Environmentalist (2012) 32:445–452
DOI 10.1007/s10669-012-9409-2

Earthworms: nature's chemical managers and detoxifying agents in the environment: an innovative study on treatment of toxic wastewaters from the petroleum industry by vermifiltration technology

Rajiv K. Sinha · Vinod Chandran · Brijal K. Soni · Upendra Patel · Ashok Ghosh

Published online: 15 July 2012

Abstract Earthworms are justifying the beliefs of Great Russian scientist Dr. Anatoly Igonin who said they are—"*disinfecting, detoxifying, neutralizing, protective and productive*". Studies indicate that some species of earthworms can "bio-accumulate, biodegrade or bio-transform" any toxic chemicals including "heavy metals", "organochlorine pesticide", "herbicides", and the lipophilic organic micro-pollutants like "polycyclic aromatic hydrocarbons" (PAHs). Worm vermicasts, due to the presence of "hydrophilic" groups in the "lignin contents" and "humus", also provide wonderful sites for "adsorption" of heavy metals and chemical pollutants in wastewater. Vermifiltration of wastewater using waste-eater earthworms is a newly conceived novel technology with several economic and environmental advantages. The earthworm's body and the their "vermicast" work as a "biofilter" removing BOD_5 by over 90 %, COD by 60–80 %, TDSS by 90–95 %, and toxic chemicals and pathogens from wastewater. This was a pioneering work done on an extremely "toxic wastewater" from the petroleum industry. It contained mixture of "aliphatic" and "aromatic" volatile petroleum hydrocarbons (C 10–C 36) and "organochlorines" originating from the cooling liquids, waste engine and gear oil, waste transmission and brake fluid, grease, spilled petrol, and diesel oil. The aliphatic fraction contained cycloalkanes as well as a complex mixture of saturated toxic hydrocarbons. The aromatic fraction mainly consisted of PAHs, which are more toxic and persistent than the aliphatic part. The chemicals of concern were the total petroleum hydrocarbons (TPH), dichloromethane (DCM), dichloroethane (DCE), and *t*-butyl methyl ether (tBME). The tBME compound has raised global concern recently due to its high mobility and persistence in the environment and possible carcinogenicity. About 1,000 earthworms (species *Eisenia fetida*) were released in the soil of vermifilter bed. They not only tolerated and survived in the toxic petroleum environment but also bio-filtered and bio-remediated the dark-brown petroleum wastewater with a pungent smell into pale-yellow and odorless water indicating disappearance of all toxic hydrocarbons. The hydrocarbons C 10–C 14 were reduced by 99.9 %, the C 15–C 28 by 99.8 %, and the C 29–C 36 by 99.7 % by earthworms.

Keywords Earthworm's body as bio-filter · Earthworms bio-accumulate, biodegrade and bio-transform toxic materials · Earthworms hosts toxic degrader microbes

R. K. Sinha (✉)
School of Environmental Engineering, Griffith University, Brisbane, Australia
e-mail: rajiv.sinha@griffith.edu.au

V. Chandran · B. K. Soni
Master of Environmental Engineering, Griffith University, Brisbane, Australia

U. Patel
Charotar University of Science and Technology, Anand, Gujarat, India

A. Ghosh
Department of Environment and Water Resources, A.N. College, Patna, India

1 Introduction

Vermifiltration of wastewater using waste-eater earthworms is a newly conceived technology with several economic and environmental advantages over the costly conventional wastewater treatment plants (Soto and Toha

1998). We have pioneered the technology in Australia. The earthworm's body works as a "biofilter" removing BOD_5 by over 90 %, COD by 60–80 %, TDSS by 90–95 %, and toxic chemicals and pathogens from wastewater and contaminated soils by over 99 % (Sinha and Bharambe 2007). Worms bio-accumulate any toxic chemicals including heavy metals and biodegrade the toxic organics, kills the pathogens in the wastewater by their anti-pathogenic ceolomic fluid, and the treated water becomes clean enough to be reused for non-potable purposes, e.g., washing, cleaning, for farm irrigation, and in industry. Earthworms in the vermifilter bed feed upon the solids in wastewater and excrete them as "vermicast". Worm vermicasts also provide wonderful sites for adsorption of heavy metals and pollutants in wastewater. This is due the presence of hydrophilic groups in the lignin contents and humus of the vermicompost. The vermicast also offers excellent hydraulic conductivity of sand (being porous like sand) and also high adsorption power of clay (Bhawalkar 1995; Sinha et al. 2008a, 2010a, b, c, 2011a, b).

