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Biomass Alteration of Earthworm in the Organic Waste-Contaminated Soil

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1. Introduction

Earthworm populations show a considerable amount of variability in time and space, with mean densities and biomass ranging from less than 10 individuals and 1 g m^{-2} to more than 1,000 individuals and 200 g m^{-2} under favourable conditions. Earthworms have been considered to play a great role in soil-formation processes and in monitoring soil structure and fertility (Lavelle & Spain, 2001) because they may increase the mineralisation and humification of organic matter by food consumption, respiration and gut passage (Edwards & Fletcher, 1988; Lavelle & Spain, 2001) and may indirectly stimulate microbial mass and activity as well as the mobilisation of nutrients by increasing the surface area of organic compounds and by their casting activity (Emmerling & Paulsch, 2001). However, within particular climatic zones, earthworm assemblages, with fairly characteristic species richness, composition, abundance and biomass, can often be recognised in broadly different habitat types, such as coniferous forest, deciduous woodland, grassland and arable land (Curry, 1998).

Agriculture is facing a challenge to develop strategies for sustainability that can conserve non-renewable natural resources, such as soil, and enhance the use of renewable resources, such as organic wastes. It has been estimated that 357,861 tons of organic sludge daily were produced in South Korea in 2009 (Anon., 2009). The production and use of organic compounds have also risen rapidly over the last four decades. Organic compounds which are released either through direct discharge into the sewer system, or indirectly through run-off from roads and other surfaces are found in sewage sludge (Halsall et al., 1993). As a suitable bioindicator of chemical contamination in soil, earthworms are easy, fast and economical merits to handle. Especially, analysis of their tissues may also provide an excellent index of bioavailability of heavey metals in soils (Helmke et al., 1979; Pearson et al., 2000).

Although the acute earthworm toxicity test developed by Edwards (1984) has been widely used and an internationally accepted protocol was also used for assaying the chemical toxicity of contaminants in soils (Organisation for Economic Cooperation and Development [OECD], 1984), the chronic toxicity test to detect subtle effects of contaminants on them by long-term exposure has not been fully achieved (Venables et al., 1992). Based upon these tests, lots of information on heavy metal uptake, toxicity and accumulation by various earthworm species have been produced. Therefore, earthworms could fill the gap by being used as potential biomarkers of ecotoxicity to various chemicals, including organic contaminants.

This chapter is particularly focused on the hazardous effects on composition, numbers and biomass of Megascolecid and Moniligastrid earthworms, which are dominant groups in South Korea, of 8 consecutive yearly applications of three levels of four different organic sludges and pig manure compost as a positive reference using field lysimeters and microcosms.

2. Legal criteria of inorganic pollutants

Many countries have been trying to prepare a regulatory limit to the use of organic wastes, such as food wastes or sludge, into crop production system in the light of their rapid increase. The regulatory system for the agricultural use of organic waste in South Korea is defined as soil concentration limits for potentially toxic elements (PTEs) to safeguard human health and crop yields. Despite legal limits, the damage of crop in the agricultural soil frequently occurs with organic waste for long-term application and with sub-quality compost made from sewage sludge.

The control system in the application of sludge to farmland varies according to country (Table 1). In South Korea, the control system for the application of sludge to farmland primarily depends upon heavy-metal concentrations that are similar to those in developed countries. Legally allowed limit values for PTEs— such as copper (Cu), zinc (Zn), chromium (Cr), cadmium (Cd), lead (Pb) and nickel (Ni) —were 400, 1,000, 250, 5, 130 and 45 mg kg^{-1}, respectively, under the Fertilizer Management Act in South Korea (Anon., 2010a).

The control system for soil intoxication limit levels primarily depends upon heavy-metal concentration. The limit levels in South Korea are Cu 50, Zn 300, Cr 4, Cd 1.5, Pb 100 and Ni 40 mg kg^{-1} under the Soil Environmental Conservation Act (Anon., 2007). In Japan, Cu must be less than 125 mg kg^{-1}, Cr 0.05 mg l^{-1} or less, Cd 0.4 mg kg^{-1} or less and Pb 0.01 mg l^{-1} or less (Ministry of the Environment Government of Japan, 1994).

In many countries, current rules for controlling the use of organic wastes on agricultural land have been criticized because they apparently do not take into consideration of the potential adverse effects of inorganic heavy metals and organic compounds produced in organic waste-treated soils on soil organisms (McGrath, 1994). The regulatory limit to the application of industrial waste on farmland only depends upon the level of PTEs in South Korea. However, PTEs limit may not be an adequate regulation protocol since organic wastes contain lots of inorganic and organic contaminants (Ministry of Agriculture, Fisheries and Food [MAFF], 1991).

An overall assessment of the soil contamination caused by inorganic and organic compounds of organic waste has been, therefore, attempted by ascribing qualitative description of the apparent risk and developing the integrated hazard assessment system (Hembrock-Heger, 1992). Available options for dealing with sludge include application to agricultural land, incineration, land reclamation, landfill, forestry, sea disposal and biogas. Of these, the application to agricultural land is the principal way for deriving beneficial uses of organic sludge by recycling plant nutrients and organic matter to soil for crop production (Coker et al., 1987). Also, agricultural use provides a reliable cost-effective method for sludge disposal. Recycling (81.7%) is the largest means of waste disposal, with 11.1% land deposition, 5.2% incineration and 2.0% sea disposal in South Korea (Anon., 2009). As an alternative way of waste disposal, the Fertilizer Management Act was revised to make it possible to apply industrial and municipal wastes into farmland in December 1996 in South Korea (Anon., 2006).

