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Desorption of pyrene was studied in freshly-amended and 120 d-aged samples of six different soils using a Tenax-assisted method in order to evaluate the influence of soil properties and aging time on desorption. The correlations between desorption percentage (P_d) , rapid desorption rate constant (k_{rap}) , and biota to soil accumulation factor (BSAF) were analyzed. Results showed that in soils with a relatively high soil organic matter (SOM) content (>1% in this study), P_d and k_{rap} decreased with the increase of SOM content both in freshly amended and aged soils. This suggests that SOM is the key component for sorption organic pollutants by providing highly active combination sites, where the combined pollutant becomes difficult to desorb. In soils with a relatively low SOM content (<1%), clay minerals played an important role through offering nanopores to entrap pollutant molecules, making it difficult for these molecules to diffuse out. Aging significantly reduced the rate and extent of pyrene desorption. It is reasonable to deduce that, during aging, some of the pyrene molecules moved from "readily desorbing sites" to "relatively slower desorbing sites," which led to a reduction of desorption. Ln P_d showed a linear relationship with In BSAF for both freshlyamended and aged soils, and In k_{rap} only in aged soils. In freshlygmended soils, rapid desorption in some soils is too quick to be the limiting step for bioaccumulation, and, therefore, the elevation BSAF became insignificant when k_{rap} was larger than $3 \times 10^{-3} h^{-1}$.

25 Keywords Desorption, biota to soil accumulation factor, aging, pyrene

Introduction

The combination state of organic pollutants in soils, which refers to the patterns they combine with or exist in the soil particles, is an important factor controlling their fate, reactivity and bioavailability in the environment. When an organic pollutant enters soil, its combination state in soil differentiates greatly due to high heterogenicity of the soil. From the viewpoint of desorption, the pollutant exists in at least three boxes: (i) rapidly desorbing; (ii) slow desorbing; and (iii) extremely slow desorbing. The pollutants in the first two boxes correspond to the rapid phase and slow phase in desorption curve, respectively (Cornelissen *et al.*, 1998; Braida *et al.*, 2002; Zhao *et al.*, 2002), while the third one is irreversible sorption

35 fraction, which is completely unavailable to desorption and organism uptake (Huang *et al.*, 2003; Burgos *et al.*, 1996; Achtnich *et al.*, 1999). Moreover, it has been reported by many references recently that desorption, mild solvent extraction and bioavailability of a pollutant

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decrease with the contact time (aging time) of the pollutant in soil (Alexander, 2000; Morrison *et al.*, 2000; Reeves *et al.*, 2001), which implies the combination state of the pollutant has changed during aging time, with some of its molecules moving from "readily desorbing and bioavailable states" to "slow desorbing and less bioavailable states," and even to "irreversible and non bioavailable states."

Bioassays are considered to be the most accurate approach to assess the bioavailability of a pollutant and its risk to the ecosystem since they integrate all of the biotic (e.g., pollutant metabolism) and abiotic factors (e.g., pollutant combination state) (Ehlers and Luthy, 45 2003). But bioassays are sometimes time-consuming, expensive, or lowly precise, and hence attempts have been made to set up abiotic approaches to predict bioavailbility; however, the results are inconsistent (Johnson et al., 2002; Tang et al., 2002). Bioaccumulation tests using earthworms are a common biological method for measuring the bioavailability of a pollutant in soils. According to Equilibrium Partition Theory (EPT), biota in soils, such as 50 earthworms, could assimilate the chemicals in soil water solution only (Lanno et al., 2004). Once pollutants enter into soils, they may adsorb immediately onto soil aggregates, and could not be uptaken by earthworms until they desorb into soil solution again. Generally, the quantity of hydrophobic organic pollutants in soil solution is extremely low, thus the pollutants adsorbed in soil solid phase could gradually be desorbed into water phase and 55 assimilated by the biota. During the initial stage of earthworm accumulation, desorption of the pollutant from soil particles was in rapid phase, and the rate of earthworm accumulation was not influenced by the rate and extent of the desorption. After the pollutants desorbed through rapid phase have been assimilated by earthworms, the diffusion rate of the pollutant from soil into water phase would become extraordinarily slow. Then the rate and extent of 60 desorption could turn into the limiting step of the bioaccumulation of the pollutant in soils. The irreversible fraction could not desorb anymore, which is completely non-bioavailable (Huang et al., 2003).

