



Assessment of tolerance limits of petroleum residues in soil organic matter: sorption of dichlorobenzene by soil

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Abstract Soil organic matter can protect plants and microorganisms from toxic substances. Beyond the tolerance limit, the toxicity of petroleum pollution to soil organisms may increase rapidly with the increase of petroleum content. However, the method for evaluating the petroleum tolerance limit of soil organic matter (SOM) is still lacking. In this study, the petroleum saturation limit in SOM was first evaluated by the sorption coefficient (K_d) of 1,2-dichlorobenzene (DCB) from water to soils containing different petroleum levels. The sorption isotherm of dichlorobenzene in several petroleum-contaminated soils with different organic matter content and the microbial toxicity test of several petroleum-contaminated soils were determined. It is found that when the petroleum content is about 5% of the soil organic matter content, the sorption of petroleum to organic matter reached saturation limit. When organic matter reaches

petroleum saturation limit, the sorption coefficient of DCB by soil particles increased linearly with the increase of petroleum content ($R^2 > 0.991$). The results provided important insights into the understanding the fate of petroleum pollutants in soil and the analysis of soil toxicity.

Keywords Petroleum-contaminated soil · Petroleum contamination intensity (PCI) · 1,2-dichlorobenzene (DCB) contamination · Petroleum saturation limit · Soil toxicity

Introduction

Pollution by petroleum residues is a worldwide problem at petroleum exploitation sites. Due to their low water solubility, most petroleum components released to the environment are eventually trapped by soil or sediment (Chen et al., 2006; Styrihave et al., 2012). Therefore, the soil/sediment is an important sink of petroleum species. A Monte Carlo simulation method was utilized to assess the total petroleum-hydrocarbon (TPH) content and distribution at the Shengli petroleum field site in China. The results showed that the average TPH was over 2100 mg kg⁻¹ based on multiple receptors and risk sources (Wu & Guo, 2020). The information issued by the Chinese government showed that 23.6% of the farmland soil was contaminated by TPHs near the petroleum field (MEE, 2014). This result is attributed to the high

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volatility and permeability of petroleum hydrocarbons in the water medium, leading to high petroleum dispersion (Karimi & Hamidi, 2021). Due to the limited sorption of petroleum hydrocarbons by soils low in organic matter, the diffusion range of petroleum pollutants extended to the farmland and drinking water source under environmental action, posing a threat to the safety of humans, animals, and plants (Noh et al., 2019; Zemanek et al. 1997). As an important soil component, organic matter could effectively regulate soil nutrients and toxicity, which also provides an effective guarantee for soil bioremediation (Vasudevan et al., 2021). However, some non-volatile or non-degradable groups in soil that have existed for a long time were immobilized by soil organic matter, especially some polycyclic aromatic hydrocarbons, which also weakened the toxicity of residual petroleum to soil organisms (Balaganesh et al., 2021; Vasudevan et al., 2016a). Therefore, many studies were conducted on the association of petroleum pollutants with soils to provide information about the relationship of pollutant migration as impacted by the SOM content and other soil properties (Fu, 2003; Ping & Luo, 2005).

It is known that when the SOM content is more than about 0.1% of the soil dry weight, the SOM becomes the major sorbent for nonionic organic compounds (Schwarzenbach & Westall, 1981). The higher the SOM content, the less toxic is the pollutant in soils (Petenello et al., 2014). But high soil petroleum content can also increase soil toxicity. Shahriari et al. (1998) found that the total dry biomass (roots and shoots) of alfalfa decreased by 60% in petroleum-contaminated soil with 1% petroleum content. The germination rate of seeds was 60% in petroleum-contaminated soil with 5% petroleum content and less than 40% at 7% petroleum content. Ogboghodo et al. (2004) found that the germination rate of maize rapidly decreased to 10% when the soil petroleum content was about 4%. The plant growth indicators are significantly reduced over a certain range of petroleum concentrations. Due to the different SOM contents and plant species involved, the physiological responses of plants to different petroleum contents in soil were also different (Dorn & Salanitro, 2000; Plaza et al., 2005). In general, as the amount of petroleum in the soil increases, the petroleum sorbed to SOM eventually reaches saturation, beyond which an additional

petroleum phase will be formed in soil (Sun & Boyd, 1991). For a soil containing 3.12% SOM, Li et al. (2010) found that when the soil oil level was < 300 mg/kg, the number of soil microorganisms did not change significantly, but when the soil oil level was > 500 mg/kg, the number of soil bacteria and actinomycetes decreased rapidly. There were differences in the petroleum tolerance of soil plants and microorganisms, and some organisms with low bioactivity were more susceptible to petroleum. The petroleum adsorbed by SOM cannot come into contact with soil microorganisms, which can effectively reduce soil biotoxicity. However, when the petroleum reached the saturation limit of SOM, the remaining petroleum can come into contact with soil microorganisms to inactivate them (Spasojevic et al., 2018; Thamaraiselvi et al., 2012).