Country	Parameter (mg kg of dry matter^{-1})							
	As	Hg	Pb	Cd	Cr	Cu	Zn	Ni
South Korea[a]	45	2	130	5	250	400	1000	45
USA[b]	75	57	840	85	3000	4300	7500	420
Canada[c]	13	0.8	150	3	210	400	700	62
EU[d]	-	1-1.5	50-300	1-3	–	50-140	150-300	30-75
Belgium[e]	-	1	120	1.5	70	90	300	20
Denmark[e]	25	0.8	120	0.8	100	1000	4000	30
France[e]	-	10	800	20	1000	1000	3000	200
Netherlands[e]	-	0.3	100	1	50	90	290	20
Sweden[e]	-	2.5	100	2	100	600	800	50
Germany[f]	-	8	900	10	900	800	2500	200
UK[g]	-	1	200	1.5	100	200	400	50
Switzerland[h]	-	1	120	1	100	100	400	30
Australia[i]	20	1	150-300	1	100-400	100-200	200-250	60
New Zealand[j]	20	2	300	3	600	300	600	60

a Anon. (2010a)
b USEPA (2000)
c Canadian Council of Ministers of the Environment [CCME] (2005)
d Anon. **(2010b)**
e Brinton (2000)
f Anon. (2010c)
g British Standards Institution [BSI] (2011)
h Anon. (2010d)
i Anon. (1997)
j New Zealand Water and Waste Association [NZWWA] (2003)

Table 1. Criteria of the inorganic pollutants in compost or sewage sludge for application to the arable land in 14 selected countries

3. Importance of earthworm

3.1 Role in soil

Earthworms have a critical influence on soil structure, forming aggregates and improving the physical conditions for plant growth and nutrient uptake. They also improve soil fertility by accelerating decomposition of plant littre and soil organic matter. Earthworms are the most important invertebrates in this initial stage of the recycling of organic matter in various types of soils. Curry & Byrne (1992) demonstrated that the decomposition rate of straw which was accessible to the earthworms was increased by 26–47% compared with straw from which they were excluded. Organic matter that passes through the earthworm gut and is digested in their casts is broken down into much finer particles, so that a greater surface area of the organic matter is exposed to microbial decomposition. Martin (1991) reported that casts of the tropical earthworms had much less coarse organic matter than the surrounding soil, indicating that the larger particles of organic matter were fragmented during passage through the earthworm gut. Earthworm species, such as *Lumbricus terrestris*, are responsible for a large proportion of the overall fragmentation and incorporation of littre in many woodlands and farmland of the temperate zone, which resulted in the formation of mulls. As a result, the surface littre and organic layers are mixed thoroughly with the mineral soil (Scheu & Wolters, 1991).

The numbers of earthworm burrows have been counted between 50 and 200 burrows m^{-2} on horizontal surfaces (Edwards et al., 1990). Earthworms not only improve soil aeration by their burrowing activity, but they also influence the porosity of soils. Earthworm burrows was found to increase the soil-air volume from 8% to 30% of the total soil volume (Wollny, 1890). In one soil, earthworm burrows comprise a total volume of 5 litres m^{-3} of soil, making a small but significant contribution to soil aeration (Kretzschmar, 1978). Water infiltration was from 4 to 10 times faster in soils with earthworms than in soils without earthworms (Carter et al., 1982). They bring large amounts of soil from deeper layers to the surface and deposit as casts on the surface. The amounts which turned over in this way greatly differ with habitats and geographical regions, ranging from 2 to 268 tons ha^{-1} (Beauge, 1912; Roy, 1957). The importance of this turnover, which was discussed first by Darwin (2009), can be seen by comparing the profile of a stratified mor soil (with few earthworms) with that of a well-mixed mull soil. Blanchart (1992) reported in a formation of aggregates that under natural conditions with or without earthworms, large aggregates (>2 mm) comprised only 12.9% of soil with no earthworms, whereas in soil with worms, large aggregates comprised 60.6% of soil after 30 months in the field. Devliegher & Verstraete (1997) introduced the concepts of nutrient enrichment process and gut associated process. They noted that earthworms are performing these two different functions that may have contrasting their effects on soil microbiology, chemistry and plant growth. Earthworms, such as *L. terrestris*, incorporate and mix surface organic matter with soil and increase biological activity and nutrient availability. However, they also assimilate nutrients from soil and organic matter as these materials pass through their gut.

3.2 Occurrence of earthworm in Korean soil ecosystem

The earthworm fauna of South Korea is dominated by the family Megascolecidae and identified 101 species, with 12 species in Lumbricidae, 9 species in Moniligasteridae and 80 species in Megascolecidae (Fig. 1) (Hong, 2000, 2005; Hong et al., 2001). In general, earthworms are classified into three types based upon life style and burrowing habit (Bouché, 1972). The epigeal forms (e.g., *Lumbricus rubellus* and *Eisenia fetida*) hardly burrow in soil at all, but inhabit decaying organic matters on the surface, including manure or compost heaps. The endogenous species (e.g., *Allolobophora chlorotica* and *Allolobophora caliginosa*) produce shallow branching burrows in the organo-mineral layers of the soil. Lastly, the anectic forms (e.g., *L. terrestris* and *Allolobophora longa*) are deep burrowing species, producing channels to a depth of one meter or more. Megascolecidae species identified in Korean ecosystem come under anectic forms. Occurrence of earthworms in agroecosystem appeared the most individuals of *Amynthas agrestis*, *Amynthas heteropodus* and *Amynthas koreanus* (Hong & Kim, 2007).

(A) (B) (C)

Fig. 1. Representative earthworms in Lumbricidae (A), Moniligasteridae (B) and Megascolecidae (C) in South Korea

3.3 Biomonitor for biological hazard assessment on soil contamination

Concerns about contamination of soil and detrimental effects of contaminants on the living environment have resulted in a strong and growing interest in soil organisms among environmental scientists and legislators. Legislation in many countries has recently focused on the need of sensitive organisms from the soil environment for environmental monitoring. Many toxic materials have been accumulated along with food webs. The decomposer levels are frequently the first to be affected since the organic matter and the soil are the ultimate sink for most contaminants. Ecologically, earthworms are near the bottom of the terrestrial tropic levels. The effects of contaminants on earthworms which were kept in soil in the laboratory have been studied (Edwards & Thompson, 1973). These tests tended to produce consistent and reproducible results because 10 individuals of *E. fetida* were used and these worms were an intimate contact with pesticides. van Hook (1974) demonstrated that earthworms could serve as useful biological indicators of contamination because of the fairly consistent relationships between the concentrations of various contaminants and mortality of earthworm. The basic requirements of finding a species easy to rear and genetically homogeneous could be fulfilled by using representatives of the species, although there have been arguments for the use of *Eisenia andrei* or a genetically controlled single strain of the *E. fetida* complex (Bouché, 1992). Callahan et al. (1994) have suggested that *E. fetida* may be a representative of the species, *Allolobophora tuberculata, Eudrilus eugeniae* and *Perionyx excavatus* based upon the concentration-response relationship for 62 chemicals when applying the Weibull function. Habitational earthworms, including *E. fetida*, are useful as biological indicator species in the ecological sense or a more useful biomonitor species. It has been proposed that *A. heteropodus* could be adopted as a bioindicator in agroecosystem because of dominant species in South Korea (Kim et al., 2009).

