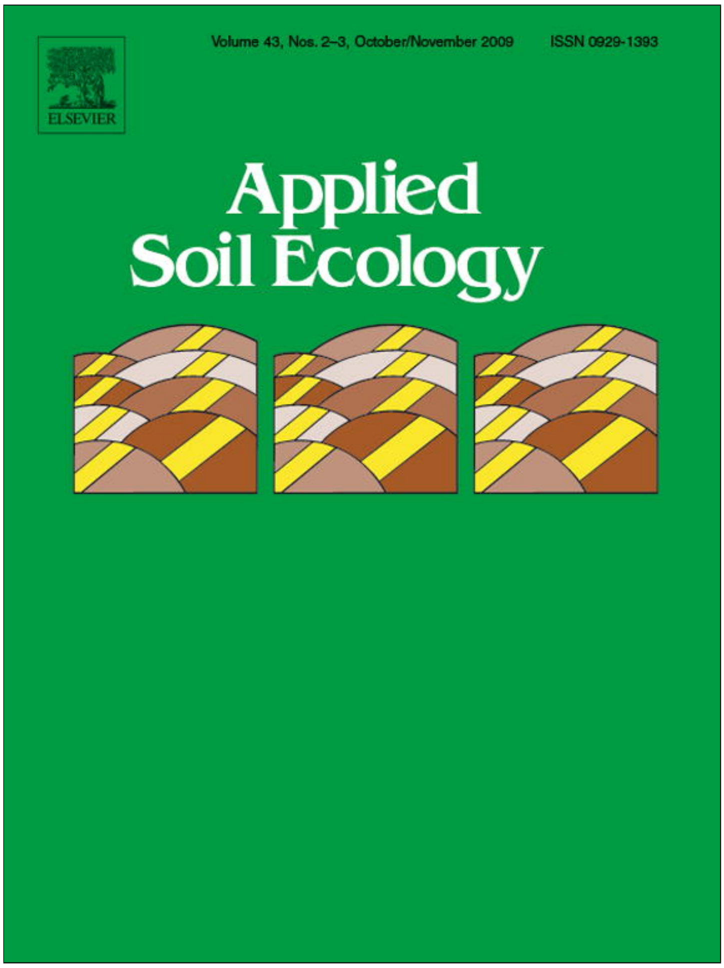
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Colonization of PAH-contaminated dredged sediment by earthworms

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ABSTRACT

In freshly deposited dredged sediment contaminated with PAHs, we followed the colonization of earthworm species by monthly monitoring over two years. Already five months after deposition the first species, *Lumbricus castaneus*, appeared, although only temporarily. The first permanent colonizing species was *L. rubellus*, soon followed by *Aporrectodea caliginosa* and *L. castaneus*, and a few months later *Eiseniella tetraeda*. At the end of the two-year observation period some first few specimen of *Allolobophora rosea* were present. These earthworm species colonized the deposited sediment apparently in succession. The colonization of each individual species did not show a gradual influx from the bordering dikes at both sides of the deposit, but a fast colonization over the whole width, presumably by surface dispersal, although at low and variable numbers, followed by a gradual increase of population numbers. Modeling the dispersal showed that diffusion was the primary driving factor. Also juvenile earthworms were observed locally in high numbers, so reproduction did occur. Total earthworm numbers in the deposit reached a maximum of 80% of the numbers in the bordering dikes consisting of loamy and clayey soils. Numbers were highest in periods with warm and rainy weather. The appearance of earthworms improved the soil development, stimulated a faster desiccation and aeration of the sediment and may have contributed to the increased degradation of PAHs, especially three- and four-ring PAHs.

1. Introduction

Jones et al. (1994) have defined the term 'ecosystem engineers' as: "organisms that directly or indirectly modulate the availability of resources other than themselves to other species, by causing physical state changes in biotic materials. In doing so, they modify, maintain and/or create habitats". This was exemplified by the way earthworms create a better growth substrate for certain plant species and micro-organisms. Lavelle et al. (1997) elaborated on this in a broader context for earthworms in general. Eijsackers (1996) did this for their possible impact on soil pollution, and suggested that earthworms could play an important role in the improvement of soil substrates with a reduced quality due to chemical compounds. Earthworms can mobilize heavy metals (Ma et al., 2002), but it was not possible to confirm this mobilization under semi-field and field conditions (Zorn et al., 2005a, b).

Eijsackers et al. (2001) showed that earthworms improve degradation of PAHs, by improving physical conditions for aerobic breakdown, by stimulating microbial processes through mixing micro-organisms with contaminated soil or by increasing the availability by decreasing the size of soil aggregates (Harmsen, 2004).

Substrates that benefit from such positive effects of ecosystem engineers are contaminated soils, like large scale industrialized areas (brownfields) or deposits of dredged contaminated sediments. These substrates normally are devoid of earthworms or have only very low populations due to toxic actions of the contaminants present. They first have to be colonized by earthworms, before these could start their engineering work. So the question is first: is colonization hampered by soil contaminants, and second: do established earthworms improve breakdown of soil contaminants either directly or indirectly.

Colonization studies have been carried out in virgin agricultural soils (Stockdill, 1982; Springett, 1972; Hoogerkamp et al., 1983), fly ash deposits (Satchell and Stone, 1977; Eijsackers et al., 1983), and sediment deposits (Vandecasteele et al., 2004). But these were all looking in retrospect at the outcome of colonization for longer periods of several decades. The studies by Satchell and Stone (1977) and Vandecasteele et al. (2004), moreover, concentrated on the relation with heavy metal loads of the sediments.