We studied the vermifiltration of petroleum wastewater obtained from the automobile service industry in Brisbane. The traditional system of treatment of effluents from petroleum refineries is based on the mechanical and physico-chemical methods followed by biological treatment. The ultra-filtration method is also used. All are highly expensive and time-consuming processes.

The wastewater used was blackish-brown with a pungent odor and contained mixture of aliphatic and aromatic volatile petroleum hydrocarbons (C 10–C 36) and organochlorines originating from the cooling liquids, waste engine and gear oil, waste transmission and brake fluid, grease, spilled petrol, and diesel oil. The aliphatic fraction contained the majority of petroleum compounds, e.g., cycloalkanes, as well as an unresolved complex mixture of saturated toxic hydrocarbons. The aromatic fraction mainly consisted of PAHs and is more toxic and persistent than the aliphatic part. The chemicals of concern in the petroleum-contaminated wastewater and the soil of vermifilter bed are the total petroleum hydrocarbons (TPH), dichloromethane (DCM), dichloroethane (DCE), and *t*-butyl methyl ether (tBME). The tBME compound has raised global concern recently due to its high mobility and persistence in the environment and possible carcinogenicity (Sinha and Valani 2011).

2 The versatility and adaptations of earthworms to tolerate and detoxify toxic chemicals in the environment: some studies

Earthworms, especially including the species *Eisenia fetida*, can tolerate high toxic chemicals in the environment. After the Seveso chemical plant explosion in 1976 in Italy, when a vast area was contaminated with extremely toxic chemicals like TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), several fauna perished, except for some species of the earthworms that survived. *E. fetida* was one of them. The earthworms that ingested TCDD-contaminated soils were shown to bio-accumulate dioxins in their tissues and concentrate it on average 14.5-fold (Satchell 1983). The World Health Organization (WHO) has not set a "safe limit" of dioxins for human beings?

Studies have indicated that earthworms can bio-accumulate, biodegrade or bio-transform heavy metals, organochlorine pesticide, herbicides, and the lipophilic organic micro-pollutants like polycyclic aromatic hydrocarbons (PAHs) residues in the medium in which it lives. It does this by enzymatic actions (Davis 1971; Ireland 1983; Haimi et al. 1992; Eijsackers et al. 2001; Gevao et al. 2001; Sinha et al. 2010a, b, c, 2011a, b).

2.1 Heavy metals

Hartenstein et al. (1980) showed that earthworms, particularly *E. fetida*, can bio-accumulate high concentrations of heavy metals like cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), manganese (Mn), calcium (Ca), iron (Fe), and zinc (Zn) in their tissues without affecting their physiology, and this particularly when the metals are mostly non-bioavailable. They can particularly ingest and accumulate extremely high amounts of zinc (Zn), lead (Pb), and cadmium (Cd). Cadmium levels up to 100 mg per kg dry weight have been found in tissues. Ireland (1983) reported that the earthworm species *Lumbricus terrestris* can bio-accumulate in their tissues 90–180 mg lead (Pb)/g of dry weight, while for *L. rubellus* and *D. rubida* it was 2,600 mg/g and 7,600 mg/g of dry weight, respectively. Metals are usually bound by a protein called metallothioneins, found in earthworms, which has a very high capacity to bind metals and render them biologically inactive.