With the above consideration, the assessment of the toxicity of petroleum-contaminated soil requires a fundamental account of the petroleum tolerance limit of SOM in soil. Currently, there was much research on the effect of SOM on soil toxicity and bioavailability of organic pollutants (Hoang et al., 2021; Kumar et al., 2022; Lin et al., 2022). However, whether soils with different organic matter content (that is, soils with different mineral content) affect the judgment of the petroleum tolerance limit of organic matter needs to be further discussed. This information is especially needed for the petroleum residue because its association and toxic effects on soils depend not only on the petroleum level but also on its state of association with soil matrices (Rasul et al., 2022; Sun et al., 2021). At a trace level, the petroleum may principally adsorb on soil minerals (if the soil is relatively dry) or partition into the SOM (if the soil is hydrated) (Chiou, 2002). At high petroleum contents exceeding the solubility in SOM of a hydrated soil, a separate petroleum phase (SPP) will be formed to greatly increase soil toxicity (Chiou, 2002). This effect calls for an in-depth understanding of how petroleum interacts with soil to get an accurate judgment. In this study, we assess the state of the residual petroleum in the different petroleum-contaminated soils by the changes of the organic-compound sorption to the soil to determine the petroleum tolerance limit of soil organic matter. It will provide a support for bioavailability evaluation of soil petroleum pollutants and the formulation of pollution remediation standards in the future.

1,2-Dichlorobenzene (DCB) is a common organic pollutant in soil and groundwater that may be used to characterize soil sorptive behavior. As a sorbate, the magnitude of its sorption coefficient with petroleum-contaminated soils should vary with the existing state of the petroleum residue in soil. In this study, the sorption isotherms of DCB from water onto the soils with different SOM contents and PCI (Hung et al., 2009 defined PCI as the ratio of petroleum content in soil to organic matter content in natural soil) were measured to yield relevant data related to the petroleum state in soil that influences the petroleum toxicity as indicated by the germination rate of seeds (Ryegrass seeds and Rape seeds) and the soil microbial count.

Materials and methods

Chemicals

1,2-Dichlorobenzene (DCB) was a chromatographic grade (Nanjing Chemical Reagent Co. LTD). The reagents include sodium azide (99%; Tianjin Catalyst Technology Development Co., Ltd, Tianjin, China), and CaCl_2 (Tianjin Zhiyuan Chemical Reagent., Ltd. Tianjin, China).

Preparation of petroleum-contaminated soil

Three uncontaminated soils with different SOM contents were used to prepare the petroleum-contaminated soils. Soil L is collected from pollution-free soil around an oil field, soil M is from farmland, and soil H is a reference peat soil of the International Humic Substances Society (IHSS) from Everglades, FL. Soils L and M are low in organic content while soil H is high in organic content. The petroleum was collected from Karamay petroleum field in Xinjiang. The petroleum was dissolved by a low boiling petroleum ether, filtered out of the mineral residue, and then volatilized the petroleum ether and the light components of the petroleum at room temperature. Three soil samples were crushed to pass through a 0.15 mm mesh sieve after being air-dried naturally and plant residues removed. A series of soil samples were accurately weighed and mixed with low-boiling petroleum ether solutions of varying petroleum content by stirring thoroughly by the drum mixer. NaN_3 was added

to the soil during the solvent volatilization process and kept stirring by the glass rod to ensure a uniform mixing. The petroleum contents in soil groups L, M, and H were set at 0–20 g/kg, 0–65 g/kg, and 0–550 g/kg, respectively. Before the sorption test, the prepared petroleum-contaminated soils were kept in brown glass bottles for three years.

Analyzes of SOM and DCB concentration in the solution

The concentration of DCB in the solution was determined using a GC-ECD (Agilent 6890 GC equipped with a $30\text{ m} \times 0.32\text{ mm} \times 0.25\text{ }\mu\text{m}$ HP-5 fused silica capillary column) based on the previous report (Zhang et al. 2013). Analytical conditions were described as follows: Oven temperature: 25 °C; transmission tube temperature: 100 °C; injection volume: 10 μL . To determine DCB, N_2 was used as carrier gas at a flow rate of 2 mL/min. The column temperature was programmed as follows: an initial 2 min at 60 °C, heated to 180 °C at 20 °C/min (holding for 2 min), then heated at 30 °C/min to 250 °C (hold for 3 min). The injection port and detector temperatures were 220 °C and 280 °C, respectively.