Desorption aided with Tenax TA has been proved to be very useful for desorption experiments because of its high sorptive rate and capacity to organic pollutants (Cornelissen *et al.* 1998). Hence, provided that the activity of earthworms have little effect on soil aggregates and that only the molecules in water phase could be accumulated, there is much similarity between Tenax-based desorption and earthworm uptake, and the bioavailability of pollutants in soil could be predicted by a rapid desorbing process assisted with Tenax. However, corresponding literatures on comparison of rapid desorption aided with Tenax and earthworm accumulation are few (Morrison *et al.*, 2000; Talley *et al.*, 2002).

Hence, in this study, desorption of a polycyclic aromatic hydrocarbon, pyrene, in different soils before and after aging, was investigated using Tenax TA-based method, and the effects of soil characteristics as well as aging on the desorbing behavior of pyrene in soils were discussed. In addition, correlations of desorption parameters and earthworm accumulation, which has been measured in literature (Sun and Li, 2005), was studied in order to evaluate the applicability of the short-term desorption experiments in estimating the bioavailability of chemicals in soils.

1. Experimental Section

1.1. Chemicals

Pyrene of 98% purity was purchased from Aldrich Chemical Company, Inc. Pyrene stock solution (1g/L) was prepared in HPLC-grade hexane. Tenax beads (35/60 mesh) were

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Soil	Content of organic matter (%)		Water content (%)	Partical size (%)			
		pН		Sand	Silt	Clay	Soil type
1	18.68	6.99	3.91	48.60	35.38	16.02	Sand loam
2	4.06	7.60	1.73	66.01	22.39	11.60	Sand loam
3	2.98	7.72	2.75	43.03	40.10	16.86	Loam
4	1.42	7.24	2.95	56.31	23.70	19.99	Sand loam
5	0.60	8.10	0.81	54.43	39.32	6.25	Sand loam
6	0.32	7.85	4.22	34.85	26.10	39.05	Loam clay

Table 1
Physicochemical characteristics of tested soils

purchased from Chromatographic Specialties Inc., USA. The beads were cleaned by sequential Soxhlet extractions with acetone/n-hexane (1:1) and acetone/methanol (1:1) for 48 h before use. Acetonitrile used for the mobile phase of HPLC analysis was of HPLC grade.

1.2. Soils

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Six soils with different sources and properties were used in this study. Soils 1–3 and 6 were collected from various districts in Tianjin, China, while soils 4 and 5 were taken from Ningxia and Hebei Province, respectively. The soils were air-dried, and then passed through a 2-mm sieve before use.

The properties of the soils are presented in Table 1. Content of organic matter in the soils was determined by potassium dichromate-outside heating method. pH was measured in a 1:1 (w/w) soil-CaCl₂ water suspension. Water content was determined by the weight loss of the samples after heating for 6–12 h at 105°C, and the particle-size distribution was measured by the densimeter method.

1.3. Preparation of Freshly-amended and Aged Soil Samples

To obtain pyrene-contaminated soil samples, 10 g samples of each soil were added to 10ml cap tubes. Specific volume of pyrene stock solution was added to the soils to make up the final pyrene concentration of 40 mg/kg (dry soil). Specific amount of 0.2% NaN₃

100 aqueous solution was added in order to inhibit microorganism activity as well as to keep soil moisture at 30% (wet weight basis). The tubes were placed into a ventilation cabinet for 4h, and the samples were vigorously mixed for 6 s on a Vortex Mixer every 30 min to accelerate hexane evaporation and to mix pyrene molecules with the soil. Then the tubes were tightly covered with silicone-backed Teflon liners and kept in the dark at $30 \pm 2^{\circ}C$ 105

overnight and for 120 days to obtain freshly-amended and aged soils, respectively.

