al*. Identification of a cytolytic protein in the coelomic fluid of *Eisenia foetida* earthworms. Immunol. Lett. 45: 123-128, 1995.

Bouche MB. Stratégies lombriciennes. In: Lohm U, Persson T (eds), Soil Organisms as Component of Ecosystems, Ecological Bulletin, Stockholm, Sweden, pp122-132, 1977.

Brown GG, Hendrix PF, Beare MH. Earthworms (*Lumbricus rubellus*) and the fate of ^{15}N in surface-applied sorghum residues. Soil Biol. Biochem. 30: 1701-1705, 1998.

Brusca RC, Brusca GJ. Invertebrates, Sinaur Associates, Sunderland, MA, 2003.

Cesarman-Maus G, Hajjar KA. Molecular mechanisms of fibrinolysis. Br. J. Haem. 129: 307-321, 2005.

Chen H, Takahashi S, Imamura M, Okutani E, Zhang Z, Chayama K, *et al*. Earthworm fibrinolytic enzyme: anti-tumor activity on human hepatoma cells *in vitro* and *in vivo*. Chin. Med. J. 120: 898-904, 2007.

Chen H. Anti-tumor effect of earthworm extracts EE2. Chin. Clin. Oncol. 6: 349-350, 2001.

Cheng NL, Wang XY, Zheng GP, Wang HZ, Niu B. The purification and characterization of fibrinolytic enzyme II from *Lumbricus bimastus*. Chin. J. Exp. Clin. Immunol. 8: 8-10, 1996.

Chevallier T, Blanchart E, Albrecht A, Feller C. The physical properties of soil organic carbon in aggregates: a mechanism of carbon storage in a Vertisol under pasture and market gardening (Martinique, West Indies). Agricul. Ecosys. Environ. 103: 375-387, 2004.

Cho JH, Choi ES, Lim HG, Lee HH. Purification and characterization of six fibrinolitic serine-proteases from earthworm *Lumbricus rubellus*. J. Biochem. Mol. Biol. 37: 199-205, 2004.

Cho JH, Park CB, Yoon YG, Kim SC. Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochim. Biophys. Acta 1408: 67-76, 1998.

Cooper EL, Balamurugan M, Huang CY, Tsao CR, Heredia J, Tommaseo-Ponzetta M, *et al*. Earthworms dilong: Ancient, inexpensive, noncontroversial models my help clarify approaches to integrated medicine emphasizing neuroimmuno systems. Evid. Based Complement. Alternat. Med. 2012: 164152, 2012. (doi:10.1155/2012/164152).

Cooper EL, Cossarizza A, Kauschke E, Franceschi C. Cell adhesion and the immune system: a case study using earthworms. Micro. Res. Techn. 44: 237-253, 1999.

Cooper EL, Hrženjak T, Grdiša M. Alternative source of fibrinolytic, anticoagulative, antimicrobial and anticancer molecules. Int. J. Immunopath. Pharm. 17: 237-244, 2004.

Cooper EL, Kauschke E, Cossariazza A. Digging for innate immunity since Darwin and Metchnikoff. BioEssays 24: 319-333, 2002.

Cortez J, Hameed RH. Simultaneous effects of plants and earthworms on mineralization of ^{15}N-labelled organic compounds adsorbed onto soil size fractions. Biol. Fertil. Soil 33: 218-225, 2001.

Cossarizza A, Cooper EL, Suzuki MM, Salvioli S, Capri M, Gri G, *et al*. Earthworm leukocytes that are not phagocytic and cross-react with several human epitopes can kill human tumor cell lines. Exp. Cell Res. 224: 174-182, 1996.

Fan Q, Wu C, Li L, Fan R, Wu C, Hou Q, *et al*. Some features of intestinal absorption of intact fibrinolytic enzyme III-1 from *Lumbricus rubellus*. Biochim. Biophys. Acta 1526: 286-292, 2001.

Fonte SJ, Kong AYY, van Kessel C, Hendrix PF, Six J. Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management. Soil Biol. Biochem. 39: 1014-1022, 2007.