We were interested in the early colonization of deposited sediments, starting immediately after deposition and in the relation between colonization and soil conditions and related conditions for organic contaminants. In a previous study (Eijsack-

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ers et al., 2001) it was shown experimentally that earthworms could improve sediment quality, especially the aeration of deposited sediment, so that aerobic degradation of PAHs was stimulated. Therefore we wanted to investigate the colonization of sediment by earthworms: (1) under specified conditions in the field, (2) directly from the moment the sediment was deposited, (3) with an intensive sampling scheme covering the first two years of colonization and (4) in combination with observations on the physical sediment conditions and the fate of the contaminants present. To this end we set up an experimental sediment deposit in which we studied:

- the colonization by earthworms from the bordering dikes (quantitatively), both in the field and by modeling;
- the colonization by other surface-active soil faunal species (quantitatively);
- the colonization or establishment of plants on the deposit as this provides cover and organic material for soil animals (semi-quantitatively);
- the development of the sediment with respect to drainage, desiccation, surface crackling, and penetration of oxygen (aeration) to deeper sediment layers (qualitative field observations);
- the fate of the contaminants, more specific PAHs (quantitatively);
- a possible relation with environmental conditions (soil temperature and soil moisture content).

2. Material and methods

2.1. Experimental field deposit and earthworm sampling

The study was carried out at the Oostwaardhoeve VOF, Ecotechnic Research Centre, at Wieringerwerf in the northwestern part of The Netherlands (52°50′N and 4°55′E). This research farm deals i.e., with research on sediment treatment by landfarming, in combination with bio energy farming.

For the experiment, a special deposit was constructed of approximately 75 m × 13 m × 1.50 m (length × width × depth) surrounded by dikes of the natural loamy soil directly available at the spot. On May 8th 2001 the deposit was filled, by pumping, with sediment from inland waters at Schagen (province, North Holland), selected because of high PAH and low heavy metals and organochlorines loads. This sediment was dredged in the end of November 2000, transported to the farm with trucks and temporarily stored in another deposit at the farm. During dredging and subsequent pumping large items were discarded from the sediment, although the deposit still contained bigger inert items (parts of bricks etc.). The deposit was filled for about two third of its length, at one end the deposit was left empty so that excess water could drain off.

For sampling the deposited area was divided in 13 sections, each 3 m wide; the two end-sections of approximately 5.5 m each at both sides of the row were excluded because they could become colonized from three directions (both dikes plus the lateral dike) or had a different drainage. Soil animals were sampled monthly for two years. Due to severe frost or excessive rain, sampling sometimes had to be postponed for some days. At each sampling date three sections were randomly chosen, and samples were taken within the deposit at 2 m intervals, starting at 50 cm from the dikes, and from the third sampling date onwards also the two bordering dikes (per section seven samples in total).

Earthworms were sampled by digging out 25 cm × 25 cm × 20 cm of soil and hand sorting at the spot. Also all other soil animals (macrofauna) found in these samples were collected. The searched soil (including earthworm cocoons) was redeposited at the sampling site afterwards, so as to minimize the depleting impact of sampling. Earthworms were brought to the laboratory for identification to species level according to Sims and Gerard (1999). During the first few months a qualitative description of the vegetation was carried out, but plant growth or organic matter production was not measured systematically. During the second year of colonization (from January to October 2003) also soil temperature and precipitation were registered to correlate (and possibly adjust) earthworm development in the deposit for possible weather impacts.

2.2. Measuring soil physical and chemical characteristics

Observations on the development of the soil profile (downward 'progression' of the aerobic soil layer) were done visually during digging for sampling earthworms, and by coring in October 2001, December 2001, February 2002 and August 2002. Visually a distinction was made between the aerated upper soil layer, the brown/black transient zone, and the lower zone of black still anaerobic sediment.

Throughout the whole observation period measurements of soil characteristics were carried out on moisture content, organic matter content, pH (H_2O). Samples were analyzed for the presence of heavy metals, PAHs and organochlorines only at the beginning. At later samplings only total and available PAH concentrations were measured together with moisture content (wet to dry weight), and organic matter content (as loss on ignition at 550 °C). Metal and organochlorine concentrations were determined by Alcontrol (Hoogvliet, The Netherlands) under certified quality assurance conditions (RVA), according to their own protocols using respectively GC/MS and ICP/AES. PAH concentration was determined for individual PAHs (naphthalene, anthracene, phenanthrene, fluoranthene, benz(*a*)anthracene, chrysene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, benzo(*ghi*)perylene and indeno(1,2,3-cd)pyrene) and for the sum of these 10 PAHs. The PAHs were measured after extraction with acetone and petroleum ether, clean up on aluminum oxide using HPLC and fluorescence detection (according to the Dutch standard method NEN 5771; NEN, 1999). The detection limit of this method is approximately 0.01 mg kg^{-1} dry weight, which is far below the measured concentrations in this investigation. Applying this method on samples of the International Sediment Exchange for Tests on Organic Contaminants (SETOC) of Wageningen University, 96% of all individual measurements had a *Z*-score <2. Available PAHs were measured using a solid phase technique described by Cornelissen et al. (1998). In the applied procedure, sediment (1 g), water (50 ml), $HgCl_2$ (1.25 g) to stop the activity of degrading organisms and 1.5 g Tenax beads were brought together in a separation funnel. Tenax TA (177–250 μm), a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide was obtained from Varian. Before use the Tenax beads were rinsed with hexane, acetone and water. The funnel was continuously shaken at room temperature (approximately 20 °C). After 20 h of shaking, the Tenax beads were separated from the slurry and the adsorbed PAH were extracted again with hexane and measured by HPLC according to the method described in NEN 5771. This amount was considered to be the fraction available for degradation. PAH remaining in the residue after removal of the Tenax beds were measured according the method for sediment. The available PAH are expressed as fraction (amount in Tenax/(amount in Tenax + amount in residue).

Because of physical and financial constraints only one deposit could be constructed and sampled. For data comparison it is assumed that the various sections of the deposit provide random samples of the total deposit, and that other factors (like weather conditions or drainage) have a similar impact on all sections. Within each section earthworm numbers in the deposit can be

Table 1
Pollutant concentrations in the sediment used for this study as sampled in November 2000. All concentrations in mg kg^{-1} dry sediment for PAH, mineral oil and heavy metals and μg kg^{-1} dry sediment for organochlorines.