4. Effects of organic waste sludge application on earthworm biology

4.1 Composition and biomass of earthworms

Four different types of organic waste sludge used in this study were as follows: municipal sewage sludge (MSS) collected from sewage treatment plants on Gwacheon (Gyeonggi Province, South Korea); industrial sewage sludge (ISS) collected from industrial complex on Ansan (Gyeonggi Province); alcohol fermentation processing sludge (AFPS) collected from Ansan industrial complex; and leather processing sludge (LPS) collected from sewage treatment plant on Cheongju (Chungbuk Province, South Korea). Pig manure compost

(PMC) was purchased from Anjung Nong-hyup, Anjung (Gyeonggi Province). These materials were collected in early March 1994 and kept in deep freezers (–60°C) to be applied annually from 1994 to 2001.

Lysimeters which composed of 45 concrete plots (1.0 m length, 1.0 m width and 1.1 m depth) (Fig. 2) were made in the upland field of Suwon (Gyeonggi Province) in March 1993. Each plot was uniformly filled with the same sandy loam soil without earthworms up to the ground surface in mid-May 1993. Three levels (12.5, 25 and 50 tons of dry matter ha^{-1} $year^{-1}$) of test materials were applied to each plot twice annually for 8 consecutive years (mid-March 1994 to mid-March 2001) and mixed into the soil of a depth of 15 cm. PMC served as a standard for comparison in lysimeter tests. A randomized complete block design with three replicates was used. Two radish, *Raphanus sativus*, cultivars (jinmialtari and backkyoung) were cultivated in every spring and autumn, respectively. Planting densities were 12 × 15 cm in spring and 25 × 30 cm in autumn with one plant. Other practices followed standard *Raphanus* culture methods without application of any mineral fertilizer and pesticide. The lysimeters were covered with a nylon net to prevent any access by birds or animals.

Fig. 2. Field lysimeters

Earthworms were collected from each of the 45 lysimeter plots from an area of 1 m^2 up to 0.3 m depth by hand sorting in mid-October 1997 and mid-October 2001 as described previously (Callaham & Hendrix, 1997). They were immediately transported to the laboratory in plastic containers and separated into juveniles and adults with a clitellum. The earthworm numbers, composition and biomass were investigated before they were fixed in a 10% formalin solution. Earthworm species identification followed Hong & James (2001), Kobayashi (1941) and Song & Paik (1969).

Pollution index (PI) was determined according to the method of Jung et al. (2005), PI = [∑(heavy metal concentration in soil tolerable level–1) number of heavy $metal^{-1}$]. Tolerable level of Cu, Zn, Cr, Cd, Pb and Ni were 125, 700, 10, 4, 300 and 100 mg kg^{-1} in Korean soil, respectively (Anon., 2007). PI values are employed to assess metal pollution in soil and indicate the average on ratios of metal concentration over tolerable level. A soil sample is judged as contaminated by heavy metal when PI value is greater than 1. Total toxic unit of PTEs was calculated by threshold level described under the Soil Environmental Conservation Act (Anon., 2007) in South Korea as follows: ∑ (Cu 50 + Zn 300 + Cr 4 + Cd 115 + Pb 100 + Ni 40). Bonferroni multiple-comparison method was used to test for significant differences among treatments in the fresh biomass of earthworms and pollution indices (SAS Institute, 2004). Correlations between accumulated pollutant contents and observed earthworm numbers and biomass were estimated from the Pearson correlation coefficients using SAS. pH values, heavy-metal contents and pollution indices of 8 consecutive yearly applications of three levels of four different organic waste materials and PMC in field lysimeters were reported previously (Na et al., 2011).

Effects on earthworm composition of 8 consecutive yearly applications of four organic waste materials and PMC were investigated using field lysimeters (Table 2). Earthworm composition in all treatments varied according to waste material examined, treatment level and application duration. Of 390 adults collected from 45 plots, earthworms were classified into 2 families (Megascolecidae and Moniligastridae), 2 genera (*Amynthas* and *Drawida*) and 5 species (*Amynthas agrestis*, *Amynthas hupeiensis*, *Amynthas sangyeoli*, *Drawida koreana* and *Drawida japonica*). The number of earthworm species in MSS-, ISS-, LPS-, AFPS- and PMC-treated soils was 2, 2, 2, 3 and 5, respectively. The dominant species were *A. agrestis*, *A. hupeiensis*, *A. sangyeoli* and *D. japonica* in the sludge treatments 4 years after treatment but was replaced with *A. hupeiensis* in all the plots 8 years after treatment. This finding indicates that *A. hupeiensis* was more tolerant to toxic heavy metals than other earthworm species. In ISS- and LPS-treated soils, the proportion of juveniles appeared was 67–100% 4 years after treatment, but no juveniles was observed 8 years after treatment.

At 4 years after treatment, effect of test waste material (F = 16.91; df = 4,44; P < 0.0001) and treatment level (F = 4.09; df = 2,44; P = 0.0268) on the number of earthworms was significant (Table 2). The material by level interaction was also significant (F = 2.63; df = 8,44; P = 0.0258). At 8 years after treatment, effect of test waste material (F = 17.33; df = 4,15; P < 0.001) and treatment level (F = 11.00; df = 3,29; P < 0.001) on the number of earthworms was significant. The material by level interaction was also significant (F = 20.53; df = 8,44; P < 0.001). The number of earthworms was significantly reduced in 25 and 50 ton MSS treatments, 25 and 50 ton AFPS treatments and 12.5 and 25 ton PMC treatments 4 years after treatments than 8 years of treatments. The total number of earthworms collected 4 and 8 years after treatment was as follows: MSS-treated soil, 66/29; ISS-treated soil, 4/2; LPS-treated soil, 15/1; AFPS-treated soil, 30/11; and PMC-treated soil, 127/439.

Earthworm biomass collected from 45 plots during the 8-year-investigation period is given in Fig. 3. The biomass in all treatments was dependent upon waste material examined, treatment level and application duration. At 4 years after treatment, effect of test waste material (F = 49.45; df = 4,44; P < 0.0001) and treatment level (F = 5.80; df = 2,44; P = 0.0074) on the earthworm biomass was significant. The material by level interaction was also significant (F = 3.88; df = 8,44; P = 0.0031). At 8 years after treatment, effect of test waste material (F = 165.13; df = 4,44; P < 0.0001) and treatment level (F = 14.39; df = 2,44; P < 0.0001) on the earthworm biomass was significant. The material by level interaction was also significant (F = 19.77; df = 8,44; P < 0.0001). Significant increase in biomass of soil treated with 50 ton PMC ha^{-1} year^{-1} was observed 8 years after treatment.