2.2 Crude petroleum oil

Eisenia fetida was used as the test organism for different soil contaminants, and several reports have indicated that *E. fetida* can tolerate up to 1.5 % crude oil (containing several toxic organic pollutants) and survived in this environment (Contreras-Ramos et al. 2006; OECD 2000; Safawat et al. 2002; Lukkari et al. 2006; Schaefer and Filser 2007). Studies with earthworm *E. fetida* on oil-contaminated soil revealed that worms significantly decreased oil contents in comparison to the control. Schaefer (2005) studied that increased microbial catabolic activity due to the presence of *E. fetida* was responsible for the loss of 91 % (1,074 mg/kg of soil to 96 mg/kg) of crude oil contamination in 56 days of treatment. It also successfully treated high molecular weight hydrocarbons "asphaltenes" from the *Prestige* oil spill in 2002. Earthworms mineralized the asphaltenes, thus eliminating them from the system. They also decontaminated complex hydrocarbon-polluted soils (Tomoko et al. 2005; Ceccanti et al. 2006; Martin-Gil et al. 2007).

2.3 Polyaromatic hydrocarbons (PAHs)

PAHs are priority pollutants and cause great concern with respect to human health and environment. They are inherently recalcitrant hydrocarbons, and the higher molecular weight PAHs are very difficult to remediate (Jager et al. 2003). Ma et al. (1995, 1998) studied the influence of the earthworm species *L. rubellus* on the disappearance of spiked PAHs phenanthrene and fluoranthene (100 μg/kg of soil) and found that the losses of both PAHs occurred at a faster rate in soils with earthworms than the soil without worms. After 56 days (8 weeks), 86 % of the phenanthrene was removed. Contreras-Ramos et al. (2006) studied the uptake of three PAHs, namely, phenanthrene, anthracene, and benzo(a) pyrene at different concentrations by *E. fetida* and measured the PAH concentrations in the soil and in the tissues of earthworms exposed to the PAHs for 11 weeks. The concentration of anthracene decreased by twofold after addition of earthworms and the average removal was 51 %, which was only 23 % by microbes alone when the earthworms were not added to the soil. On average, the concentration of benzo[*a*]pyrene decreased by 1.4-fold, and the average removal was 47 %, which was only 13 % by microbes when earthworms were not present. Phenanthrene was completely removed (100 %) by earthworms when the amount of the chemical was <100 mg/kg of soil, while only 77 % was removed by microbes in the absence of earthworms.

We also studied the remedial action of earthworms on PAH-contaminated soils obtained from a former gas-works site in Brisbane, Australia, where gas was being produced from coal. The initial concentration of total PAHs compounds in the soil at site was greater than 11,820 mg/kg of soil. The legislative requirements for PAHs concentration in soil in Australia is only 100 mg/kg for industrial sites and 20 mg/kg for residential sites. Worms removed nearly 80 % of the PAHs as compared to just 47 % where worms were not applied and only microbial degradation occurred. This was just in a 12-week study period. It could have been removed by 100 % in another few weeks. More significant was that the worm-added soil became odor-free of chemicals in a few days and were more soft and porous in texture (Sinha et al. 2008b).

2.4 Organochlorine pesticides and herbicides

Several studies have found a definite relationship between organochlorine pesticide residues in the soil and their amount in earthworms *E. fetida*, with an average concentration factor (in earthworm tissues) of about 9 for all compounds and doses tested. Studies indicated that the earthworms bio-accumulate or biodegrade organochlorine pesticides and PAH residues in the medium in which it lives (Davis 1971; Haimi et al. 1992; Ireland 1983).

Bolan and Baskaran (1996) studied the effect of the earthworm species *Lumbricus rubellus* and *Allobophora callignosa* vermicast on the sorption and movement of herbicides C^{14}–metsulfuron methyl, C^{14}–atrazine, C^{14}–2,4 dichlorophenoxyacetic acid (2,4-D) in soil. Worm vermicasts sorbed higher amount of herbicides from the contaminated soil than the control soil.

2.5 Polychlorinated biphenyls (PCBs)

PCBs are categorized as unusually toxic and persistent organic pollutant (POPs). They have serious adverse effects on human health as well as the environment. They constitute a family of chemicals with over 200 types, and are used in transformers and power capacitors, electrical insulators, as hydraulic fluids and diffusion pump oil in heat-transfer applications, and as plasticizers for many products. Singer et al. (2001) studied the role of the earthworm species *Pherertime hawayana* in mixing and distribution of PCB-degrader microorganisms when added to Aroclor 1242 contaminated soil (100 mg/kg of soil) over an 18-week period. The contaminated soil treated with earthworms resulted in significantly greater PCB losses (average 52 %) when compared to the soil without earthworm treatment, which was 41 %.