PAH		Heavy metals		Organochlorines	
Naphthalene	0.1	Cadmium	1.1	∑Chlorobenzenes	16.0
Anthracene	1.0	Mercury	0.6	Hexachlorobenzene	16.0
Phenanthrene	2.0	Copper	49.0	∑Drins	25.0
Fluoranthene	7.9	Nickel	13.0	∑HCHs	<70.0
Benz(*a*)anthracene	2.8	Zinc	290	∑Pesticides	90.0
Chrysene	2.4	Chromium	24.0	∑7PCBs	105
Benzo(*k*)fluoranthene	1.3	Arsenic	1.0	Mineral oil	1200
Benzo(*a*)pyrene	3.2				
Benzo(*ghi*)perylene	1.6				
Indenopyrene	1.6				
∑10PAH	23.8				

compared with the source populations in the bordering dikes at both sides. As it was not possible to distinguish the impact of both source dikes from each other we compared the mean numbers of the five sub-samples in the deposit with the mean numbers found in the samples from the dikes; all in triplicate. Because of the limited duplication and the inherent differences between sampling plots at different distances form the source dikes, it was not realistic to calculate statistical variabilities within or between the sections.

3. Results

3.1. Soil development

The sediment as analyzed directly after dredging and deposition at the Oostwaardhoeve in November 2000 contained 7.6% organic matter, 13% clay (<2 μm), 20% fine silt (2–16 μm) and 67% silt + sand (>16 μm), and had a pH-H_2O of 7.1. Metal, PAH and organochlorine concentrations in the sediment are summarized in Table 1.

According to Dutch policy, on the basis of the ∑10PAH level of 23.8 mg kg^{-1} dry weight, the sediment has to be classified as medium contaminated. For none of the other pollutants, concentrations exceeded the Intervention Value defined by the Dutch government (VROM, 2000).

Three months after pumping the sediment into the deposit (July 2001), the sediment had settled, with a cracked surface (including big cracks of over 1 cm wide) and a dried upper layer of approximately 10 cm thick. Below 10 cm there was a compact moist anaerobic soil layer. There were no visible signs of layered sedimentation, but the surface was irregular with height differences up to 10 cm and dry and moist spots.

In September 2001, five months after deposition, the total thickness of the sediment layer had diminished from 1.0 to 0.9 m, and the transition zone between aerobic and anaerobic soil was lowered to 30–40 cm below the soil surface.

The dry matter content of the soil was only registered extensively and in relation to the analyses of PAH concentrations. Compared to the original sediment the dry matter content increased in time as an effect of settling and ripening. The moisture content observed in the upper layer did approach field capacity. When we sampled one section of the sediment in three layers (2.5–7.5; 7.5–12.5 and 12.5–17.5 cm below surface level) in December 2001, the mean moisture content increased with depth, for the three layers respectively mean 58.2%, min 53.4%–max 64.5%; mean 60.9%, 42.5–71.5%; mean 68.1%, 49.3–83.1%), but the total pattern was highly heterogeneous. The dry matter content of the lower layer (moist, anaerobic, not ripened) stayed at a higher level (approximately 40%), without clear trend in time. Neither was there a clear trend visible in the measured organic matter contents, which ranged between 7% and 8%.

3.2. PAH concentrations

The concentrations of phenanthrene (three-ring), fluoranthene (four-ring), benzo(*a*)pyrene (five-ring) and indenopyrene (six-ring) are plotted against time in Fig. 1. Concentrations of the two three- and four-rings PAHs showed a fast decline in the first few

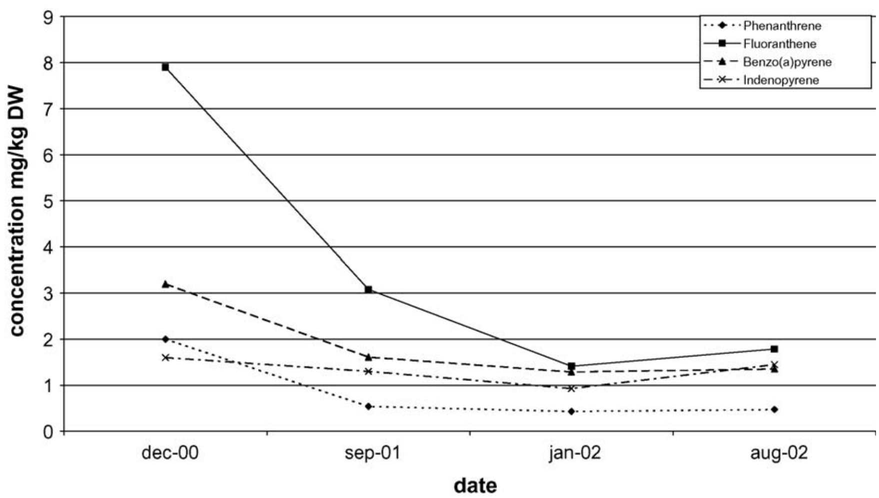

Fig. 1. Decrease in the course of time (months) of the concentrations of phenanthrene (three-ring PAH), fluoranthene (four-ring), benzo(*a*)pyrene (five-ring) and indenopyrene (six-ring) in the experimental dredged sediment deposit.

Table 2
Mean PAH concentrations and percentage available PAHs in the sediment of the deposit in September 2002, 16 months after depositing. A distinction is made between the upper (0–10 cm; aerobic) and lower (10–20 cm; anaerobic) sediment layer, and expressed as the ratio of lower divided by upper. Available concentrations were measured with the Tenax-method (see Harmsen, 2004).

	Total mg kg^{-1}dry weight			Percentage available	
	Upper	Lower	l/u	Upper	Lower
Anthracene	0.33	0.94	2.86	6.8	26.2
Phenanthrene	0.54	1.26	2.36	9.7	17.1
Fluoranthene	3.08	4.47	1.45	10.6	37.4
Benz(*a*)anthracene	1.38	1.96	1.42	7.2	22.8
Chrysene	1.68	2.71	1.61	7.4	19.5
Benzo(*k*)fluoranthene	1.35	2.03	1.50	7.0	16.6
Benzo(*a*)pyrene	1.61	2.74	1.70	5.6	13.0
Benzo(*ghi*)perylene	1.35	2.12	1.57	6.5	6.8
Indenopyrene	1.30	2.08	1.60	3.5	6.3
∑PAH[a]	12.0	19.5	1.62	7.2	19.1

[a] Pyrene is not included because of difficulties in analysis, hence, ∑PAH includes only nine PAHs here.

months, while the two five- and six-rings PAHs showed only limited decrease. Six months after deposition (October 2001), the concentrations of phenanthrene and fluoranthene (three- and four-ring) in the surface layer (0–10 cm) were much lower than in the layer directly below (10–20 cm). For benzo(*a*)pyrene and indenopyrene the differences in concentration between 0–10 and 10–20 cm were much smaller or negligible.