1.4. Desorption Experiment

In desorption experiment, 1 g freshlyamended or aged soil samples were suspended in a glassy tube containing 10 ml electrolyte solution (5 mmol·L⁻¹ CaCl₂), and then 0.2 g Tenax beads were added. The tube was capped and shaken horizontally in a shaker at 120 rpm for a designated period. Desorption tests were conducted in duplicate. At regular intervals



0:17

Figure 1. Desorption dynamics of pyrene from freshlyamended and aged soils.

(as shown in Figure 1), the tubes were taken out from the shaker, and centrifuged for 15 min at 3000 rpm. Tenax beads were then carefully taken out with a clean stainless steel spatula and transferred to a 10 ml clean glass tube. Another 0.2 g clean Tenax beads were then added into the tube to continue the desorption. Desorption was carried out by 6–8 consecutive steps over a total desorption time of 12 days. Pyrene absorbed on Tenax was eluted by shaking 115 with 10 ml of acetone/hexane (1:1, v:v) for 24 h in two successive extractions. The eluted solutions were combined and concentrated to near dryness under a stream of nitrogen. The residue was dissolved in 10 ml acetonitrile. This solution was passed through a 0.45 μ m Teflon filter to remove particulate matter prior to HPLC analysis. The recovery of pyrene from Tenax was determined by directly adding specific amount of pyrene onto Tenax beads, 120 and the recovery was found to be over 98%.

1.5. Determination of Pyrene

Concentration of pyrene in acetonitrile was determined by HPLC (Waters 1525 Binary HPLC Pump, Waters 2475 Multi λ Fluorescence Detector, Millinium 32 Station, USA) fitted with a Waters Symmetry C₁₈ column (Ireland). Acetonitrile:water (80:20) was used **125** as the mobile phase with a flow rate of 1 ml/min. Pyrene was detected by excitation at 333 nm and emission at 390 nm.

1.6. Desorption Data Interpretation

Cornelissen *et al.* (1998) have described desorption of organic pollutants from the soils by the following first-order two-compartment model assuming $k_{slow} \ll k_{rap}$. 130

$$\frac{S_t}{S_0} = F_{rap} e^{(-k_{rap}t)} + F_{slow} e^{(-k_{slow}t)}$$

where S_t and S_0 are the pollutant amounts sorbed on soil at time t and the beginning of the desorption experiment; F_{rap} and F_{slow} are the desorption percentage during rapid and slow desorption phase; k_{rap} and $k_{slow}(h^{-1})$ are the first order desorption rate constant of rapid and slow desorption.

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135 To simplify calculation, we suppose that the slow-desorption fraction is negligibly small compared to that of rapid desorption fraction during rapid desorption phase. Therefore, we could calculate k_{rap} by the slope of the following equation based on the desorption data during the rapid desorption phase of 1d.

$$Ln(S_t/S_0) = LnF_{rap} - k_{rap}t$$

1.7. Bioaccumulation in Earthworm

- 140 Bioaccumulation of pyrene in earthworms using the same freshly, amended and aged soil samples has been reported in literature (Sun and Li, 2005). Briefly, five adult earthworms (*Eisenia foetida*) were placed in 10 g of pyrene-amended soil at a moisture content of 80%. The bioaccumulation tests were conducted in the dark at $28 \pm 2^{\circ}$ C for 10 days. Then the worms were removed, rinsed with water, and placed on moist filter paper in a glass petri
- 145 dish for 24 h to allow for depuration. Then the worms were sequentially washed with water, dried with paper towels, weighed, and freeze-dried. The worms were ground in a mortar with pestle and mixed with three times their weight of anhydrous Na₂SO₄. Then pyrene in the samples was extracted by Soxhlet extraction with dichloromethane, concentrated to suitable volume and analyzed by HPLC.

150 2. Results and Discussion

2.1. Desorption of Pyrene from Freshlyamended Soils

Desorption dynamics of pyrene in the six freshlyamended soil samples are shown in Figure 1a. Desorption could be divided into two phases: rapid and slow phase. Desorption percentage achieved at 2.6, 5.7, 8.3, 14.1, 28.8 and 32.7% within the first day, and afterwards desorption became slow, however did not reached equilibrium at 12 d. At the end of the experiment, there are 6.7, 19.4, 31.6, 35.8, 49.4 and 49.2% of the sorbed pyrene

desorbing into water phase, respectively.

