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Earthworms - the Miracle of the Earth

Sometimes we could not think of importance of different organisms. We just feel them as the living entities on the earth. We, sometimes ignore them. Our planet is very rich in having a great diversity of animals and plants. Every living creature has its own place and own role in the nature. Shape and size of the individuals are futile components. All are equally vital in making our planet - The Earth in a balanced state. We must honor them, salute them, save them, protect them, conserve them and allow them free to do their own natural roles. Natural balance is a cumulative phenomenon of the roles of all the living entities. Missing of any entity may disbalance the static state of our wonderful planet.

Some creatures have sundry and magic roles. Earthworms are one of them, although most of them are small creatures ranging from 2″ to 6″ but simple, soft, segmented animals and boon to the whole humanity. They make our soil porous, airy, and enrich its natural nutritional status and transform it into more fertile and least toxic by remediating persistent pollutants (agro chemicals and pesticidal residues) from it. Some of them have wonderful potentiality to transform various organic wastes, liable to aquatic and air pollution, into bioavailable form. Even, body secretions of earthworms like, their fibrinolytic enzymes have been known as very good remedial agents in the medication of strokes.

VERMECO recently discussed the role of earthworms in removing non-essential toxic chemicals and synthetic pesticides from the soil with Prof. M. Vikram Reddy of Pondicherry University; in transforming of fly-ash - a major waste product of coal mines in to bio available form with Prof. GN Chattopadhyay of Visva-Bharati University, Shantiniketan and as bio-therapeutic agents for the stroke with Dr. S. Umamaheswari *et al.* of Manonmaniam Sundaranar University, Alwarkurichi (Tamilnadu). The articles must reflect a beam to the researchers in searching more unknown facets of the earthworms and proving them as a **Miracle of Earth** in the New Year' 2014.

"VERMECO" wishes to all its readers "A Very Happy New Year' 2014…!

Editor

Message from the President

Survival of whole of the biodiversity is threatened today. The pace of such state is fast in those which are very sensitive even to slight change in the environment. We, the humans, are the main culprits.

Due to speedy urbanization, our agricultural land is transforming into buildings. The rate of such process is very fast especially in the developing countries, like India, where we do not have any control over the human population leading to shrinking of farming land.

In such circumstances, our farmers have no other options expect to use the chemical fertilizers and pesticides regularly in the crops to increase their yields. Although, all such chemicals do have carcinogenic and persistent toxic metals turning our precious soil highly contaminated and poisonous. These chemicals disbalance its nutritional homeostatic condition too. Food products produced from such soils have toxic effects in our bodies when taken up by us and are the major causal factors for raising various curable and non-curable diseases in us.

We should work hard in investigating new and easy bio-technologies in boosting- up nutritional status of the soil without using chemical fertilizers and pesticides, reducing its toxicity by minimizing poisonous contents, transforming various organic wastes into nutritionally rich manure and searching vermi-medicines. Besides, awareness programmes should also be organized amongst the farming communities all over the country to teach them about the consequences of chemical farming on the human health in the years to come and to guide them to opt organic farming and about the technologies for the soil enrichment.

I hope the contents of the bulletin will be useful and motivating to the scientific community to work more in these areas and come forward to solve the related problems of the farmers in a sustainable way by unfolding more facts on earthworms. I wish every success of the collaborative work of the Academic Forum, SEEER and the Centre for Vermiculture and Vermicomposting of the University.

Prof. Mushahid Husain
Vice Chancellor
Mahatma Jyotiba Phule Rohilkhand University, Bareilly (India)
Former Director, Centre for Nano Science and Technology,
Jamia Millia Islamia, New Delhi