Table 2 lists the mean concentrations of the various PAHs in the upper and lower layer and the ratio between these two. Next to this, it is of interest to look at the decrease of the three-ring versus the four-ring PAHs. For most of the PAHs the ratio of concentrations in the upper and lower layer was approximately the same (1.4–1.8), but for anthracene and phenanthrene this ratio was much greater. This may indicate that degradation of these two PAHs did occur, but caution is needed as the range in data is fairly large. The concentrations of PAHs in the dikes around the deposit were at background level (<1 mg kg^{-1} dry weight)

The available PAH fraction, measured with Tenax extraction, in the lower part of the sediment in direct contact with the water phase, varied from around 30% for the three + four-ring PAHs to 6% for six-ring PAH. Available PAH fractions in the aerobic upper layer were all lower (range 5–10%).

3.3. Vegetation development

The vegetation development was 'luxuriant' from the beginning. Three months after pumping the sediment into the deposit (July 2001), the surface was covered with *Persicaria maculosa* (70% coverage, about 1 m high), *Phragmites australis*, *Capsella bursa-pastoris*, *Chenopodium album*, *Epilobium hirsutum* and *Schoenopletus triquetes*. The latter species indicates for saline influences. At later stages the vegetation developed to approximately 1.25 m high, became denser and covered the total surface of the deposit, although between the bushes there were still bare soil spots available to sample soil animals.

This development resulted in a layer of fresh dead plant material at the end of the first sampling year (autumn and winter 2001), gradually degrading and forming a litter and raw humus layer.

3.4. Earthworms

3.4.1. Total numbers and reproduction ratio

Data on total earthworm numbers are plotted in Fig. 2. From the very first sampling, five months after pumping the sediment into the deposit, earthworms were found in the deposited sediment, albeit only very few, scattered individuals. From the third sampling onwards also the two bordering dikes were sampled. The numbers of earthworms in the clayey dike (right hand side in Fig. 2) were much higher than in the sandy loam dike (left hand side in Fig. 2). The pattern of population development

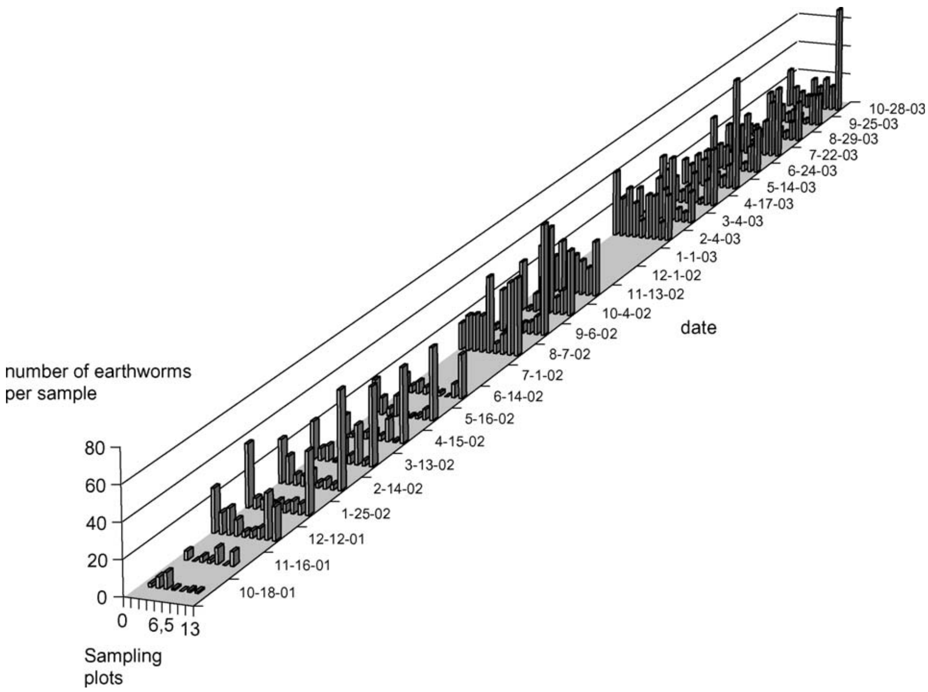

Fig. 2. Total earthworm numbers per sample (0.0625 m^2) in an experimental dredged sediment deposit in the course of time (months). For each sampling date (day–month–year) the mean numbers are given for three sampling series, and going from one source dike (most left hand bar) to the other source dike (most right hand bar). Distances between sample sites were 2 m.

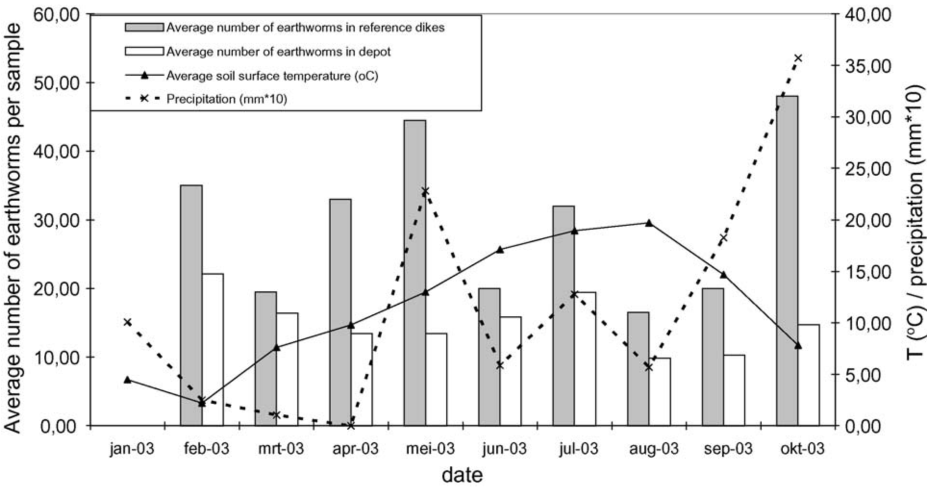

Fig. 3. Mean total numbers of earthworms per sample (0.0625 m^2) in an experimental dredged sediment deposit in the course of time (months) in relation to the temperature in °C and rainfall in mm.