Engelmann P, Farkas K, Kis J, Richman G, Zhang Z, Liew CW *et al*. Characterization of human invariant natural killer T cells expressing FoxP3. Int. Immunol. 23: 473-484, 2011.

Gailit J, Clark RAF. Wound repair in the context of extracellular matrix. Curr. Opin. Cell Biol. 6: 717-725, 1994.

Gao Y, Qin M. Lumbrokinase in treatment of patients with hyperfibrinogenemia of coronary atherogenesis disease. J. Cap. Univ. Med. Sci. 20: 264-269, 1999.

Grdiša M, Mikecan AM, Knežević N. Fibrinolytic enzymes from earthworms. Dyn. Soil Dyn. Plant 3: 61-63, 2009.

Grdiša M, Popović M, Hrženjak T. Glycoprotein extract of *Eisenia foetida* exerts some antioxidative activity. Comp. Biochem. Physiol. 128A: 821-825, 2001.

Grdiša M, Popović M, Hrženjak T. Stimulation of growth factor synthesis in skin wounds using tissue extract (G-90) from the earthworm *Eisenia foetida*. Cell Biochem. Funct. 22: 373-378, 2004.

Hasanuzzaman AF, Hossian SZM, Das M. Nutritional potentiality of earthworm (*Perionyx excavates*) for substituting fishmeal used in local feed company in Bangladesh. Mesopot. J. Mar. Sci. 25: 25-30, 2010.

Haynes RJ, Fraser PM. A comparison of aggregate stability and biological activity in earthworm casts and uningested soil as affected by amendment with wheat or Lucerne straw. Eur. J. Soil Sci. 49: 629-636, 1998.

Hirigoyenberry F, Lassale F, Lassègues M. Antibacterial activity of *Eisenia foetida Andrei* coelomic fluid: transcription and translation regulation of lysozyme and proteins evidenced after bacterial infection. Comp. Biochem. Physiol. 95B: 71-75, 1990.

Hong HN, Rumpel C, des Tureaux TH, Bardoux G, Billou D, Duc TT *et al*. How do earthworms influence organic matter quantity and quality in tropical soils? Soil Biol. Biochem. 43: 223-230, 2011.

Hori M, Kondon K, Yoshida T, Konishi E, Minami S. Studies of anti-pyretic components in the Japanese earthworm. Biochem. Pharmacol. 23: 1582-1590, 1974.

Hrženjak M, Kobrehel Đ, Levanat S, Jurin M, Hrženjak T. Mitogenicity of earthworm (*Eisenia foetida*) insulin-like proteins. Comp. Biochem. Physiol. 104B: 723-729, 1993.

Hrženjak T, Popović M, Božić T, Grdiša M, Kobrehel Đ, Tiška-Rudman Lj. Fibrinolytic and anticoagulative activities from the earthworm *Eisenia foetida*. Comp. Biochem. Physiol. 119B: 825-832, 1998.

Hu YL, Xu JM, Zhang SQ, Huang WH, Kang SH, Wang QX. Study of earthworm extracts on the immunologic activity and anti-tumor effects. Biotech. (Chin) 12: 9-10, 2002.

Ismail SA, Pulandiran K, Yegnanarayan R. Anti-inflammatory activity of earthworm extracts. Soil Biol. Biochem. 24: 1253-1254, 1992.

Jones CG, Lawton JH, Shachak M. Organisms as ecosystem engineers. Oikos 69: 373-386, 1994.

Kauschke E, Eue I, Lange S, Mohrig W, Cooper EL. Immune proteins in earthworms. Recent Res. Develop. Comp. Biochem. Physiol. 1: 105-122, 2000.

Kauschke E, Mohrig W, Cooper EL. Coelomic fluid proteins as basic components of innate immunity in earthworms. Eur. J. Soil Biol. 43: S110-S114, 2007.

Kauschke E, Pagliara P, Stabili L, Cooper EL. Characterization of proteolytic activity in coelomic fluid of *Lumbricus terrestris, L.* Comp. Biochem. Physiol. 118B: 235-242, 1997.