2.6 Endocrine-disrupting chemicals (EDCs)

Earthworms have also been reported to bio-accumulate endocrine-disrupting chemicals (EDCs) from sewage that is otherwise not removed by conventional wastewater-treatment systems. Markman et al. (2007) has reported significantly high concentrations of EDCs (dibutylphthalate, dioctylphthalate, bisphenol-A and 17 β-estradiol) in tissues of earthworms (*E. fetida*) living in sewage-percolating filter beds and also in garden soil.

3 The mechanism of worm action in vermifiltration and toxicity removal

1. The twin processes of microbial stimulation and biodegradation, and the enzymatic degradation of waste solids by worms as discussed above simultaneously work in the vermifiltration system, too.
2. Vermifilters provide a high specific area—up to 800 m^2/g and voidage up to 60 %. Suspended solids are

trapped on top of the vermifilter and processed by earthworms and fed to the soil microbes immobilized in the vermifilter.

3. Earthworms intensify the organic loadings of wastewater in the vermifilter soil bed by the fact that it granulates the clay particles thus increasing the hydraulic conductivity of the system. They also grind the silt and sand particles, thus giving high total specific surface area, which enhances the ability to adsorb the organics and inorganic from the wastewater passing through it.
4. Vermicompost (produced by earthworms in the vermifilter bed through feeding of solids in wastewater and their excretion as vermicast) provides wonderful sites for adsorption of heavy metals and pollutants in wastewater. This is due to the presence of hydrophilic groups in the lignin contents and humus of the vermicompost. The vermicast also offers excellent hydraulic conductivity of sand (being porous like sand) and also high adsorption power of clay (Bhawalkar 1995).
5. The chloragogen cells in earthworms appear to mainly accumulate heavy metals and their immobilization in the small spheroidal chloragosomes and debris vesicles that the cells contain. Some metals are bound by a protein called metallothioneins found in earthworms, which has a very high capacity to bind metals and render them biologically inactive.
6. The microbial flora associated with the intestine and the vermicasts of the earthworms also degrade several categories of organics including the toxic organics in wastewater. Species like *Pseudomonas, Mucor, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes,* and *Acidobacterium* have been reported. *Acaligenes* can even degrade PCBs and *Mucor* can degrade dieldrin (Singleton et al. 2003). Under favorable conditions, earthworms and microbes act "symbiotically" to accelerate and enhance the decomposition of the organics through enzymatic actions.
7. Earthworms also graze on the surplus harmful and ineffective microbes in the wastewater selectively, prevent choking of the medium, and maintain a culture of effective bio-degrader microbes to function.

4 Materials and methods

The study was carried out in a vermicomposting (VC) bin, which was made to work as the vermifiltration (VF) kit. The temperature in the lab was maintained at 21.5 °C, with 50 % humidity. The vermifiltration kit contained about 30–40 kg of gravels with a layer of garden soil on top. This formed the vermifilter bed. It has provisions to collect the filtered water at the bottom in a chamber, which opens out through a pipe fitted with a tap. Above the chamber lies the net of wire mesh to allow only water to trickle down while holding the gravel above. The bottommost layer is made of gravel aggregates of size 7.5 cm and it fills up to the depth of 25 cm. Above this lies the aggregates of 3.5–4.5 cm sizes filling up to another 25 cm. On the top of this is the 20-cm layer of aggregates of 10–12-mm sizes mixed with sand. The topmost layer of about 10 cm consists of pure soil in which the earthworms were released. The worms were given around 1-week settling time in the soil bed to acclimatize in the new environment.

4.1 The control kit without earthworms: the geofilter

A control kit (exact replica of vermifilter kit but devoid of earthworms) was also organized for reference and comparison. The control system had same components of filtration, e.g., different grades of pebbles at the bottom with soil on top, but no earthworms. This is called a "geofilter", as soils and pebbles also aid in filtration of wastewater. It is important to note that the soil and sand particles and the gravels in the kit also contribute in the filtration and cleaning of wastewater by adsorption of the impurities on their surface. They provide ideal sites for colonization by decomposer microbes, which also help in the treatment of wastewater.