A Noble Role of Earthworms in Soil

Earthworms-the ecosystem engineers, enrich the surface soil by augmenting its physical, chemical and microbiological properties. In the process, they turn over and mix it with organic matter and transformed into a nutrient-rich soil. The process of castings-egestion involves ingestion of soil along with organic matter and litter particles (Marinissen *et al.*, 1996).Though the nutrient level of worm-castings has been known, very little information is documented on the insecticidal residues of worm-castings and in their body tissues in relation to their concentration in the surrounding soil (Singh, 2009; NSEEE-3, 2012). Use of biomarkers is becoming increasingly important for the evaluation of effects of soil pollutants like pesticide residues on earthworms. Research of novel biomarkers in earthworms is receiving increasing attention for its potentiality in soil pollution monitoring and assessment. Acetyl cholinesterase biotransformation enzyme is amongst the most used biomarkers due to its crucial role in neurocholinergic transmission in cell homeostasis preventing toxic actions of chemicals (Novais *et al.*, 2011). Presence of relatively lower concentration of persistent pollutants such as pesticide residues and heavy metals in the castings as compared to that of the underlying soil is probably because of the fact that the earthworms absorbed and accumulated higher concentration of pollutants in their body tissues with consequent reduction in their concentration in the worm-castings (Hartenstein and Hartenstein, 1981). Soil and organic matter ingested by earthworms undergoes considerable chemical changes while passing through the alimentary canal and organic carbon is converted into soluble forms that are more available to the organism; part of the organic matter of the soil is digested in the gut. As a result, the possibility for metals and synthetic pesticide residues bound to ions and carbonates that are in more soluble fractions, increases in the digested material (Morgan and Morgan, 1999). These soluble fractions can be accumulated in the body tissues during transit of ingested soil through the worm's gut, resulting in the reduction of pollutants in the worm-castings. Variation in dietary intake of essential heavy metals and absorption of non-essential trace metals and pesticide residues could be an important factor contributing to differences in the availability of these pollutants with reduced concentrations in the surface deposited castings (Pattnaik and Reddy, 2011). Variation in percentage remediation of persistent pollutants (like pesticide residues and heavy metals) in the castings of *Drawida willsi* as compared to that of *Glyphidrilus tuberosus,* was probably because of specific differences in the gut morphology of earthworms and its influence on assimilatory efficiency as typhlosole of soil/humus consuming earthworm species is structurally more elaborate and different from the other species. Relative amount of metals and pesticide residues absorbed and remediated from the diet (ingested soil) of different species of earthworms may depend up on the relative surface area of the typhlosolar epithelia though both of the species are endogeic. The persistent pollutants present are normally bound with the humus substances in the form of organo-metallic and organo synthetic mineral complexes; earthworm gut could modify the mobility of metals splitting the aggregates into simple molecules due to action of various digestive enzymes in the gut. The chloragosomes of earthworms consisting of modified epithelial cells, the eleocytes of the gut containing constituents of ion exchange compounds – phosphoric acid, carboxyl, phenolic hydroxyl and sulphonic acid groups acted as a cation exchange system capable of taking up and accumulating heavy metals, thus probably resulting in the remediation and lowering of pollutant concentrations in the worm-castings and vermicompost (Cooper, 1996). Corroborating the present findings, Dominguez (2004) reported 35 to55 % reductions in total amount of heavy metal pollutants in vermicompost attributing that the decrement is due to the chemical binding of the metals with metal binding protein granules present in the worm's gut region. Non-enzymatic metalloprotein granules or metallothioneins present in the earthworm's gut most likely played a significant role in the remediation of heavy metals and other such pollutants from the soil (Pattnaik and Reddy, 2012). In farming land, the non-essential toxic heavy metals like Cd and synthetic pesticide residues were recorded along with the essential metals. It was most probably because of indiscriminate usages of agrochemicals including pesticides in the agricultural fields (Reddy *et al.*, 2013). More removal of persistent pollutants like heavy metals and pesticide residues by the earthworms has already been estimated. In a study conducted at Pondicherry University, the non-essential heavy metal Cd was found about 48.4% and the least in case of Pb. In the agricultural fields, the % removal of heavy metals reported was in the order of- Cd>Cr>Mn>Zn>Pb. It showed worm's enigmatic role in the soil in eliminating highly toxic heavy metals like Cd from their bodies as early as possible as compared to the essential trace elements like Zn.

M. Vikram Reddy- Prof. of Ecology, Pondicherry University, Puducherry

Vermicomposting: a viable technology for recycling fly ash

With the consistently increasing numbers of coal-fired plants, generation of large scale of fly ash is posing an acute waste management problem not only in India but in different parts of the world. This coal residue has created environmental hazards and requires vast space for disposal too. Amongst different pathways suggested for re-use of fly ash, it's utilization for crop production and as a soil conditioner forms an important but less attended component. Fly ash contains different plant nutrients in considerable amount, except nitrogen and organic carbon. However, the major constraint for the maximum utilization of the nutrient supplying potential of this material is the low bio-availability of the plant nutrients. This behaviour has primarily been attributed to the poor availability of different microorganisms and, thereby restricted transformation of the plant nutrients to the available forms in the fly ash. Since positive effects of organic matter in improving microbiological activities and availability of different nutrients are widely documented, incorporation of adequate amount of organic materials along with fly ash has been considered to be a useful proposition for increasing efficiency of this waste material in providing plant nutrition. The maximum benefits from such organic materials may be achieved if such waste be composted properly. Vermicomposting - is an emerging biotechnology for accelerating the composting process and also for the production of better quality compost. It may be applied to assess the effectiveness of different plant nutrients from fly ash. In view of the competence of vermicomposting technology in decomposing wide range of organic wastes with the help of different microorganisms, a series of studies were carried out at the Institute of Agriculture, Visva-Bharati University to investigate the effects vermicomposting on the extraction of different plant nutrients from an inorganic material, fly ash into bio-available form. It was thought that simultaneous vermicomposting of a mixture of organic wastes and fly ash will help to intensify the microbiological activity in fly ash and thus will help to extract various plant nutrients from this inert material into bio-available form.