Lal R. Soil carbon sequestration to migrate climate change. Geoderma 123: 1-22, 2004.

Lavelle P, Martin A. Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soil of the humid tropics. Soil Biol. Biochem. 24: 1491-1498, 1992.

Lavelle P, Spain AV. Soil Ecology, Kluwer Academic Publicher, Dordrecht, Netherlands, 2001.

Li D, Wang P, Zeng Y. Study on *Lumbricus* in promoting ligation hemorroidectomy postoperative wound healing. Zhongguo Zhong Xi Yi Jie He Za Zhi 20: 899-902, 2000. (PMID 11938859).

Li L, Zhao J, He RQ. Isolation and some characterizations of a glycosylated fibrinolytic enzyme of earthworm *Eisenia foetida*. Prot. Pept. Lett. 10: 183-190, 2003.

Li G, Wang KY, Li D, Wang N, Liu D. Cloning, expression and characterization of a gene from earthworm *Eisenia foetida* encoding a blood-clot dissolving protein. PLoS ONE 7: e53110, 2012.

Lijnen OH, Clooen D. Fibrinolytic agents: mechanisms of activity and pharmacology J. Thromb. Haem. 74: 387-390, 1995.

Lin SQ, Yu P, Lan RF. Application of affinity chromatography for purification of fibrinolytic enzyme from earthworm. Pharma. Biotech. (Chin) 7: 229-233, 2000.

Lu YH, Jin RC, Wu YW. Separation and purification of fibrinolytic enzyme from earthworm. J. Biochem. (Chin) 4: 166-172, 1988.

Mataušić-Pišl M, Čupić H, Kašuba V, Mikecin AM, Grdiša M. Glikolipoprotein extract (G-90) from *Eisenia foetida* accelerated the rats wound healing. Eur. Rev. Med. Pharmacol. Sci. 14: 177-184, 2010.

Mataušić-Pišl M, Tomičić M, Micek V, Grdiša M. Influence of earthworm extract G-90 on the haemostasis in Wistar rats. Eur. Rev. Med. Pharmacol. Sci. 15: 71-78, 2011.

Medina AL, Cova JA, Vielma RA, Pujic P, Carlos MP, Torres JV. Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. Food Agricul. Immunol. 15: 255-263, 2003.

Mihara H, Sumi H, Akazawa K, Yoneta T, Mizumoto H. Fibrinolytic enzyme extracted from the earthworm. Thrombosis and Haemostasis 50: 258-263, 1983.

Milochau A, Lassègues M, Valembois P. Purification, characterization and activities of two haemolytic and antimicrobial proteins from coelomic fluid of the annelid *Eisenia foetida Andrei*. Biochim. Biophys. Acta 1337: 123-132, 1997.

Mohrig W, Eue I, Kauschke E, Hennicke F. Crossreactivity of haemolytic and hemagglutinating proteins in the coelomic fluid of earthworms. Comp. Biochem. Physiol. 115A: 19-30, 1996.

Mohrig W, Eue I, Kauschke E. Proteolytic activities in the coelomic fluid of earthworms (*Annelida, Lumbricidae*). Zool. Jahr. Physiol. 93: 303-317, 1989.

Mora P, Miambi E, Jiménez JJ, Decaëns, Rouland C. Functional complement of biogenic structures produced by earthworms, termites and ants in the neotropical savannas. Soil Biol. Biochem. 37: 1043-1048, 2005.

Mora P, Seugé C, Chotte JL, Rouland C. Physico-chemical typology of the biogenic structures of termites and earthworms: a comparative analysis. Biol. Fertil. Soils 37: 245-249, 2003.

Moreno AG, Paoletti MG, *Andiorrhinus (Amazonidrilus) kuru* sp. nov. (*Ologocheta: Glassoscolecidae*), a giant earthworm as food resource for Makiritate Indians of the Alto Rio Padamo, Amazonas, Venezuela. Can. J. Zool. 82: 1000-1004, 2004.