5 The experimental procedures

Around 5–6 l of petroleum wastewater was kept in calibrated a 10-l PVC drum. These drums were kept on an elevated platform just near the vermifilter kit. The PVC drums had a tap at the bottom to which an irrigation system was attached. The irrigation system consisted of a simple 0.5-in. polypropylene pipe with holes for trickling water that allowed uniform distribution of wastewater on the soil surface (vermifilter bed). Wastewater from the drums flowed through the irrigation pipes by gravity.

The wastewater was fed to the vermifilter kit from the top, which percolated down through various layers in the vermifilter bed, passing through the soil layer inhabited by earthworms, the sandy layer, and the gravels and at the end was collected in a chamber at the bottom of the kit. On the next day, this treated wastewater from both kits was collected and sent to Queensland Laboratories, Brisbane, for chemical analysis. The hydraulic retention time (HRT) was kept uniformly between 1 and 2 h in all experiments (Figs. 1, 2, 3, 4).

The vermifilter bed consisted of an additional layer of vermicompost on the top covered by a layer of soil with earthworms. Vermicompost also aids in filtration (by adsorption), as it is rich in humus. About 1,000 earthworms

(*Eisenia fetida*) were released in this soil with feed materials, which mainly consisted of lettuce leaves. This was necessary to keep the worms healthy as the petroleum wastewater does not contain any biodegradable organic matter that can be consumed by earthworms as feed materials.

5.1 Results and findings

Worms not only tolerated and survived in the toxic environment but also bio-filtered and bio-remediated the dark-brown petroleum wastewater with a pungent smell into pale-yellow and odorless water indicating disappearance of all toxic and complex hydrocarbons. The hydrocarbons C 10–C 14 were reduced by 99.9 %, the C 15–C 28 by 99.8 %, and the C 29–C 36 by 99.7 % by earthworms. In the control system, termed as a geo-filtration system by soil (soil microbes, organics, and inorganic), pebbles and gravels, it was only reduced by 9.75, 2.35, and 4.22 %, respectively. This implies that earthworms in soil played a very important and critical role, and significantly reduced the toxic hydrocarbons from the petroleum wastewater by over 99 % within some hours. If the vermifiltered wastewater was again subjected to further treatment by earthworms, it could be completely free of any toxic chemicals.

The volatile hydrocarbons from the soil in the vermifilter bed were also removed within 2–3 days, as indicated by the complete disappearance of any odor. The worms bio-accumulated, biodegraded, and bio-transformed all the toxic chemicals from the wastewater and the contaminated soil in the vermifilter bed (Fig. 5). The pungent odor continued for several days from the soil of control geofilter bed without worms (Chandran 2010). Results are given in Table 1.

The experiment was repeated again in 2012 with same source of wastewater from the automobile industry, with same constitution of vermifilter bed and with same species and number of worms to verify and testify the previous results of 2010. There were slight differences in the concentrations of hydrocarbons (C 10–C 36) in the raw wastewater but the percentage reduction was very much similar in both systems-control (geofiltration) and those with worms (vermifiltration). Results have been given in Table 2.

Fig. 1 Different sizes of gravels used in preparation of the vermifilter bed (bottom layer gravel sizes 7.5 cm)

Fig. 2 Different sizes of gravels used in preparation of the vermifilter bed (middle layer gravel sizes 3.5–4.5 cm)

Fig. 3 Different sizes of gravels used in preparation of the vermifilter bed (upper layers gravel sizes 10–12 mm)

6 Conclusions and remarks

Our vermifiltration experiments with extremely toxic petroleum wastewater have once again proved that earthworms, especially *E. fetida* and some other chemically tolerant and adapted species, can detoxify and neutralize diverse types of toxic and hazardous industrial wastewaters more economically and in a more environmentally sustainable manner without the use of any other neutralizing chemicals. The traditional systems of treatment of effluents

Fig. 4 Soil bed with earthworms in the uppermost layer & the device of wastewater discharge and distribution through perforated PVC pipes