Material[a]	Rate[b]	Species	Individuals of species		Total number[d]		P-value[e]
			4 YAT[c]	8 YAT	4 YAT	8 YAT	
MSS	12.5	*A. sangyeoli*	3	1	10	16	0.0132
		A. hupeiensis	3	8			
		Juvenile	4	7			
	25	*A. sangyeoli*	4	0	22	8	0.0006
		A. hupeiensis	5	5			
		Juvenile	13	3			
	50	*A. sangyeoli*	4	0	34	5	0.0038
		A. hupeiensis	5	4			
		Juvenile	25	1			
ISS	12.5	*A. agrestis*	1	0	3	2	0.7247
		A. hupeiensis	0	2			
		Juvenile	2	0			
	25	Juvenile	1	0	1	0	0.3739
	50		0	0	0	0	
LPS	12.5	*D. japonica*	1	0	8	0	0.0907
		Juvenile	7	0			
	25	*A. hupeiensis*	0	1	5	1	0.2302
		Juvenile	5	0			
	50	Juvenile	2	0	2	0	0.1161
AFPS	12.5	*A. sangyeoli*	3	0	10	9	0.9019
		A. hupeiensis	3	4			
		D. japonica	0	2			
		Juvenile	4	3			
	25	*A. sangyeoli*	4	0	9	0	0.0065
		A. hupeiensis	1	0			
		Juvenile	4	0			
	50	*A. sangyeoli*	5	0	11	2	0.0031
		A. hupeiensis	2	1			
		Juvenile	4	1			
PMC	12.5	*A. agrestis*	2	1	24	63	0.0069
		A. hupeiensis	6	40			
		D. japonica	4	2			
		Juvenile	12	20			

Table 2 (Continued)

Material[a]	Rate[b]	Species	Individuals of species		Total number[d]		P-value[e]
			4 YAT[c]	8 YAT	4 YAT	8 YAT	
PMC	25	*A. agrestis*	0	1	34	117	0.0054
		A. sangyeoli	7	0			
		A. hupeiensis	14	84			
		D. japonica	3	2			
		D. koreana	0	2			
		Juvenile	10	28			
	50	*A. sangyeoli*	0	2	69	259	0.2066
		A. hupeiensis	24	70			
		D. japonica	10	19			
		D. koreana	7	18			
		Juvenile	28	150			

[a] Abbreviations are same as in the text
[b] Tons of dry matter ha^{-1} $year^{-1}$
[c] Years after treatment plots
[d] The combined number of earthworms in the three replicate plots
[e] *t*-test

Table 2. Earthworm numbers and composition of 4 and 8 consecutive yearly applications (twice annually) of three levels of four different organic waste materials and pig manure compost using field lysimeters

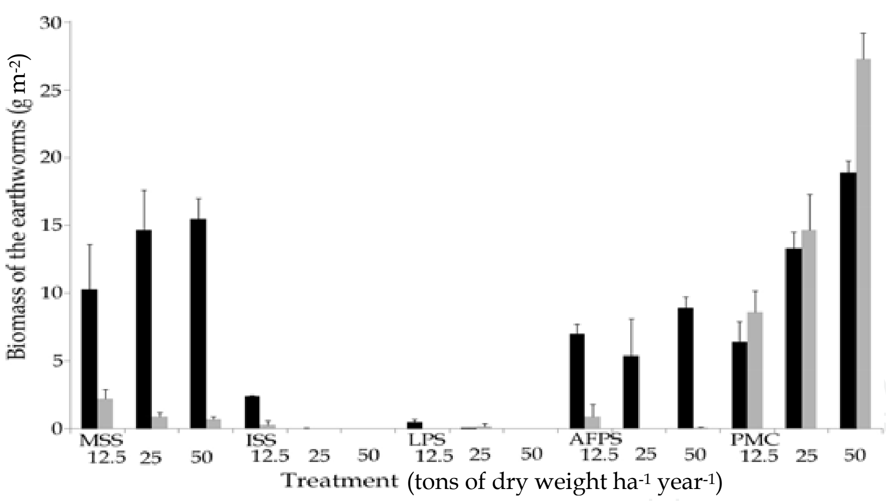

Fig. 3. Earthworm biomass of 4 (■) and 8 (▪) consecutive yearly applications (twice annually) of three levels of four different organic waste materials and pig manure compost using field lysimeters.

To evaluate potential toxic effects of residual heavy metals, total toxic units of PTEs were determined (Fig. 4). The total toxic units in all treatments varied with waste material examined, treatment level and application duration. At 4 years after treatment, effect of test waste material (F = 34872.4; df = 4,44; P < 0.0001) and treatment level (F = 60.24; df = 2,44; P < 0.0001) on the the total toxic units of PTEs was significant. The material by level interaction was also significant (F = 2601.2; df = 8,44; P < 0.0001). At 8 years after treatment, effect of test waste material (F = 52439.5; df = 4,44; P < 0.0001) and treatment level (F = 28451.0; df = 2,44; P < 0.0001) on the the total toxic unit of PTEs was significant. The material by level interaction was also significant (F = 13057.2; df = 8,44; P < 0.0001).