The content of organic matter in soil was determined by the $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method (Gerenfes et al., 2022). When the soil was weathered, the total carbon content of natural soil and petroleum-contaminated soil was determined by the carbon and sulfur analyzer (LECO CS-344).

Sorption isotherms

0.200 g of non-contaminated or petroleum-contaminated soil was weighed and added to the 25–150 mL sample bottles. Then 10.0 mL of 0.01 mol L^{-1} CaCl_2 aqueous solution containing 400 mg L^{-1} NaN_3 were added to the sample bottles. Initial DCB concentrations ranged from 30 to 100 mg L^{-1} prepared by adding small amounts of the DCB stock solution in ethanol (volume <0.2%) to the sample bottle. Each isotherm consisted of 5–6 points, and each point was run in duplicate and each data point was the average of three values (Tang et al., 2022; Qian et al., 2011; Zhuang et al., 2016). The bottles were sealed and shaken at 120 rpm and 25 °C for 48 h (Kile et al., 1995a, 1995b). After reaching sorption equilibrium, DCB in the headspace air phase was measured with

a GC, and then the DCB concentration in solution was calculated with a standard curve corrected for the DCB loss to the headspace using the established Henry's law equation. The soil and water ratio used for groups L and M was 1:50 and that for group H was 1:250. The sorption isotherm was drawn based on the amount (Q) of DCB sorbed to the uncontaminated soil or petroleum-contaminated soil and the corresponding DCB residue (C_e) in the solution.

In order to determine the recovery rate, the blank experiment was performed by adding DCB at different concentrations to the same sample bottles without soil. Experimental errors are corrected by Henry's law.

Determination of plant seed germination percentage and microbial biomass

The tenfold dilution for the soil sample was prepared to start with 90 mL of sterilized phosphate buffered saline added to 10.0 g of soil sample and the contents stirred for 30 min. The phosphate buffered saline was inoculated into LB medium with inoculating ring and shaken at 180 rpm and 36 °C for 18 h. LB medium was centrifuged and the centrifuged bacteria were diluted with glucose inorganic salt medium to prepare a bacterial solution with $OD_{600}=0.5$ (Bhuyan and Pandey 2022).

The non-polluted sandy soil sterilized by high-pressure steam sterilization (126 °C /30 min) was used to prepare the soil with different PCIs (4%, 5%, 6%, and 7%) in the sterile room. When all soils were sealed and frozen for half a year, 100 g of soil with different PCIs was placed in each petri dish for observation of soil microbial biomass and plant germination rate, respectively. The bacterial solution with $OD_{600}=0.5$ was added to different soils with different PCIs to keep 70% of the soil-saturated water content. After culturing each experimental group for 48 h, 5 g soil was added into 95 mL normal saline to shock for 30 min. 1 mL supernatant was diluted step by step to 10^{-7} , and the soil microbial biomass was determined using the plate counting method as described by Patowary et al. (2018). Ryegrass seed (*Lolium perenne* L.) and rape seed (*Brassica napus*) were selected as experimental subjects. 20 plant seeds with a germination rate greater than 95% were planted in soils with different PCIs, and the germination status of soil plants was evaluated after 5 days. Plant germination

Table 1 Analysis of petroleum composition

Density (20 °C) g/cm ³	Petroleum hydrocarbon, %	Pectin, %	Asphalt, %	Wax, %	Organic carbon, %
0.83	83.3	10.0	0.26	5.3	81.2%

Table 2 The soils properties

Soil type	Sand, %	Silt, %	Clay, %	f_{om} , %	f_{oms} , %
L ₀	55.4	27.7	16.9	0.55	0.33
M ₀	38.5	31.7	29.8	1.11	1.11
H ₀	–	–	–	85.00	84.49

f_{om} , represents the organic matter content in natural soil; f_{oms} , represents the SOM content after the addition of organic solvents

rate is the ratio of the germinating number of plant seeds to the total number of plant seeds (Guo et al., 2022). Three parallel experiments were set up for each experimental group, and water was supplemented to each experimental group every 8 h.