Fig. 5 The dark-brown raw petroleum wastewater (right) color changes into a pale yellow (left) after vermifiltration (Color figure online)

from petroleum refineries are highly expensive and time-consuming processes. Moreover, they all produce "sludge" (a biohazard), which requires safe disposal in secured land-fills. There is no sludge formation in the vermifiltration system, as the worms eat the solids and convert them into soft and porous detoxified vermicast, which can be used in farms. If successful (as the preliminary studies indicate), vermifiltration can be a highly cost-effective, much quicker, and also environmentally sound and sustainable technology for the treatment of effluents from petroleum refineries.

Earthworms have been found to remove very high BOD loads (10,000–1,00,000 mg/l often found in wastewater from food processing industries) within 4–10 h of HRT, suggesting that they possess the necessary enzymes that can degrade high amounts of "waste organics". More significantly, they can also significantly reduce COD (chemical oxygen demand) loads of wastewater. This strongly suggests that earthworms also posses the necessary catabolic enzymes that can degrade even complex chemicals in the wastewater.

As earthworms play a critical role in wastewater purification, their number and population density (biomass) in the soil of vermifilter bed, maturity, and health are important factors. This may range from several hundred to several thousand. There are reports about 8–10,000 numbers of earthworms per cubic meter of the vermifilter bed or in biomass as 10 kg of earthworms per cubic meter (cum) of soil in the vermifilter bed for optimal function of the vermifilter bed (Komarowski 2001).

Moreover, in all vermiculture technologies, "earthworms biomass" comes as a valuable by-product, which is finding new applications in several industries, including the poultry, fishery, and dairy industries (as raw materials for production of nutritive feeds due to high protein contents); pharmaceutical industry (for production of modern life-saving medicines for several ailments and more specifically for cardiovascular diseases and stroke problems and combating cancers from their bioactive compounds); and in the detergent and lubricant industries. If the research into their bioactive compounds is successful in "waging a war on cancer", it will be the biggest scientific achievement of the new millennium for mankind, much bigger than the moon landing of 1969 and a great tribute to the "father of modern biology", Sir Charles Darwin (Sinha and Valani 2011).

Table 1 Efficiency of earthworms in removal of toxic hydrocarbons from petroleum-contaminated wastewater generated in the automobile industry

Parameters studied	Raw wastewater (in μg/l)	Geofiltered water (control) (in μg/l)	Reduction (%)	Vermifiltered water (by earthworms) (in μg/l)	Reduction (%)
Hydrocarbons (C 10–C 14)	41,000,000	37,000,000	9.75	2,500	99.93
Hydrocarbons (C 15–C 28)	170,000,000	130,000,000	23.52	22,000	99.87
Hydrocarbons (C 29–C 36)	71,000,000	68,000,000	4.22	16,000	99.77

Source: Chandran (2010)

N.B. μg/l, Micrograms per liter; geofiltered water, wastewater filtered by soil, pebbles and microbes; vermifiltered water, wastewater filtered by soil, pebbles, microbes, earthworms and vermicompost)

Table 2 Efficiency of earthworms in removal of toxic hydrocarbons from petroleum contaminated wastewater generated in automobile industries (in µg/L)

Parameters studied	Raw wastewater	Geofiltered water (Control)	Reduction (%)	Vermifiltered water (by earthworms)	Reduction (%)
Hydrocarbons (C 10–C 14)	42,000,000	38,000,000	9.52	3,700	99.91
Hydrocarbons (C 15–C 28)	168,000,000	132,000,000	21.42	24,400	99.85
Hydrocarbons (C 29–C 36)	72,000,000	67,000,000	6.94	18,600	99.74

Source: Sinha and Soni (2012)

N.B. µg/l, micrograms per liter; geofiltered water, wastewater filtered by soil, pebbles and microbes; vermifiltered water, wastewater filtered by soil, pebbles, microbes, earthworms, and vermicompost

The earthworm truly combines the attributes of both "civil, chemical, and environmental engineers" and a "producer and protector" for human civilization.