Rapid desorption rate constant, k_{rap} was calculated based on linear correlation of the data within 1 day. The values are 1.04, 1.98, 3.17, 4.96, 15.5, 12.9 (×10⁻³ h⁻¹) for soils

- 160 1 to 6, respectively. Both desorption percentages (P_d) and k_{rap} varied greatly among the six soils. It is generally accepted that soils with high soil organic matter (SOM) content possess a large amount of active combination sites, where the combined pollutant is more difficult to desorb. This could be supported by our above data for soil 1 to 5. However, in some cases, other constitutes of soil particles may also play a great effect. Unexpected
- 165 results occurred for soil 6 in this study. Although the 0.3% SOM content in soil 6 is lower than the 0.6% in soil 5, P_d of the two soils at 12th day is almost the same, and k_{rap} of soil 6 was $12.9 \times 10^{-3} h^{-1}$, which is lower than $15.5 \times 10^{-3} h^{-1}$ of soil 5. This phenomenon is likely due to the difference of clay content in these soils. Clay content in soil 6 is 39.5%, whereas those of other soils are below 20%. Nam *et al.* (1998) reported that clay content
- 170 plays a relatively important role in the availability of chemicals in soils when the content of organic carbon is below 2%. To elucidate why clay mineral decreases the bioavailability of pollutants, Alexander (2000) pointed out that there are lots of pores or voids with diameter of 0.3–1.0 nm in aggregates of soil minerals, whose size is in the range of the molecular size of organic pollutants. Once a pollutant molecule enters into the nanopores, it is difficult

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for it to diffuse out. Soils with high clay content possess more nanopores, which leads to 175 slow desorbing rate constant of the pollutant.

2.2. Influence of Aging

After aging for 120d, desorption decreased greatly (Figure 1b), with 12 d- P_d being 2.2, 5.4, 8.4, 10.5, 15.5, and 12.5% in soil 1 to 6, respectively. Based on the data in desorption curve during one day, the k_{rap} in soil 1 to 6 was calculated as 0.53,1.02, 1.57, 1.83, 3.65 180 and 2.30 (×10⁻³ h⁻¹), respectively. 12d- P_d decreased by 67.2–74.6%, and k_{rap} decreased by 48.5–82.2% (Figure 2). Reduction extent of P_d did not vary significantly among the different soils, while the change of k_{rap} decreased with increase of SOM content. This implies that aging plays much more effect on the soils with relatively low organic matter. Soil is a heterogeneous medium, where combination state of molecules of the same pollutant 185 is highly different. It has been postulated that SOM could be regarded as glassy region and rubber region, and diffusion rate constant of pollutant molecules combined in glassy organic matter is markedly slower than those in rubbery (Pignatello and Xing, 1996). Moreover, as described above, pores in clay minerals might also play an important effect on the availability of pollutants in soils. It is plausible to think that molecules entrapped in smaller micro- or 190 nano-pores are less available than those in relative larger macropores (compared with the molecular size of the pollutant). Hence, it is reasonable to attribute the further reduction of availability during aging to that some of the pyrene molecules moved or transferred from those "rapidly desorbing sites" (the rubbery SOM or macropores of the clay minerals) to relatively "slower desorbing sites" (the glassy region of SOM or micro- or nano-pores of the 195 clay minerals). For soils with high SOM, there are lots of "slow desorbing sites," and many of the pollutant molecules are associated with these sites even without aging, and therefore aging effect is not so significant as those in soils with low SOM content, where aging time is needed to allow the pollutant molecules moving from those "rapidly desorbing sites" to those " slower desorpbing sites." 200



Figure 2. Effect of aging on desorption of pyrene P_d is desorption percentage at 12th day; K_{rap} is first order desorbing rate constant during the rapid desorption period of 1 day.