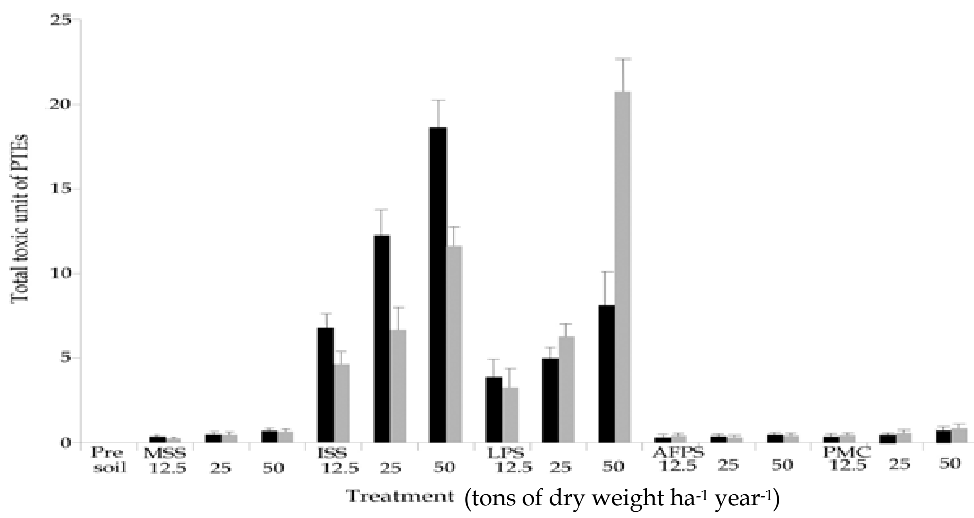

Fig. 4. Total toxic units of potentially toxic elements (PTEs) of 4 (■) and 8 (■) consecutive yearly applications (twice annually) of three levels of four different organic waste materials and pig manure compost using field lysimeters. Abbreviations are same as in the text

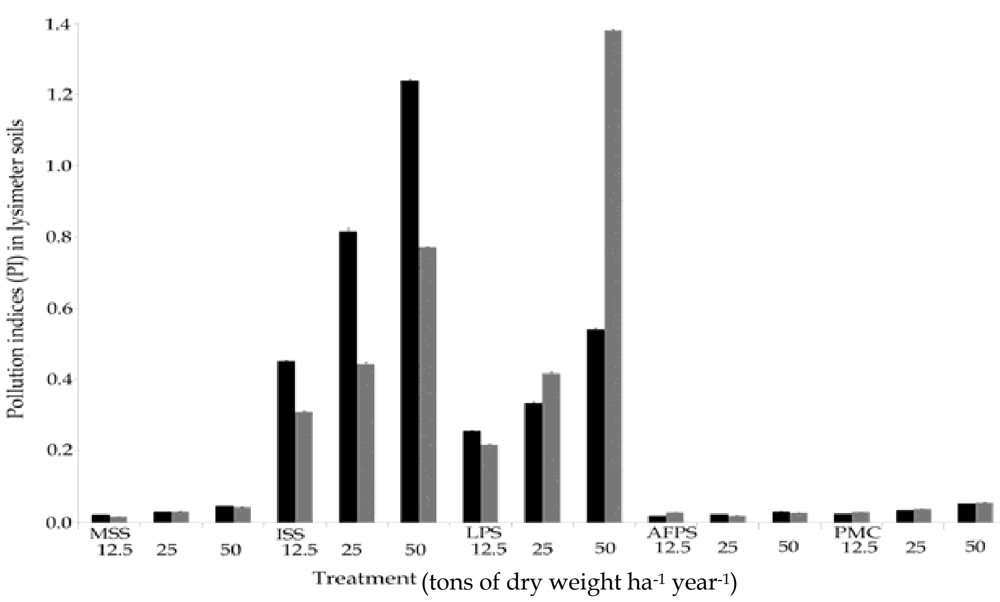

Fig. 5. Pollution indices of 4 (■) and 8 (■) consecutive yearly applications (twice annually) of three levels of four different organic waste materials and pig manure compost using field lysimeters. Abbreviations are same as in the text

PI values of lysimeter soils sampled during the 8-year-investigation period are reported in Fig. 5. At 4 years after treatment, effect of test waste material (F = 34047.6; df = 4,44; P < 0.0001) and treatment level (F = 5957.3; df = 2,44; P < 0.0001) on the the total toxic unit of PTEs was significant. The material by level interaction was also significant (F = 2505.3; df = 8,44; P < 0.0001). At 8 years after treatment, effect of test waste material (F = 48793.6; df =

4,44; P < 0.0001) and treatment level (F = 26515.1; df = 2,44; P < 0.0001) on the the total toxic unit of PTEs was significant. The material by level interaction was also significant (F = 12190.9; df = 8,44; P < 0.0001). There was significant difference in PI values between the treatment duration. Particularly, PI value of ISS-treated soil was higher 8 years after treatment than 4 years after treatment, while PI value of LPS-treated soil was higher 4 years after treatment than 8 years after treatment.

Correlation between total toxic unit of PTEs and PI and earthworm individuals and biomass was determined (Table 3). At 4 years after treatment, earthworm individuals were correlated negatively with the total toxic unit of PTEs (r = –0.509) and PI (r = –0.508). At 8 years after treatment, earthworm individuals were correlated negatively with the total toxic unit of PTEs (r = –0.265), but were not correlated negatively with PI.

At 4 years after treatment, earthworm biomass was correlated negatively with the total toxic unit of PTEs (r = –0.673) and PI (r = –0.672) (Table 3). At 8 years after treatment, earthworm biomass was correlated negatively with the total toxic unit of PTEs (r = –0.308), but were not correlated negatively with PI.

Parameter	Correlation coefficient (r)			
	Earthworm individuals		Earthworm biomass	
	4 YAT[a]	8 YAT	4 YAT	8 YAT
Total toxic unit of PTEs	-0.509	-0.265	-0.673*	-0.308
PI	-0.508*[b]	-0.265	-0.672*	-0.280

[a] Years after treatmen

[b] *0.001<P<0.05 treatment

Table 3. Correlation between total toxic unit of potentially toxic elements (PTEs) and pollution indicies (PI) and earthworm individuals and biomass 4 and 8 years after treatment

The impact of heavy metals and sludge on lumbricid earthworms, particularly *E. fetida* and *L. terrestris*, has been well noted. Heavy metals cause mortality and reduce fertility, cocoon production and viability, growth, composition and biomass, and bioaccumulation and bioavailability of earthworms. The toxic values of heavy metals to earthworms vary according to an earthworm acute toxicity test. Based upon an artificial soil test, Spurgeon et al. (1994) determined no observed-effect concentrations (NOECs) for *E. fetida* exposed to heavy metals. The estimated NOEC values were 39.2 mg Cd kg^{-1}, 32 mg Cu kg^{-1}, 1,810 mg Pb kg^{-1} and 199 mg Zn kg^{-1}. In soil contaminated by effluent containing Cr, the rate of 10 mg kg^{-1} was fatal to *Peretima posthuma* and other species (Abbasi & Soni, 1983). Copper caused higher mortality than Pb or Zn against *E. fetida* at the same rate and the LC_{50} and NOEC values for Cd could not be determined since no significant mortality was observed at the highest test rate (300 µg g^{-1}) (Spurgeon et al., 1994).