Results and discussion

The SOM content of uncontaminated soil

Table 1 listed the basic physical and chemical properties of petroleum. The physical properties of three soils with different organic content are listed in Table 2. The petroleum content and organic carbon content of soils are listed in Table 3. The soil in group L is a sandy clay loam with f_{om} of 0.55%, in group M is a silty clay with f_{om} of 1.11%, and in group H is a peat with f_{om} of 85%. The higher the SOM content, the more organic pollutants can be adsorbed and fixed by the soil. When the partition of petroleum into SOM reached tolerance limit, additional petroleum could be coated on the soil mineral particles to form a petroleum thin film (Hakim et al., 2017), which was called the separated petroleum phase (SPP). The mechanism of petroleum adsorption on the porous surface of minerals includes capillary action connected with filling the available pores and oily layer (film) formation on the external surface and around the adsorbent

Table 3 The petroleum content and organic carbon content in each experimental group

Soil classification	Experimental group	C_p , g/kg	PCI, %	f_{oc} , %
L	L ₀	0	0.00	0.32
	L ₁	0.10	3.03	0.19
	L ₂	0.34	10.30	0.21
	L ₃	1.10	33.33	0.25
	L ₄	1.90	57.58	0.41
	L ₅	4.99	151.21	0.79
	L ₆	15.2	460.61	0.94
M	M ₀	0	0.00	0.64
	M ₁	0.57	5.18	0.66
	M ₂	1.65	15.00	0.68
	M ₃	2.51	22.82	0.77
	M ₄	11.23	102.09	0.84
	M ₅	25.46	231.45	1.29
	M ₆	50.41	458.27	2.57
H	H ₀	0	0.00	49.30
	H ₁	8.8	1.04	49.92
	H ₂	33.2	3.93	51.14
	H ₃	43.4	5.14	51.9
	H ₄	172.8	20.45	59.31
	H ₅	430.6	50.96	74.14

C_p , petroleum content of soil; f_{oc} , organic carbon content of the soil; PCI, petroleum-contaminated intensity

grains, but petroleum substances cannot penetrate into narrow micropores of mineral adsorbents (Bandura et al., 2017; Vasudevan et al., 2016b). Polar sites on the mineral surface caused petroleum to diffuse as much as possible to the mineral surface. It was found that some dissolved organic matter coated on the surface of soil minerals to form a compact structure, which weakened the sorption of organic matter for foreign hydrophobic organic compounds (Murphy et al., 1990; Liu et al., 2008; Wang & Xing, 2005). Therefore, it was shown that a small amount of petroleum coated on the mineral surface might also form a compact petroleum film, which weakened the partition of DCB in the petroleum film. But as the petroleum content increased, excess petroleum continued to coat the petroleum film, resulting in weak interaction between the outer petroleum film and the mineral surface. Therefore, the more petroleum adsorbed on the surface of the mineral, the easier it was to migrate in the soil and poison soil organisms.

The sorption of DCB to the SOM with petroleum

As indicated in Table 4, the sorption isotherms of DCB on the three natural soils and petroleum-contaminated soils followed the linear sorption model ($R^2=0.92-0.99$), which was the same as the adsorption model of DCB in PCB-contaminated soil by Sun and Boyd (1991). The K_{oc} values of the three natural soils were analyzed ($K_{ocL}=760$, $K_{ocM}=1600$, $K_{ocH}=330$), and the variation coefficient of the mean K_{oc} was 58.8%, which was more than 50% (Standard test method for determining a sorption constant (K_{oc}) for an organic chemical in soil and sediments HJ-201). It was because the organic carbon of standard organic soil (H soil) was higher (49.92%), which was also not available in conventional soil. Grathwohl (1990) found that due to their origin and geological history, organic matter differs in composition and structure, which had a significant impact on the sorption affinity for nonionic organic molecules. Therefore, higher organic matter content may lead to structural differences of organic matter and affect the adsorption capacity of organic compounds. Considering the low polarity of DCB, one expects DCB to associate readily with the petroleum components in petroleum-contaminated soil (Schaeffer & Anke, 2001). However, the K_d values of DCB with three soils at low PCIs were not closely related to the PCI (Fig. 1). This result may reflect the different SOM-petroleum mixed phases at different PCIs. When the petroleum contents in soil exceed certain values (presumably, the petroleum solubility limits in SOM), the fitted DCB sorption coefficients and the petroleum contents conformed to a close linear relation. This suggests that the sorption of DCB is more favorable with the excess petroleum phase than with the SOM phase.