in the deposit with time can be deduced from Fig. 2. If the increase would be due to dispersal (influx) from the populations in the dikes into the deposit, the distribution pattern from one dike to the other should have a U-shape with a gradual decrease going from the dike into the deposit and lowest numbers in the middle. For the total numbers this was not the case. Moreover, the figure shows clearly that there was a seasonal fluctuation of the earthworm numbers in the dikes. This fluctuation is plotted in Fig. 3 together with the seasonal fluctuations in the deposit itself. Until mid 2002, earthworm numbers in the deposit stayed low with some slight seasonal fluctuation, only after that (lag)period numbers started increasing consistently. The fluctuation in earthworm numbers was related to both soil temperature and precipitation, with highest numbers especially found when high temperatures and high precipitation occurred in the same period (Fig. 3).

Next to adult worms also sub-adult and juvenile worms were sampled. The ratio between adult worms and sub-adult + juvenile worms, as indicated in Fig. 4, was much lower in the deposit than in the dikes for the period July 2001–August 2002. In the autumn of 2002 and the winter of 2002/2003 there was an impressive reproduction in the dikes, and to a lesser extent in the deposit. From spring 2003 onwards the ratio fluctuated, with the ratio in the deposit being both higher and lower than in the dikes.

3.4.2. Numbers per species

The pattern of earthworm abundances in the deposit becomes more diversified when looking at the numbers of the various species (Fig. 5). *L. rubellus* and *L. castaneus* were the first colonizers, found from the first sampling in October 2001 onwards. The distribution pattern over the deposit of the epigeic *L. rubellus* (=active in the surface litter layer) was irregular, and no seasonal pattern could be observed. Only few individuals of *L. castaneus* were found and this species disappeared at later sampling dates. The next species that established was *Aporrectodea caliginosa*. Here the distribution pattern was different, not only because there were high numbers in the dikes, but also due to low numbers in the middle parts of the deposit, causing a more or less a U-shaped distribution that lasted until April/May 2002. A few weeks later *L. castaneus* settled again. The distribution pattern was slightly irregular (and non-U-shaped). *Eiseniella tetraeda* appeared next, for the first time in November 2002. As last settler *Allolobophora chlorotica* was observed from February 2003 onwards. Further there was a sparse observation of *A. rosea* in this last period (single specimen in April and July 2003), and there was an observation of the relatively rare species *Dendrobaena pygmea*.

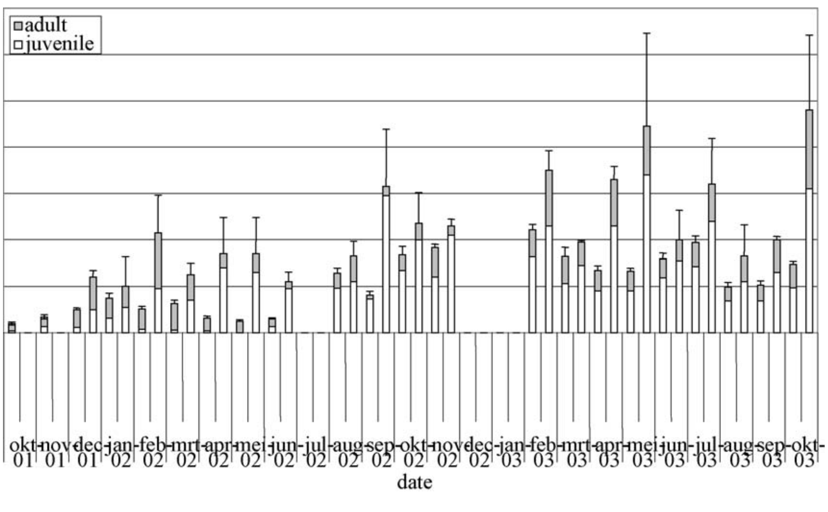

Fig. 4. Mean adult (hatched) and juvenile (non-hatched) numbers (total numbers ± s.e.) of earthworms per sample (0.0625 m^2) in an experimental dredged sediment deposit in the course of time (months).

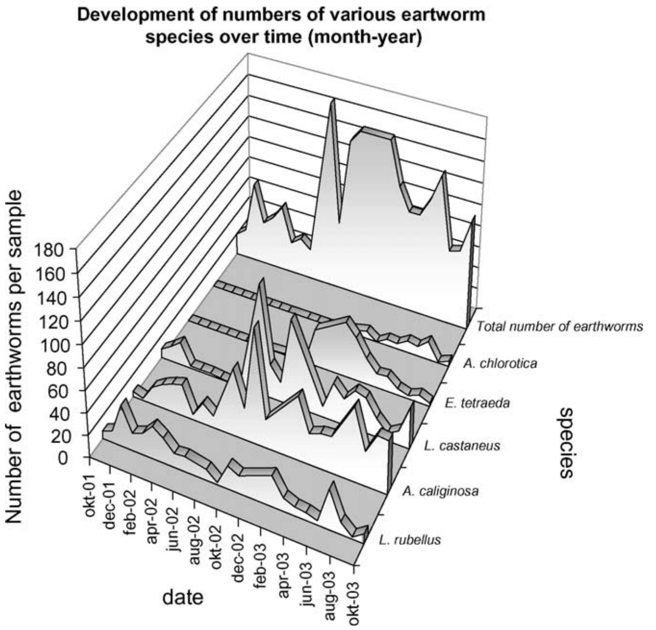

Fig. 5. Mean numbers of different earthworm species per sample (0.0625 m^2) in an experimental dredged sediment deposit in the course of time (months).