Although heavy metals did not show direct lethal effects to earthworms, they can sensitively cause their reproduction and sperm count reduction and low hatching success of cocoons. *Lumbricus terrestris* worms exposed in artificial soil to sublethal concentrations of technical chlordane (6.25, 12.5 and 25 ppm) and cadmium nitrate (100, 200 and 300 ppm) exhibited significant reduction in spermatozoa from testes and seminal vesicles (Cikutovic et al., 1993). *Eisenia fetida* worms grew well in the lead-contaminated environment and produced cocoons at the same rate as the control worms, but the hatchability of these cocoons was much lower, indicating that lead toxicity affects reproductive performance by major spermatozoa damage (Reinecke & Reinecke, 1996). In addition, Zn, Mn and Cu produced slower growth, later maturation and fewer or no cocoons. Reinecke and Reinecke (1997) have shown the structural damage of spermatozoa, including breakage and loss of nuclear and flagellar membranes, thickening of membranes, malformed acrosomes and loss of nuclear material, and the results are associated with heavy metals, such as Pb and Mn. The toxicity order of metals on reproduction in earthworms is Cd, Cu, Zn and Pb. Similar results have been found in *E. fetida* exposed to a geometric series of concentrations of Cd, Cu, Pb and Zn in artificial soil and the effects of Cd and Cu on the reproductive rate were particularly acute (Spurgeon et al., 1994).

It has been well known that earthworms are able to inhabit soils contaminated with heavy metals (Becquer et al., 2005; Li et al., 2010; Maity et al., 2008) and can accumulate undesirably high concentration of heavy metals (Cu, Zn, Pb and Cd) that may give adverse effects on livestock (Hobbelen et al., 2006; Oste et al., 2001). Earthworms (*L. rubellus* and *Dendrodrilus rubidus*) sampled from one uncontaminated and 15 metal-contaminated sites showed significant positive correlations between earthworm and total (conc. nitric acid-extractable) soil Cd, Cu, Pb and Zn concentrations (Morgan & Morgan, 1988). The important factor in the accumulation of heavy metals in earthworms is bioavailability by uptake (Dai et al., 2004; Spurgeon & Hopkin, 1996) because there are significant correlations between the concentrations of heavy metal accumulated in earthworms and bioavailable metal concentrations of field soils (Hobbelen et al., 2006). Earthworm metal bioaccumulation and bioavailability have been well reviewed by Nahmani et al. (2007). There were positive relationships between earthworm tissue and soil metal concentrations and also earthworm tissue and soil solution metal concentrations with slightly more significant relationships between earthworm tissue and soil metal concentrations 42 days after treatment. Recently, Li et al. (2010) reported the positive logarithmic relationship between the bioaccumulation factors of *E. fetida* to heavy metals and the exchangeable metal concentration of pig manure. The differences in these accumulation and availability among earthworms may, in part, play a role in affecting their population density and genetic adaptation living in metal-contaminated soils.

However, Lee (1985) suggested that the differences in the relative toxicity of compounds may explain some of the conflicting data in the literature on the concentrations which have deleterious effects on earthworms. For instance, very high concentrations of lead that influence growth and reproduction of earthworms may be attributable more to the very low solubility of lead compounds that are found in soils and the ability of earthworms to sequester absorbed lead than to any lower toxicity of lead compared with other heavy metals. It has been suggested that *E. fetida* may regulate the concentration of zinc in their body tissue through allowing rapid elimination by binding zinc using metallothioneins in their chloragogenous tissue (Cotter-Howells et al., 2005; Morgan & Morris, 1982; Morgan & Winters, 1982; Prento, 1979). High tolerance of earthworms to cadmium poisoning may also result from detoxification by metallothionein proteins in the posterior alimentary canal (Morgan et al., 1989). In addition, heavy metals have high affinity for glutathione, metallothioneines and enzymes of intermediary metabolism and heme synthesis (Montgomery et al., 1980). The metals Zn, Pb, Bi and Cd which are not consistently prevailing toxicants were most accessible to earthworms and Cu, Zn and Cr were also accumulated in earthworm tissue and the contaminated soils imparied earthworm reproduction and reduced adult growth, while elevated superoxide dismutase activity suggested that earthworms experienced oxidative stress (Berthelot et al., 2008).

Lead, copper and zinc may inhibit *d*-aminolevulinic acid dehydratase (*d*-ALAD) which is a key enzyme in heme synthesis by lowering haemoglobin concentration in earthworm blood. Replacement of zinc, a protector of the active site of *d*-ALAD, by lead may result in its inhibition.

Soil pH has been comprehensively identified as the single most important soil factor controlling the availability of heavy metals in sludge-treated soils (Alloway & Jackson, 1991). Soil pH is also one of the most important factors that limit the species, numbers and distribution of earthworms (Dunger, 1989; Edwards & Bohlen, 1996; Satchell & Stone, 1972) because it may affect the survival of adults and thus production and avoidance behaviour of juveniles (Aorim et al., 1999, 2005). van Gestel et al. (2011) reported that soil pH and organic matter content determine molybdenum toxicity to enchytraeid worm, *Enchytraeus crypticus* A higher pH resulted in a decreased sorption of the molybdate anion, and it caused increased bioavailability and toxicity.

A lot of studies concerning the effects of heavy metals on earthworms in terms of mortality, loss of weight, fertility, cocoon production, cocoon viability and growth were carried out during short-term experiments (14 or 21 days) in artificial soils contaminated with metal solution containing a single metallic element. Recently, Na et al. (2011) studied the effects of long-term (8 years) application of four organic waste materials on earthworm numbers and biomass. They reported that earthworm individuals were correlated positively with pH (r = 0.37) and negatively with heavy metals (r = –0.36 to –0.55) with the exception of Zn 4 years after treatment, while earthworm individuals were correlated positively with pH (r = 0.46) and negatively with Pb (r = –0.41) but positively with Zn (r = 0.59) 8 years after treatment. Earthworm biomass was correlated negatively with heavy metals (r = –0.43 to –0.72) with the exception of Zn 4 years after treatment, while earthworm biomass was correlated positively with pH (r = 0.57) and negatively with Pb (r = –0.50) and Ni (r = –0.30) but positively with Zn (r = 0.68) 8 years after treatment.