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2.3. Relationship Between Desorption and Bioavailability of Pyrene

When the change of molecular interaction forces is similar during the partition of an organic pollutant between different phases, the partition coefficients are linearly correlated, which is called linear free–energy relationship (LFER) (Schwarzenbach *et al.*, 2003). Hence, it is

- thought that LFER should be exist between Tenax-assisted desorption and earthworm assimilation because the two processes are highly analogous. Both P_d and k_{rap} have been used to predict bioavailability in literature. For example, Lamoureux and Brownawell (1999) found that there was a good linear relationship ($r^2 = 0.78$) between biota-sediment accumulation factors (BSAF) of PAHs and PCBs in sediments and their desorption quantities within 48 h. Kraaij *et al.* (2001) obtained a similarly good linear relationship between the BSAF
- of PAHs to amphipod in sediments and rapidly desorbing rate constant.

In this study, we analyzed whether or not linear relationship exists between P_d , k_{rap} , and bioaccumulation. Biota to soil accumulation factor (BSAF) in the earthworms were reported in literature by Sun and Li (2005), which was obtained through a 10-day accumulation test using the same freshlyamended and aged soils. The BSAF values were 0.052, 0.317, 0.366,

215 using the same freshlyamended and aged soils. The BSAF values were 0.052, 0.317, 0.366, 0.391, 0.452, and 0.418 in freshlyamended soil 1 to 6. When the soils were aged, the values decreased to 0.036, 0.057, 0.083, 0.097, 0.114 and 0.074. After aging, pyrene became less bioavailable. According to the theory of LFER (Schwarzenbach *et al.*, 2003), we analyzed



Figure 3. Correlation relationship between $\ln P_d$, $\ln k_{rap}$, and \ln BSAF of pyrene in freshlyamended and aged soils P_d is desorption percentage at the 12th day; k_{rap} is first order desorbing rate constant during the rapid desorption period of 1 day. The dots in the figures are data observed using different soils; whereas the lines represent correlation relationship, and their corresponding correlation equations are also given.

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the correlations using the logarithm of the three variables (P_d , k_{rap} and BSAF) rather than using the original values, which has been usually used in other studies. Linear relationships 220 between the logarithm of P_d and BSAF, and between the logarithm of k_{rap} and BSAF are shown in Figure 3. It can be seen that a good relationship between $\ln P_d$ and $\ln BSAF$ exists for both freshlyamended and aged soils with the correlation relationship coefficients (r^2) being 0.8953 and 0.8855, respectively. However, for $\ln k_{rap}$, a good relationship only exists for aged soils with r^2 being 0.8762, whereas linear relationship could not be achieved for 225 freshlyamended soils. BSAF did not increase significantly when k_{rap} became larger. We explain this by noting that when k_{rap} is large enough, desorption from soil particles into soil solution loses its limiting effect on bioaccumulation, while bioaccumulation rates are controlled by bio-uptake itself. k_{rap} is a dynamics-relating constant, and BSAF is a thermodynamic partitioning constant, which was acquired at the steady state; a good relationship 230 exists only when desorption is the limiting step for bioaccumulation. After aging, desorption became slow enough to be the limiting step, and a good relationship was found between ln k_{rap} and ln BSAF for aged soils.

3. Conclusion

The properties of soil particles determine combination state of pyrene in soils, and therefore 235 its desorption and bioavailability. SOM is the key factor that desorbing percentage (P_d) and rapid desorbing rate constant (k_{rap}) decreased with increase of SOM content. However, when SOM content is low enough (<1%), the effect of clay took up. SOM and clay hold the pollutant and hinder its desorption by providing active combination sites and nanopores with similar size than that of organic molecules. Moreover, aging significantly reduced the desorption of pyrene in soils. It is reasonable to attribute the further reduction of desorption during aging to some of pyrene molecules moved or transferred from those "rapidly desorbing sites" to relatively "slower desorbing sites."

A logarithmic approach should be used when the linear free energy relationship is studied. The ln P_d correlated well with ln BSAF both in freshlyamended soils and aged 245 soils. Hence, it could be accepted as a predictive approach for bioavailability of pollutants in soils. k_{rap} is more convenient to measure than P_d , however, ln k_{rap} had linear correlation relationship with ln BSAF in aged soils only. This is because, when soils are not aged, the k_{rap} is too large in some soils to be the limiting factor for bioaccumulation.

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