Inclusion of epigeic earthworms in mixtures of fly ash and organic wastes resulted in significant improvement in various microbiological activities in the substrates. This improvement was evidenced by increased population of total bacteria, fungi and actinomycetes, nitrogen fixing bacteria, phosphate solubilizing bacteria and also the values of microbial biomass carbon and basal respiration in the system. Such increments in the population of beneficial microorganisms in an inert material like fly ash were attributed to simultaneous use of organic waste which has increased activity of different microorganisms in the earthworm's gut. This increased microbial activity, in turn, helped in the release of different nutrients in fly ash to bio-available forms. Concentrations of nitrogen and organic carbon have also increased. In addition, solubility of various heavy metals was decreased under vermicomposting due to formation of metalothionein by the earthworms. Field level assessment of the efficiency of transformed fly ash showed that this material to be highly effective for integrated nutrient management and increased the yield rates of different agricultural crops like rice, mustard, tomato, potato etc.

Vermicomposting of fly ash is simple and easily adoptable biotechnology which can be taken up both at big and small scales even at house hold level too. Huge amount of foreign exchange is being spent every year to import millions of tons of potassium and phosphorus fertilizers for our agricultural cultivation. Both plant nutrients are present in considerable amount in the fly ash. Large scale adoption of this technology in rural sectors will not only help in recycling of good amount of fly ash for productive purpose along with the generation of colossal rural employment opportunities but also provide substantial savings of precious foreign exchange of the country.

GN Chattopadhyay - Former Professor at the Institute of Agriculture, Visva- Bharati University, Shantiniketan (West Bengal)

Indian Earthworm Biologist-5

Prof. Bikram K. Senapati (b.1948)

Dr. Senapati - a former Professor of Ecology & Environmental Biotechnology and Head, School of Life Sciences, Sambalpur University, Sambalpur (Odisha), India, is one of the illustrious personalities of Earthworm Biotechnology. He did his PG and PhD from Sambalpur University, Sambalpur (Odisha) and has gained P.G. teaching and research experience of more than 32 years especially on Environmental Biotechnology with special emphasis on Applied Bio-system (macro fauna), Bio-organic (Biodiversity) Management, Restoration of Degraded Systems and Biomass Waste Management with local resources.

He served as a Visiting Professor at the University of Paris (France) in 2000, Utkal University (Odisha) during 2010-2011 and Ravi Shankar University, Raipur during 2012-2013.

He has undertaken Research projects from various International Agencies, National Government Agencies and Private Agencies and evaluated research projects from different funding agencies like, DST, DBT, CSIR, UGC, ICAR etc. He carried out collaborative research at the International level with IRD, Univ. of Paris (France), Soil Science Institute of Mexico and Embrapa (Brazil) and National level with JNU, ZSI, and DST.

Prof. Senapati supervised 20 M. Phil. and 3 Ph.D. students and published 23 Review Articles and Book Chapters (in CABI, CAB (U.K.), Elsevier, UNESCO, FAO, Science Publishers Inc., (USA), IRD (France); Edited Books and Proceedings and 12 Consultancy Reports and 90 Research papers (International- 60%, National- 40%).

He has gained such an amazing recognition as one of the field ecologists that a French Group of Scientists have given the name of a newly invented genus of the earthworm as *Senapatiella* after his name with three new species from Western Ghats of India.

He has been conferred Dr. M. R. Bhiday Memorial Award for pioneering research and development work in Earthworm Biotechnology from INORA in 1998, Pune; Samanta Chandra Sekhar Award for outstanding work in Life Sciences in 2002 by the Government of Orissa and Gold Medal for the contribution in Environmental Sciences by the Zoological Society in 2004.

Prof. Senapati has patented "Fertilisation Bio-organique Dans les Plantations de the" or "Bioorganic Fertilization for Plantations" through the Ministry of Agriculture, France (Patent No. 11034) granted to Institut francais de recherche scientifique pour le development en cooperation of rule 213 rue la Fayette, 75480 Paris, France on 17th July, 1997, Protected in 24 different countries, applicable for plantation crops, agro-industries and waste biomass management for assessment and management of degraded land, organic farming, soil conservation and sustainability, technology transferred to China and updated for application since 2003, approved through field updating in October 2006 with 72% enhancement in profit. 'FBO' is already in application as an entrepreneurship model in Applied Soil Biology and Environmental Biotechnology. Ministry of Agriculture, France, is dealing with International Transfer Mechanism.