4.2 Effects of hexane extractable material on composition and biomass of earthworm

United States Environmental Protection Agency [USEPA] 9071B method (1998) was used to extract relatively non-volatile hydrocarbons from 45 lysimeter soils treated twice annually with three levels of four different organic waste materials and pig manure compost tested for 8 consecutive years, as stated in section 4.1. The extracts were generally designated hexane extractable material (HEM) because the solvent used was hexane. Soils were acidified with 0.3 ml of concentrated HCl and dried over magnesium sulfate monohydrate. After drying in a fume hood, HEM was extracted for 4 hr using a Soxhlet apparatus which was attached a 125 ml boiling flask containing 90 ml of hexane. Solvent was then concentrated under vacuum for less than 30 min at 35°C. The extracts were cooled in a desiccator for 30 min, and HEM concentrations were calculated by the formula, HEM (mg kg of dry weight^{-1}) = (A × 1000)/BC, where A is gain in weight of flask (mg), B is weight of wet solid (g) and C is dry weight fraction (g of dry sample g of sample^{-1}).

HEM amounts varied with treatment level and organic waste examined (Fig. 6). At 8 years after treatment, effect of test waste material (F = 49.45; df = 4,14; P < 0.001) and treatment level (F = 4.09; df = 2,30; P = 0.028) on the HEM was significant. The material by level interaction was also significant (F = 2.63; df = 8,44; P = 0.0258). Particularly, the amount of HEM in PMC-treated soil was the lowest of any of test materils at all treatment levels.

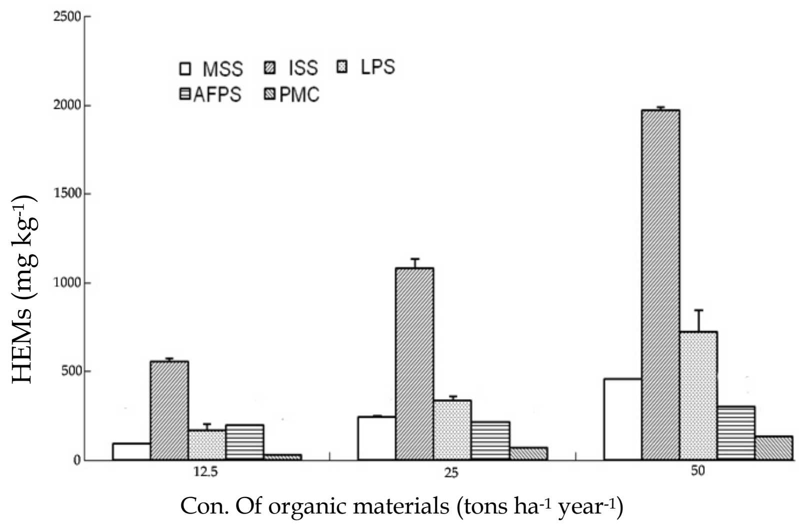

Fig. 6. Hexane extractable material (HEM) contents of 8 consecutive yearly applications (twice annually) of three levels of four different organic waste materials and pig manure compost using field lysimeters. Abbreviations are same as in the text

Correlation between HEM content (Fig. 6) and earthworm individuals and biomass (Table 2) was determined. At 8 years after treatment, earthworm individuals were negatively correlated with HEM (r = -0.313) and earthworm biomass (r = -0.335).

In general, organic compounds existed in sewage sludge have been potentially transferred to sludge-amended agricultural soils, and most organic compounds have been solved in hexane solvent. HEMs from sewage sludges contain a variety of contaminants, such as hydrocarbons, grease, plant or animal oils, wax, soap, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Hua et al., 2008; Stevens et al., 2003). Drescher-Kaden et al. (1992) reported that 332 organic contaminants (e.g., pyrene, benzo(*a*)pyrene, benzene and toluene) with potential to exert soil contamination were identified in German sewage sludges. Hembrock-Heger (1992) found that the concentrations of PAHs and PCBs appeared to be highest in soils treated with sewage sludge for 10 years. According to the United Kingdom Water Research Centre Report No. DoE 3625/1 on the occurrence, fate and behaviour of some of organic pollutants in sewage sludge (Sweetman et al., 1994), there was no evidence of any significant problems arising from organic contaminants in sludges applied to agricultural land.

Of some waste sludge and PMC applied into red pepper fields in South Korea from 2003 to 2004, the highest contents of HEM and PAHs were observed in cosmetic and pharmaceutical industy sludge, respectively, and the cosmetic industry sludge affected remarkedly growth of red pepper, which resulted in 25-60% of yield reduction (Lee, 2006). These results indicate that PMC may contain a lot of polar compounds with functional groups, such as COO^-, O^-, NR_2H, COOH or OH, to be more easily metabolized by various soil-born organisms, including earthworm. Water drained from processing of ISS, LPS, MSS and AFPS may contain more non-soluble compounds than that of PMC. Considering the hexane fraction obtained from PMC containing plentiful P or N atom (Na, 2004), it may be biodegradable by long-term exposure to a variety of soil organisms owing to biological uses. In general, most hydrophobic compounds are accumulative and difficult to biodegrade them introducing into environments because most aliphatic hydrocarbons retain unfavorable large ΔG (minus value) with increase in chain length.

4.3 Toxicity of soil contamination level to *E. fetida* in microcosms

Each microcosm was made of commercially available high-density stable polyethylene container (14 cm length, 14 m width and 7 m depth) with 36 pores (1 mm diameter) of lid. Soils sampled in microcosms treated with three levels (12.5, 25 and 50 tons of dry matter ha^{-1} year^{-1}) of MSS, ISS, LPS, AFPS and PMC for 4 consecutive years (twice annually) were sieved gently through a 2 mm mesh sieve. In a preliminary experiment, ~40% of water holding capacity was optimal for microcosm test. Amount of 300-g fresh soil was hydrated to ~40% of water holding capacity. Hydrated water required to achieve the desired hydration was calculated according to the method of Greene et al. (1988). Ten earthworms were placed into each microcosm. The microcosms were kept in the controlled chamber at 20°C and 60±5% relative humidity under a 16:8 h light:dark cycle. Mortalities were assessed by emptying the test soil onto a tray and sorting the worms from the soil. Earthworms were considered to be dead if their bodies and anterior did not move or respond when they prodded with fine wooden dowels. Live worms were placed back into their original microcosms. The numbers of live and dead worms in each microcosm were recorded every 2 weeks and the dead worms were discarded. A randomized complete block design with three replicates was used. Mortality percentages were transformed to arcsine square root values for analysis of variance. The Bonferroni multiple-comparison method was used to test for significant differences among the treatments (SAS Institute, 2004).