Nakajima N, Mihara H, Sumi H. Characterization of potent fibrinolytic enzymes in earthworm, *Lumbricus rubellus*. Biosci. Biotech. Biochem. 57: 1726-1730, 1993.

Nakajima N, Sugimoto M, Ishihara K. Stable earthworm serine proteases: application of the protease function and usefulness of the earthworm autholysate. Biotech. Bioeng. 90: 174-179, 2000.

Pan R, Zhang ZJ, He RQ. Earthworm protease. Appl. Environ. Soil Sci. 2010: Article ID 294258, doi:10.1155/2010/294258

Paoletti MG, Buscardo E, Vanderjagt DJ, Pastuszyn A, Pizzoferrato L, Huang YS *et al.* Nutrient content of earthworms consumed by de Ye Kuana Amerindians of the Alto Orinoco of Venezuela. Proc. Roy. Soc. 270: 249-257, 2003.

Parmelee RW, Bohlen PJ, Blair JM. Earthworms and nutrient cycling processes: integrating across the ecological hierarchy. In: Edwards C (ed), Earthworm ecology, St. Lucie Press, Boca Raton, Fla, USA, pp 179-211, 1998.

Parthasarathi K, Ranganathan LS, Anandi V, Zeyer J. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J. Environ. Biol. 28: 87-97, 2007.

Pechenik JA. Biology of the Invertebrates. 5th edition McGraw-Hill Higher Education, Boston, 2009.

Popović M, Grdiša M, Hrženjak T. Glycolipoprotein G-90 obtained from the earthworm *Eisenia foetida* exerts antibacterial activity. Vet. Arch. 75: 119-128, 2005.

Popović M, Hrženjak T, Babić T, Kos J, Grdiša M. Effect of earthworm extract (G-90) on formation and lysis of clots originated from venous blood of dogs with cardiopathies and with malignant tumors. Pathol. Oncol. Res. 7: 1-6, 2001.

Postma-Blaauw MB, Bloem J, Faber JH, van Groenigen JW, de Goede RGM, Brussaard L. Earthworm species composition affects the soil bacterial community and net nitrogen mineralization. Pedobiologia 50: 243-256, 2006.

Prakash M, Balamurugan M, Parthasarathi K, Gunasekaran G, Cooper EL, Ranganathan LS. Anti-ulceral and anti-oxidative properties of earthworm paste of *Lampito mauritii, Kinberg* on *Rattus norvegicus*. Eur. Rev. Med. Pharmacol. Sci. 11: 9-15, 2007.

Procházková P, Šilerová M, Felsberg J, Josková R, Beschin A, De Baetselier P, *et al.* Relationship between hemolytic molecules in *Eisenia foetida* earthworms. Dev. Comp. Immunol. 30: 381-392, 2006.

Ranganathan LS. Vermitechnology: from soil health to human health, Agrobis, India, 2006.

Reynolds JW, Reynolds WM. Earthworms in medicine. Am. J. Nursing 72: 1273-1283, 1972

Righi G, Araujo Y. *Andiorrhinus (Amazonidrilus) motto* n. sp. *Rhindrilus appuni pavoni* n. subsp. (*Ologocheta: Glassoscolecidae*) from the Venezuelan Amazonia. Misc. Zool. (Barc.) 22: 93-100, 1999.

Roubath-Sadiqui L, Marcel R. Analysis of proteinic nutrients in clitellum and cocoon's albumen in *Eisenia foetida Sav*. (*Annelide, Oligocheta*). Evidence for a vitellogenin-like glycoprotein. Reprod. Nutr. Dev. 35: 491-501, 1995.

Roubath-Sadiqui L, Marcel R. Glucids and lipids of clitellum and cocoon's albumen in *Eisenia* *foetida Sav*. (*Annelide, Oligocheta*). Reprod. Nutr. Dev. 35: 537-548, 1995.

Ryu GH, Park S, Kim M, Han DK. Antithrombogenicity of lumbrokinase-immobilized polyurethane. J. Biomed. Mat. Res. 28:1069-1077, 1994.