References

Bhawalkar US (1995) Vermiculture eco-technology. Publication of Bhawalkar Earthworm Research Institute (BERI), Pune

Bolan NS, Baskaran S (1996) Characteristics of earthworm casts affecting herbicide sorption and movement. Biol Fertil Soils 22:367–372

Ceccanti B, Masciandaro G, Garcia C, Macci C, Doni S (2006) Soil bioremediation: combination of earthworms and compost for the ecological remediation of a hydrocarbon polluted soil. J Water Air Soil Pollut 177:383–397

Chandran V (2010) Study of vermifiltration of fruit juice industry and petroleum industry wastewater and vermiremediation of soil contaminated by petroleum products using earthworms (Eisenia fetida). 40 CP Research Project for the fulfilment of Master Degree in Environmental Engineering, Griffith University, Australia. (Supervisors: Dr. Rajiv K. Sinha & Dr. Sunil Herat)

Contreras-Ramos SM, Alvarez-Bernal D, Dendooven L (2006) *Eisenia fetida* increased removal of polycyclic aromatic hydrocarbons (pahs) from soil. Environ Pollut 141:396–401

Davis B (1971) Laboratory studies on the uptake of dieldrin and DDT by earthworms. Soil Biol Biochem 3:221–223

Eijsackers H, Van Gestel CAM, De Jonge S, Muijis B, Slijkerman D (2001) PAH—polluted dredged peat sediments and earthworms: a mutual inference. Ecotoxicology 10:35–50

Gevao B, Mordaunt C, Semple KT, Piearce TG, Jones KC (2001) Bioavailability of non-extractable (bound) pesticide residues to earthworms. Environ Sci Technol 35:501–507

Haimi J, Salminen J, Huhta V, Knuutinen J, Palm H (1992) Bioaccumulation of organochlorine compounds in earthworms. J Soil Biol Biochem 24(12):1699–1703

Hartenstein R, Neuhauser EF, Collier J (1980) Accumulation of heavy metals in the earthworm *E. foetida*. J Environ Qual 9:23–26

Ireland MP (1983) Heavy metals uptake in earthworms; earthworm ecology. Chapman & Hall, London

Jager T, Baerselman R, Dukeman E, De Groot AC, Hogendoorn EA, Jong AD, Kruitbosch J, Peijn W (2003) Availability of PAH to earthworms (*Elsenia andrei*) in field polluted soil and soil-sediment mixtures. J Environ Toxicol Chem 22(4):767–775

Komarowski S (2001) Vermiculture for sewage and water treatment sludges. Water, Publication of Australian Water and Wastewater Association, July, pp 39–43

Lukkari T, Teno S, Vaisanen A, Haimi J (2006) Effect of earthworms on decomposition and metal availability in contaminated soil: microcosm studies of populations with different exposure histories. Soil Biol Biochem 38:359–370

Ma WC, Imerzeel J, Bodt J (1995) Earthworm and food interactions on bioaccumulation and disappearance of PAHs: studies on phenanthrene and fluoranthene. Ecotoxicol Environ Saf 32:226–232

Ma WC, van Kleunen A, Immerzeel J, De-Maargd G-J (1998) Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: assessment of equilibrium partitioning theory in in situ studies and water experiments. Environ Toxicol 17:1730–1737

Markman S, Guschina IA, Barnsleya S, Buchanan KL, Pascoe D, Muller CT (2007) Endocrine disrupting chemicals accumulate in earthworms exposed to sewage effluents (also garden soils). Cardiff School of Biosciences, Cardiff University, Cardiff, UK. J Chemosphere 70(1):119–125

Martin-Gil J, Navas-Gracia LM, Gomez-Sobrino E, Correa-Guimaraes A, Hernandez-Navarro S, Sanchez-Bascones M (2007) Composting and vermicomposting experiences in the treatments and bioconversion of asphaltens from the *Prestige* oil spill. Bioresour Technol 99:1821–1829

OECD (2000) Guidelines for testing organic chemicals. Proposal for new guidelines: earthworms reproduction tests (*E. fetida andrei*). Organization for Economic Co-operation and Development (www.oecd.org)

Safawat H, Hanna S, Weaver RW (2002) Earthworms survival in oil-contaminated soil. J Plant Soil 240:127–132