Toxic effects of MSS, ISS, LPS, AFPS and PMC treatments on *E. fetida* in microcosm tests were evaluated (Table 4). All treatments did not affect any adverse effects on the organisms 2 weeks after treatment. At 4 weeks after treatment, effect of test waste material (F = 3.73; df = 4,44; P = 0.0141) on the mortality was significant but that of treatment level (F = 1.83; df = 2,44; P = 0.1785) was not significant. The material by level interaction was also significant (F = 2.34; df = 8,44; P = 0.0436). At 8 weeks after treatment, effect of test waste material (F = 200.90; df = 4,44; P < 0.0001) and treatment level (F = 5.37; df = 2,44; P = 0.0101) on the the mortality was significant. The material by level interaction was also significant (F = 9.49; df = 8,44; P < 0.0001). After 16 weaks after treatment, effect of test waste material (F = 124.11; df = 4,44; P < 0.0001) and treatment level (F = 9.73; df = 2,44; P = 0.0006) on the mortality was significant. The material by level interaction was also significant (F = 63.42; df = 8,44; P < 0.0001).

Heimbach et al. (1992) demonstrated that there is a good correlation (r = 0.86) between LC_{50} values of pesticides from an artificial soil test and the number of earthworms collected from a standardized field test. Our present and previous studies indicate that microcosm soil test using earthworms can predict results from a field test for assessing side effects occurred by long-term exposure of soil contaminants. Burrows & Edwards (2002) have been tried to use integrated soil microcosm based upon earthworms to predict effects of pollutants on soil ecosystems.

5. Future perspectives

Due to the predicted impacts of climate change, many farmers are increasingly concerned about severe soil compaction and water stagnation on their fields. To prevent the deterioration of arable soils, appropriate soil management stratigies have to be developed. Earthworms are an important component of the soil biodiversity and their positive effects on soil structure are well-known. A variety of functional groups of earthworms can be restored through decrease in a soil disturbance and occurence of crop residues in the upper soil. Investigating earthworm biomass and population is a very complex process and therefore consists of various methods of sampling. However, it is difficult to conduct efficient investigations due to horizontally aggregated earthworm populations and their complex phenologies. Of the vaiorus methods, hand sorting which involves sorting through soil samples by hand is one of the most earliest popular sampling methods. The soil washing method is more effective in sorting out cocoons and smaller earthworms. This method consists of a combination of washing and sieving soil samples, with a possible flotation stage. Another method that is used for soil sampling is the electrical method which consists of inserting an electrode into the groud causing earthworms to surface due to the electrical pulse in the soil. These methods, however, usually result in disrupting earthworm biomass and population, killing and injuring them and affecting their habitats. Considering the relationships between heavy metals and earthworms inhabiting in contaminated soils, it needs to adjust study focus on the long-term effects of multiple elements, not one heavy metal, to earthworms. However, these methods make it difficult to consistantly study and investigate a selective biomass during long periods.

With future developements in terms of remote sensing used for detecting the small- or large-scale acquisition of information of an object or phenomenon, these issues will no longer serve as a problem because biomass can be studied without any need of disruption. Although underground remote sensing technologies are in use, they have not yet been applied to the investigation of living organisms, such as earthworms. For that reason, we believe that scientists and remote sensing developers should put their heads together to optimize remote sensing equipment to the investigation of underground living organisms. These advancements will significantly help researchers to consistantly study a select biomass and calculate the amount of toxic materials that are being inserted into the soil more accurately.

Treatment[a]	Rate[b]	% mortality (mean ± SE) at weeks after treatment		
		4	8	16
MSS	12.5	0	6 ± 3.3	53 ± 6.0
	25	3 ± 3.3	7 ± 6.7	93 ± 6.0
	50	10 ± 10.0	13 ± 3.3	70 ± 5.2
ISS	12.5	37 ± 18.6	60 ± 5.8	87 ± 6.0
	25	7 ± 6.7	97 ± 3.3	100
	50	0	97 ± 3.3	100
LPS	12.5	0	30 ± 5.8	97 ± 3.0
	25	0	3 ± 3.3	97 ± 3.0
	50	0	27 ± 8.8	100
AFPS	12.5	0	0	33 ± 6.0
	25	0	17 ± 3.3	20 ± 5.2
	50	0	0	90 ± 9.0
PMC	12.5	0	0	17 ± 7.9
	25	0	0	20 ± 0.0
	50	3 ± 3.3	3 ± 3.3	3 ± 3.0

[a,b] Tons of dry matter ha^{-1} $year^{-1}$

Table 4. Accumulative mortality of *Eisenia fetida* earthworms in microcosm soils treated twice annually with three levels of four different organic waste materials and pig manure compost tested for 4 consecutive years

6. Conclusion

The long-term applications of organic waste materials containing heavy metals and HEMs affected the establishment of Megascolecid and Moniligastrid earthworms in field. The biomass of earthworms in lysimeter and microcosm soil tests would provide valuable tools for establishing the integrated hazard assessment system for organic wastes. Future research is needed to establish additional soil physico-chemical characteristics, particularly those that might influence heavy-metal bioaccumulation and bioavailability and physical habitat such as compaction and soil water holding capacity across treatment through time course.

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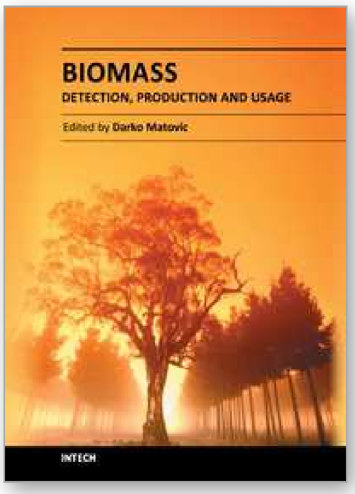

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Biomass has been an intimate companion of humans from the dawn of civilization to the present. Its use as food, energy source, body cover and as construction material established the key areas of biomass usage that extend to this day. Given the complexities of biomass as a source of multiple end products, this volume sheds new light to the whole spectrum of biomass related topics by highlighting the new and reviewing the existing methods of its detection, production and usage. We hope that the readers will find valuable information and exciting new material in its chapters.

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