3.4.3. Modeling the dispersal of the earthworms

To estimate the rate at which the earthworms colonize the deposit from the surrounding dikes and the rate at which they disperse through the deposit, a model application was developed and dispersal parameters were estimated by fitting this model to the data. A diffusion model was chosen because of its relative simplicity and because it can be applied to any initial distribution of organisms. It was useful for the studied deposit, since at the start of the experiment, no earthworms were present in there.

It was assumed that colonization was only possible from the bordering dikes, but once present in the deposit, individual species were assumed to disperse in all directions. The model was designed to capture both migration to and from neighboring plots and local population dynamics (i.e., reproduction and mortality), the two processes that determine earthworm abundance at any plot in the deposit. For the population dynamics component of the model, we assume that at any point within the deposit the population grows logistically towards a (deposit-specific) carrying capacity.

The local change in earthworm population size was described by the partial differential equation based on the well known Fisher–Kolmogorov equation (Murray, 2004):

$$\frac{\partial w}{\partial t}(x,t) = D\frac{\partial^2 w}{\partial x^2}(x,t) + w(x,t)r\left(1 - \frac{w(x,t)}{K}\right), \tag{1}$$

$w(x,t)$ = earthworm population density (number of individuals per sample) at distance x from the first dike (ranging from 0 to 13 m) and time t, D = diffusion constant (m^2/d), r = maximum specific population growth rate (d^{-1}), and K = carrying capacity (number of individuals per sample).

Population densities at the deposit boundaries, i.e., $w(0,t)$ and $w(13,t)$, were fixed; they were taken equal to linearly interpolated population densities observed in the bordering dikes (see Figs. 2 and 4). This makes sure that temporal density fluctuations observed in the field situation are also implemented in the model. Earthworm densities at time 0 throughout the deposit were taken equal to the linearly interpolated values observed during the first sampling day.

Eq. (1) was solved numerically by discretizing it in time and space, allowing us to express the worm density at location m and time $n+1$ as:

$$W_{m,n+1} = W_{m,n} + \Delta t\left[D\frac{2}{\Delta x_m}\left(\frac{W_{m+1,n} - W_{m,n}}{\Delta x_{m+1} + \Delta x_m} - \frac{W_{m,n} - W_{m-1,n}}{\Delta x_m + \Delta x_{m-1}}\right) + W_{m,n}r\left(1 - \frac{W_{m,n}}{K}\right)\right],$$

with $W_{m,n}$ = earthworm population size at grid cell m and time index n, Δx_m = width of grid cell m, and F_n = parameterization of the population change due to reproduction and mortality at time point n.

For the sake of simplicity we used a spatial grid that corresponds to the plot division in the field study, i.e., seven grid cells (m = 1–7) with cell centers taken equal to the sampling locations (1.25 m for the first and last cell, and a width of 2 m for the cells 2–6). This allows for direct comparisons between model result and observation, without requiring additional interpolation. The time step is set to seven days; smaller steps did not noticeably affect the results.

The model contains three parameters characterizing dispersal and population growth: the diffusion constant D (m^2/d), which relates to the rate of earthworm dispersal, the maximum specific growth rate r (d^{-1}) and the carrying capacity K (expressed as individuals per sample).

Eq. (2) was solved numerically using the principle of maximum likelihood.

Optimal parameter values equalled $D = 0.0016 \pm 0.0014$ m^2/d, $r = 0.0204 \pm 0.0056$ d^{-1}, $K = 14.3 \pm 1.27$ individuals per sample. This value of diffusion constant D implies that 0.16%, or one in every 625 worms, crosses over to a bordering plot a day. By using the Fisher–Kolmogorov equation it is possible to deduce the wave propagation (the speed by which the fore front of the earthworms disperse); this is 4.2 ± 1.33 m $year^{-1}$. The resulting model description is shown together with observations in Fig. 6.

Fig. 6 shows that there is a reasonably good fit between model predictions and observations, certainly when we take into account that only three parameters were estimated from the data. There are some points where considerably more worms were observed than expected from lateral dispersal.

4. Discussion

4.1. Dispersal of earthworms and colonization of the deposit

Gradual dispersal from a source area through the soil will result in a diffusive process, which can be described as a random process (see Oude Voshaar and Eijsackers, 1983, Annex to Hoogerkamp et al., 1983). This will result in a distribution pattern with a steep slope at the start that with proceeding colonization gradually flattens out, until after full colonization numbers in source and colonization area are similar. Such pattern has only been observed in this study for *A. caliginosa*. The other earthworm species dispersed faster, presumably by surface dispersal as described by Mather and Christensen (1988, 1992, 1998). They observed surface dispersal over several meters per night, for all three earthworm eco-types (anecic and endogeic species like *L. terrestris*, *A. longa*, *A. caliginosa*, *A. rosea* and *A. chlorotica*, and more limited for epigeics like *L. rubellus* and *L. castaneus).* Already in the first samplings, a few months after deposition of the sediment, specimens of *L. rubellus*, *L. castaneus* and some very few *A. caliginosa* were found, and already in the middle part of the deposit at 6.5 m from the bordering dikes. Normal dispersal rates by burrowing in and through the soil are 7 to >10 m $year^{-1}$. Surface dispersal may result in a more randomized arrival of individual specimens.