Scheu S. Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia 47: 846-856, 2003.

Scott MD, Lubin BH, Zuo AL, Kuypers FA. Erythrocite defence against hydrogen peroxide: preeminent importance of catalase. J. Lab. Clin. Med. 18: 7-16, 1991.

Sherman M, Takayama Y. Screening and treatment for hepatocellular carcinoma. Gastroent. Clin. North Am. 33: 671-691, 2004.

Sinha B, Bhadauria T, Ramakrishnan PS, Saxena KG, Maikhuri RK. Impact of land scape modification on earthworm diversity and abundance in the Hariyali sacred landscape, Garhwal Himalaya. Pedobiologia 47: 357-370, 2003.

Söderhäll K, Cerenius L. Role of prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunol. 10: 23-28, 1998.

Stein EA, Cooper EL. The role of opsonins in phagocytosis by coelomocytes of the earthworm *L. terrestris.* Dev. Comp. Immunol. 5: 415-425, 1981.

Stein EA, Wojdani A, Cooper EL. Agglutinins in the earthworm *Lumbricus terrestris*: Naturally occurring and induced. Dev. Comp. Immunol. 3: 173-178, 1982.

Stevenson J. Oligochaeta, Claredon Press, Oxford, 1930.

Taboga L. The nutritional value of earthworms for chickens. Br. Poultry Sci. Abington 21: 405-410, 1980.

Tisdall JM, Oades JM. Organic matter and water-stable aggregates in soil", J. Soil Sci. 33: 141-163, 1982.

Valembois P, Roch P, Lassègues M, Cassand P. Antibacterial activity of haemolytic system from the earthworm *Eisenia foetida Andrei*. J. Invertebr. Pathol. 40: 21-27, 1982.

Vassilli JD, Saurat JH. Cuts and scrapes? Plasmin heals! Nature Med. 2: 284-285, 1996.

Wang F, Wang C, Li M, Giu L, Zhang J, Chang W. Purification, characterization and crystallization of a group of earthworm fibrinolytic enzymes from *Eisenia foetida*. Biotech. Lett. 25: 1105-1109, 2003.

Wang F, Wang C, Li M, Giu L, Zhang J, Chang W. Purification, characterization and crystallization of a group of earthworm fibrinolytic enzymes from *Eisenia foetida*. Biotech. Lett. 25: 1105-1109, 2003.

Wardle DA. Communities and ecosystems: linking the aboveground and belowground components, Princeton University Press, Oxford, UK, 2002.

Weil RR, Magdoff F. Significance of soil organic matter to soil quality and health. In: Weil RR, Magdoff F. (eds), Soil organic matter in sustainable agriculture, CRC Press, pp 1-44, 2004.

Xie JB, He WG, Weng N, Guo ZQ, Yu MM, Liu NN. Extraction and isolation of the anti-tumor protein components from earthworm (*Eisenia foetida Andrei*) and the anti-tumor activity. Chin. J. Biochem. Mol. Biol. 19: 359-366, 2003.

Xu YH, Liang GD, Sun ZJ, Chen F, Fu SH, Chai YB, *et al*. Cloning and expression of the novel gene PV242 of earthworm fibrinolytic enzyme. Prog. Biochem. Biophys. 29: 610-614, 2002.

Yuan L, Xu JM, Zhou YC. Effects of the purified earthworm extracts on various hemal tumor cells. Clin. Med. J. China. 11: 177-179, 2004.

Zheng HJ, Xu JM, Huang ZH. Lumbrokinase capsule *vs* ticlopidine in treating coronary artery disease with angina pectoris. Chin. J. New Drugs Clin. Remed. 5: 406-408, 2000.

Zhenjun S, Xianchun L, Lihui S, Chunyanq S. Earthworm as a potential protein resource. Ecol. Food Nutr. 36: 221-236, 1997.

Zhenjun S. Nutritive value of Earthworms. In: Einfield NH (ed), Ecological implication of Miniliveestock, Science Publisher, Inc, pp 491-236, 2005.