Satchell JE (1983) Earthworm ecology—from Darwin to Vermiculture. Chapman and Hall Ltd., London

Schaefer M (2005) Earthworms in crude oil-contaminated soils: toxicity tests and effects on crude oil degradation. J Contam Soil Sediment Water 35:708

Schaefer M, Filser J (2007) The influence of earthworms and organic additives on the biodegradation of oil-contaminated soil. J Appl Soil Ecol 36:53–62

Singer AC, Jury W, Leupromchai E, Yahng C-S, Crowley DE (2001) Contribution of earthworms to PCB bioremediation. J Soil Biol Biochem 33:765–775

Singleton DR, Hendrix BF, Coleman DC, Whitemann WB (2003) Identification of uncultured bacteria tightly associated with the intestine of the earthworms *Lumricus rubellus*. Soil Biol Biochem 35:1547–1555

Sinha RK, Bharambe G (2007) Removal of high bod and cod loadings of primary liquid waste products from dairy industry by vermifiltration technology using earthworms; Indian J Environ Prot (IJEP) 27(6):486–501, ISSN 0253-7141; Regd. No. R.N. 40280/83; Indian Institute of Technology, BHU, India

Sinha RK, Soni Bk (2012) Experimental studies on treatment of petroleum-contaminated wastewater from automobile industry in Brisbane. Griffith University, Brisbane

Sinha RK, Valani D (2011) Vermiculture revolution: the technological revival of Charles Darwin's unheralded soldier's of mankind. NOVA Science Publication, NY, p. 328; ISBN 978-1-61122-035-3

Sinha RK, Bharambe G, Chowdhary U (2008a) Sewage treatment by vermi-filtration with synchronous treatment of sludge by earthworms: a low-cost sustainable technology over conventional systems with potential for decentralization. Environmentalist 28:409–420, Published Online 8 April 2008, Springer, USA

Sinha RK, Bharambe G, Ryan D (2008b) Converting wasteland into wonderland by earthworms: a low-cost nature's technology for soil remediation: a case study of vermiremediation of PAH-contaminated soil. Environmentalist 28:466–475, Published Online 14 May 2008, Springer, USA

Sinha RK, Valani D, Chauhan K, Chandran V, Soni BK, Patel V (2010a) Earthworms: Charles Darwin's 'unheralded soldiers of mankind': protective & productive for man & environment. J Environ Prot 1:251–260, Scientific Research Publishing, USA

Sinha RK, Agarwal S, Chauhan K, Chandran V, Soni BK (2010b) Vermiculture technology: reviving the dreams of Sir Charles Darwin for scientific use of earthworms in sustainable development programs. J Technol Invest 1:155–172, Scientific Research Publications, USA (www.SciRP.org/journal/ti)

Sinha RK, Herat S, Valani D (2010c) Earthworms–the environmental engineers: review of vermiculture technologies for environmental management and resource development. In: Sinha RK, Herat S and Agarwal S (eds). Special Issue on 'Vermiculture Technology for Environmental Management and Resource Development. Int J Global Environ Issues, Inderscience Pub. USA 10:265–292. Nos. 3/4

Sinha RK, Herat S, Valani D, Chandran V, Soni BK (2011a) Earthworms—the Great Environmental & Civil Engineers of 21st Century: vermiculture technology—a Soft bioengineering alternative to the hard civil engineering methods for sustainable development & waste management with potential to mitigate global warming. Int J Environ Prot (in press)

Sinha RK, Herat S, Valani D, Chandran V, Soni BK (2011b) Earthworms (Charles Darwin's Children) in new role for mankind: some recent developments in vermiculture research. In: Suthar S (ed) Vermitechnology: Recent Advances. Springer Publications (in press)

Soto MA, Toha J (1998) Ecological wastewater treatment: advanced wastewater treatment, recycling and reuse. AWT 98, Milan, September 14–16, 2008

Tomoko Y, Koki T, Hiroaki S (2005) Enhanced bioremediation of oil-contaminated soil by a combination of the earthworm (*Eisenia fetida*) and tea extraction residue. Edaphologia 77:1–9