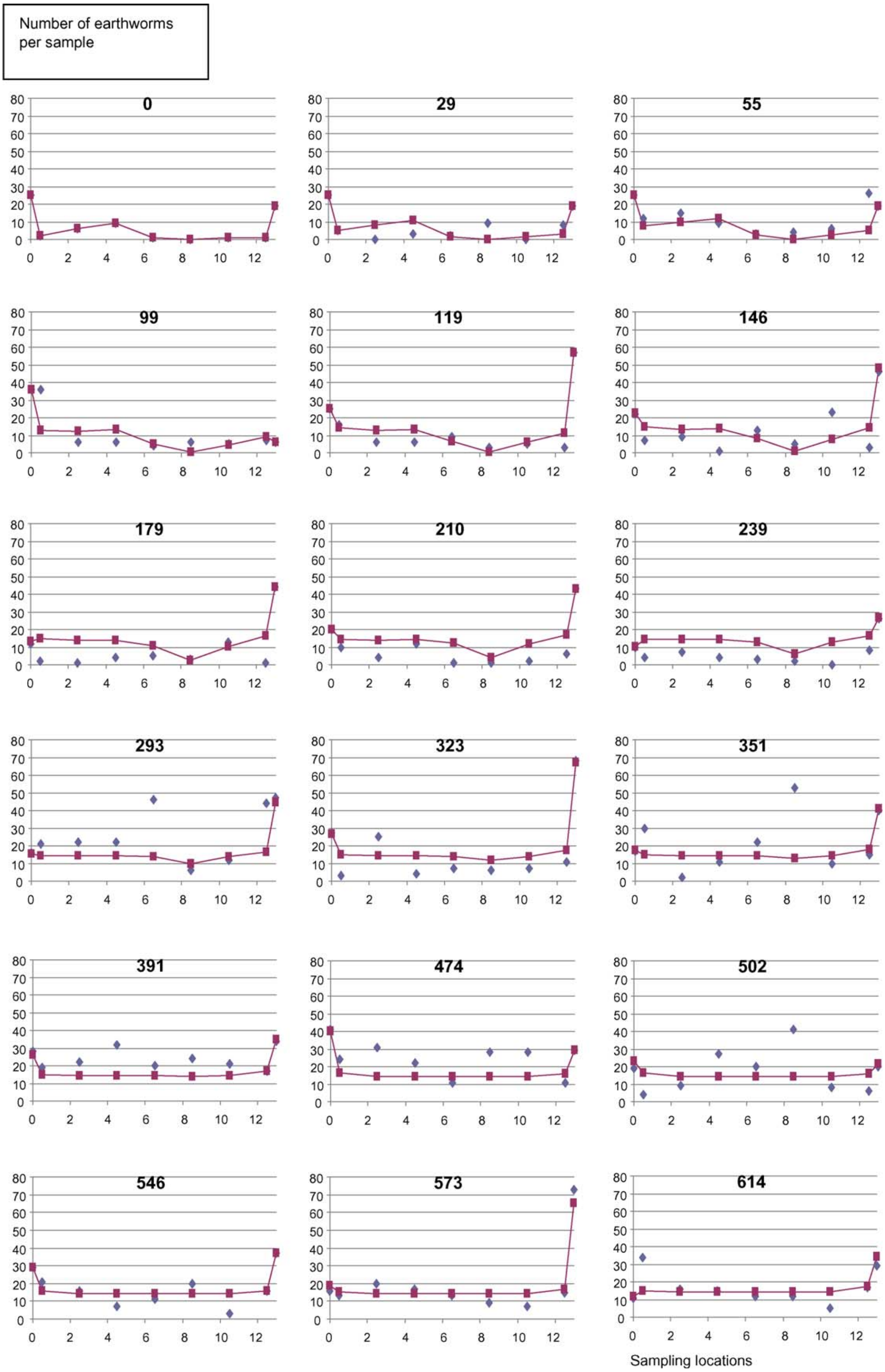

Fig. 6. Comparison of model output with interpolated data of worm numbers in the field ($D = 0.0016$, $r = 0.020$, $K = 14$) on different sampling times ($t = 0–614$ days) and at different locations in the site (1–12). The dots are the field data of number of worms per sample and the line with squares is the model prediction of the numbers of worms per sample.

These specimens established and reproduced as shown from numbers of cocoons and juveniles and sub-adults, although within the deposit with a retardation of about a year compared to the bordering dikes. Establishment and population development were stimulated by favorable weather conditions (high temperatures in combination with precipitation resulting in high soil moisture content). During field visits we did not observe indications of stagnant rainwater that may have resulted in anaerobic soil conditions. The luxuriant vegetation development illustrated the nutrient richness of the sediment which is not surprising given the usual high nutrient value (both N and P) of these inland water sediments (Van Puijenbroek and Kampf, 1998).

Nevertheless the earthworm numbers never reached the population levels in the surrounding dikes. The highest numbers we observed were just over 80% of those in the dikes. In general the maximum numbers in the dikes at favorable periods reached 40 specimens per sample (with extreme numbers of over 60), corresponding with 640 worms per m^2 (and over 960 per m^2), which are high numbers for grasslands (Curry, 1994), in our case including adult, sub-adult and juvenile worms. Also the highest numbers within the sediment deposit (80% of the dikes, hence 512 respectively 768 worms per m^2) are well within this normal range (Edwards, 1983). The chemical quality of the deposit (concentrations of pollutants) at the beginning of the sampling five months after deposition did not prevent earthworms from entering the deposit. The general rate of diffusion was, however, somewhat low compared to other studies in non-polluted virginal, clayey soils.

4.2. Modeling dispersal

When we modeled these results on dispersal we found a much lower dispersal rate of the worm front, a population growth rate that is comparable to normal conditions and a carrying capacity that is clearly lower than of the surrounding dikes.

For an initially empty area that is invaded from a single boundary (here: reference dikes), the estimated diffusion constant implies a rate of propagation of the population front of $2\sqrt{rD} = 4.2\,\mathrm{m\,year^{-1}}$ (Murray, 2004). This is at the low end of a range of dispersal front rates of 9–14.5 m $year^{-1}$ reported in other Dutch studies on virginal clayey soils that did not contain earthworms before (Hoogerkamp et al., 1983; Stein et al., 1992 and Eijsackers, unpublished data.). Modeling the results assuming a Poisson instead of a normal distribution (not reported above), resulted in a front dispersal rate of 8.5 m $year^{-1}$ instead of 4.2 m $year^{-1}$, this is more in agreement with rates observed in unpolluted virgin soils. Additionally, the estimated maximum specific population growth rate approximates the value of 0.014 d^{-1} reported by Klok (2007). The local higher earthworm numbers compared to the model output can be explained by reproduction hotspots from earthworms that arrived earlier over the surface.

The field situation differs from the model assumptions, as not all plots are equally suitable for worms, and different species of worms have different habitat preferences and colonization rates as shown above. Moreover, seasonal influences and soil type are not incorporated in the model. The correspondence with estimated parameters and reported values suggests that the present model aptly captures the behaviour of the earthworm population during colonization of the deposit. From these results it seems that especially carrying capacity is negatively affected by the composition of the sediment, while the front dispersal rate and population growth rate are less affected. However, the colonization period was only two years, while Hoogerkamp et al. (1983) observed in well-drained clayey soil that build-up to a stabilized population level took 7–8 years.