Presently, Professor Emeritus at the Sambalpur University (Odisha) and is offering consultancy services at International and National levels. He is engaged in Extension and Awareness activity on Science and Spirituality and Reviewer of several reputed International and National Scientific Journals.

Eudrilus eugeniae-A better therapeutic agent for stroke than *Lampito mauritii*

Earthworms have been widely used in India, China and Japan to treat various diseases as anticoagulant and fibrinolytic medicine from the ancient times. In the beginning, deep exploration has been made to reveal the use of earthworms as anticoagulant, antimicrobial, anti-inflammatory and anticancer agents. In 1991, Mihara et al found that the earthworms from Lumbricidae family could directly dissolve fibrin activated plasminogen. The active constituents of earthworm have been characterized and known to have fibrinolytic enzymes (EFEs) and are composed of several isoenzymes which can be transported into blood through the intestinal epithelium. Its lowest blood viscosity markedly reduces platelet aggregation and thrombus degradation in the blood. Due to these properties, earthworm fibrinolytic enzyme has been extensively studied as an oral thrombolytic drug. *Lumbricus rubellus, Eisenia fetida* and *Pheretima* sp. serve as a reservoir of fibrinolytic enzymes. The activities of earthworms differ across species due to the variations in their coenzyme constituents. Haemostasis- the arrest of bleeding from an injured blood, requires combined activities of vascular, platelet and plasma factors, counter balanced by mechanisms to limit the accumulation of platelets and fibrin to the area of the injury. Fibrinolytic enzymes dissolve fibrin, the main component of blood clots. Medications incorporating the enzymes are the most effective methods used for the thrombosis. A variety of therapeutically important fibrinolytic enzymes such as Streptokinase, Urokinase, tissue type Plasminogen Activator (t-PA), Staphylokinase and Recombinant Prourokinase have been extensively investigated and used clinically as thrombolytic agents eventhough they are expensive. This on administration intravenously expressed a number of significant limitations including fast clearance during circulation in the blood prior to expression of their therapeutic effects and lack of resistance to reocculusion as well as bleeding complications may appear. In addition, various side effects such as anaphylaxis and immunoreactions are often caused by the administration of foreign proteins. In the present study, antithrombic and proteolytic enzymes were purified from two earthworm species, viz *Eudrilus eugeniae* and *Lampito mauritii*. The crude and purified extract of *E. eugeniae* was found to be enriched with proteolytic activity whereas high thrombolytic ability was noticed to encounter in the extract of *L. mauritii*. The crude extract of both worms was extracted separately and their proteolytic activity was determined. The proteolytic activity of *E. eugeniae* and *L. mauritii* was 124 ± 4.8 and 63.05 ±1.4 µg/ml, respectively at 30 min. The proteolytic activity of different segments of the earthworm was estimated and it was found to be higher in the below part of the clitellum. With the doubt of the microbes of the gut region responsible for the proteolytic activity, the organisms were isolated and assessed for their proteolytic activity by biochemical tests and it was confirmed by the usage of selective media. Cultures from the gut region of *E. eugeniae* indicated the presence of Escherichia coli with the development of metallic sheen colonies developed on the Mac Conkey media. In order to identify the particular localization of the proteolytic activity, organisms cultured from the interior region of the gut were mass cultured. These cultures were centrifuged in order to separate the supernatant and the pellet and their proteolytic activity was determined. The supernatant of the bacterial culture isolated from *E. eugeniae* rendered a proteolytic activity of 86.02 ± 0.79 µg/ml whereas it was 70 ± 0.58 µg/ml in its castings. The supernatant of the bacterial culture isolated from *L. mauritii* exhibited a proteolytic activity of 79.76 ± 5.57 µg/ml whereas it was 74.35 ± 0.50 µg/ml in their castings. It indicates existence of high proteolytic activity in the supernatant of the culture than the respective worm-castings and confirms the extracellular nature of the proteolytic enzymes. With a view to analyze the haemolytic effect of the earthworm extracts, an experimental trial was set up with sheep RBC (SRBC) mixed with water (control). SRBC with the extract of the individual test earthworms are subjected to observation in the haemocytometer. These samples did not show any remarkable change in the intact RBC count. With a view to study the thrombolytic activity of the earthworm extracts, the sheep blood was mixed with the test extracts and the delay in blood clotting was detected. About 70% of inhibition of blood clot delay was observed in 1.75 ml of sheep blood mixed with 0.25 ml of *E. eugeniae* extract whereas it was 60% in case of *L. mauritii* extract. Purification of the enzyme was carried out by adopting ammonium sulphate precipitation followed by membrane dialysis. This purified enzyme could be administered to experimental animals for effective in vivo thrombolytic analysis and for further usage in treating human intravenous blood clots especially during the stroke.

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READER'S OPINION IS SOLICITED

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