4.3. Sequential appearance of earthworm species

We observed a clear sequence (succession) in appearance of different earthworm species on a short term of various months to some years, which to our knowledge never has been reported before. *L. rubellus*, *L. castaneus* and *A. caliginosa* were the first arrivers with some few months in between, *E. tetraeda* and *A. chlorotica* came as second shift (10 respectively 13 months later), while the anecic earthworm species are still to come; although *A. rosea* was already observed incidentally. *L. rubellus*, *L. castaneus* and *A. caliginosa* are well known as first colonizers from the literature (see for instance Pizl, 1992, 1999), although it is remarkable that the first two species, which are known as typical colonizers of more acid sandy soils, also appeared first here. The incidental presence of *L. castaneus* could be due to small pockets of organic matter in the freshly deposited sediment. After these pockets had been consumed, the species disappeared to return only after new organic matter (from the newly grown vegetation) had become available. The endogeic *A. caliginosa* burrows through the soil, and may take more time to enter the deposit subsoil from the dikes. *A. chlorotica* is mentioned as colonizer too, but especially in more clayey and moist substrates, *E. tetraeda* is especially observed in moist conditions at later stages, and is reported from wetter locations. October 2002 effectively had a very high precipitation level (Fig. 4). As the bottom part of the sediment was still wet, it is reasonable to assume that the top layer could quickly become saturated after heavy rain. *A. rosea* is a representative of the deeper burrowing anecic species appearing when a well-developed aerobic deeper topsoil is available, apparently at the end of the two-year experimental period. Another typical representative of the anecic group – *L. terrestris* – has not been observed yet.

Vandecasteele et al. (2004, 2005) did an inventory of sediment deposits between 6 and 70 years old. They found mainly *L. rubellus* and *L. castaneus* as first colonizers and dominating in these deposits. Especially in moister deposits with willows, *E. tetraeda* was found. They related total earthworm biomass to grain size and time since disposal, soil pollution status was of lesser importance. Ma and van den Ham (personnel communication) inventoried Rotterdam harbor sediment deposits approximately 15 years after deposition. Single individuals of *L. castaneus* and *D. rubida* had dispersed farthest, at 110 and 85 m from the source dike, *E. tetraeda* was found some 60 m from the dike, but the majority of the worms was within the first 30–40 m from the dike and consisted only of *L. rubellus*. Front dispersal rates were low: mean rates <4 m $year^{-1}$ over a 15 years period, although limited drainage capacity and saturated soil conditions may have hampered dispersal.

4.4. Chemical and physical quality of the sediment, and impact of earthworm activities

With respect to the chemical quality of the sediment in the deposit, the levels of the different available organic compounds and heavy metals did not cause serious intoxication. For copper which is very toxic for earthworms, the concentrations in the sediment were much lower than the approximate value for field effects of 100 mg kg^{-1} (established by Ma (2005). Also for other heavy metals present the threshold value was not surpassed. Harmsen (2004) measured the ecotoxicity of landfarmed sediments with bioassays with *L. rubellus*. Negative impacts on weight increase and reproduction were observed in sediment sampled between four and nine years after deposition, with total PAH-contents of 30–50 mg kg^{-1} dry weight, but also with considerable amounts of mineral oil and salt.

Earthworm activity improves soil quality by providing a better soil texture and structure by formation of stable aggregates

(Marashi and Scullion, 2003; Van Delft et al., 1999) and formation of desiccation cracks in recently inundated farmlands (Kazanci et al., 2001).

At Oostwaardhoeve surface drainage was possible from the start of the experiment, and dry matter content increased in time as an effect of settling and ripening. The moisture content observed in the upper layer did approach field capacity. The dry matter content of the lower layer (moist, anaerobic, and not ripened) stayed at approximately 40%. There was no clear trend in the measured organic matter contents, which ranged between approximately 7% and 8%. The sediment layer had shrunken from 1.0 to 0.9 m and the aerobic/anaerobic transition zone was found at 30–40 cm below the soil surface, this took one year longer at the Kreekraksluizen landfarm (Harmsen, 2004).

In the deposit, concentrations of the lighter PAHs (phenanthrene and fluoranthene) disappeared quickly from the sediment, directly after depositions and some few months before any earthworm had appeared. This reduction could be largely ascribed to sediment drainage and evaporation resulting in an aerobic top layer, and the development of a vegetation cover. The first plant growth started shortly after deposition, covered the total surface after five months) and rooted the upper sediment layer. This may have contributed to further aeration of that layer, while root turnover may stimulate biodegradation of PAHs according to Leigh et al. (2002).

In laboratory and field experiments, Eijsackers et al. (2001) observed that introduced earthworms increased degradation of PAHs, either by improving aeration of the sediments or by stimulating microbial activities. Earthworms do not have the capability to degrade PAHs by themselves (Stroomberg et al., 2004). Haimi et al. (1992) observed in the laboratory no influence of earthworms (*L. rubellus* and *A. caliginosa*) on chlorophenol (2,3,4,6 TeCP, PeCP and metabolites) concentration in soil. The function of earthworms in degradation of PAHs will be indirect. After dredging, the supply of oxygen is the most limiting condition for degradation. Earthworms have a positive effect on the change of anaerobic sediment into well structured aerobic soil, thereby stimulating the aerobic degradation of PAHs. We speculate that the role of the earthworms in the degradation of PAHs will be more prominent in the next phase of slower degradation (Harmsen, 2004). Next to optimizing soil structure and aeration, earthworms have a major impact on the size of soil aggregates. Breaking of soil aggregates will increase the availability of PAHs for degradation by micro-organisms. Already after two years of observation the quality of the sediment was greatly improved. Such fast improvement of the chemical sediment quality combined with the fact that no adverse impacts on earthworms were observed, allows for a lower environmental risk assessment classification of the sediment and creates possibilities for reuse of the obtained soil.

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