The sorption equations are listed in Table 4. Going from L₀ to L₁, the small decrease in K_d was caused possibly by the loss of some unstable SOM components upon their contact with solvents during the sample preparation, and there was practically no loss of organic components in groups M and H (Table 3). The K_d of PAH in fresh soil with low petroleum content did not change significantly with the petroleum content in the experiment of Jonker et al. (2003), but the sorption trend of PAH in petroleum-contaminated soil aged for 27 months was consistent with the sorption trend of DCB of this experiment. This also

Table 4 Isothermal sorption of DCB in petroleum-contaminated soil and uncontaminated soil

Soil types	Samples	PCI (%)	Regression equation C_e (mg/L), Q (mg/kg)	Sorption coefficient (K_d)	Correlation coefficient (R^2)
S	L ₀	0.00	$Q=2.34 C_e+0.66$	2.34	0.96
	L ₁	3.03	$Q=2.01 C_e-1.91$	2.01	0.98
	L ₂	10.30	$Q=2.34 C_e-0.86$	2.34	0.98
	L ₃	33.33	$Q=2.47 C_e-2.53$	2.47	0.98
	L ₄	57.58	$Q=4.56 C_e+0.13$	4.56	0.97
	L ₅	151.21	$Q=11.98 C_e-6.33$	11.98	0.92
	L ₆	460.61	$Q=31.37 C_e+13.86$	31.37	0.92
B	M ₀	0.00	$Q=10.26 C_e+0.17$	10.26	0.97
	M ₁	5.18	$Q=12.01 C_e-5.35$	12.01	0.99
	M ₂	15.00	$Q=12.38 C_e-5.44$	12.38	0.92
	M ₃	22.82	$Q=14.20 C_e-4.5$	14.20	0.96
	M ₄	102.09	$Q=26.54 C_e-4.2$	26.54	0.96
	M ₅	231.45	$Q=52.20 C_e-4.56$	52.20	0.96
	M ₆	458.27	$Q=81.93 C_e+7.15$	81.93	0.99
H	H ₀	0.00	$Q=161.67 C_e+1.46$	161.67	0.98
	H ₁	1.04	$Q=172.62 C_e+17.63$	172.62	0.97
	H ₂	3.93	$Q=233.10 C_e+15.36$	233.10	0.94
	H ₃	5.14	$Q=299.41 C_e+22.95$	299.41	0.97
	H ₄	20.45	$Q=499.60 C_e-9.40$	499.60	0.98
	H ₅	50.96	$Q=775.56 C_e+0.93$	775.56	0.97

C_e , DCB content present in solution in soil–water system; Q , the amount of DCB adsorbed by soil in soil–water system

indicated that the petroleum in fully aged oil-contaminated soil had entered the organic matter, which also ensured the accurate analysis of subsequent studies on the behavior of petroleum in the soil (Jonker et al., 2003; Sun & Boyd, 1991). When petroleum was released into the soils, it took more than a few months to achieve dynamic equilibrium in the SOM–mineral system (Xing et al., 1996; Weber & Huang, 1996). When the soil is aged for enough time, the petroleum entering the SOM can change the sorption capacity of the SOM, which leads to no linear relationship between K_d value of DCB and PCI in the soil with low PCI.

In L₁–L₆, M₀–M₆, and H₀–H₅, a similar trend in K_d with the petroleum content indicated that DCB sorption to low petroleum levels in soils was also normal. When the PCIs of L₁ soil increased from 3.03% to 10.30% (L₂), the DCB K_d increased rapidly from 2.01 to 2.3. But the PCI of L₂ soil increased from 10.30% to 33.33% (L₃), the DCB K_d increased slowly from 2.3 to 2.4. When the PCI increased to 151.21% for L₅, the DCB K_d was 5.96 times that of L₁, indicating that the partition effect of petroleum on DCB

is greater than that of SOM. Compared with L₁, the petroleum content of L₂ and L₃ increased by 10 and 19 times, and the K_d only increased by 29% and 37%, respectively. However, petroleum and DCB, as non-polar compounds, also have competitive effects on partition into the SOM (Lu & Pignatello, 2004). The petroleum occupied the sorption site of SOM, resulting in the reduction of the DCB partition. Compared with L₁–L₂ and L₃–L₆, the increase of petroleum content in L₂–L₃ did not effectively improve the partition of DCB on soil (Fig. 1-L). It was inferred that the sorption of petroleum in SOM may reach tolerance limit in the L₂ soil, and the partition of DCB in a small amount of petroleum coated on minerals may be weaker than that of SOM and petroleum in organic matter.

Compared to the M₀ soil, the K_d value of the M₁ soil increased by about 10% with an increase of PCI from 0 to 5.18%. For M₂ with a PCI 2.9 times that of M₁, the K_d was only 3.08% higher than that of M₁ soil, which was similar to the phenomenon of L₂–L₃. However, in group H, as the PCI increased from 0 (H₀) to 1.04% (H₁), 3.93% (H₂), and 5.14%

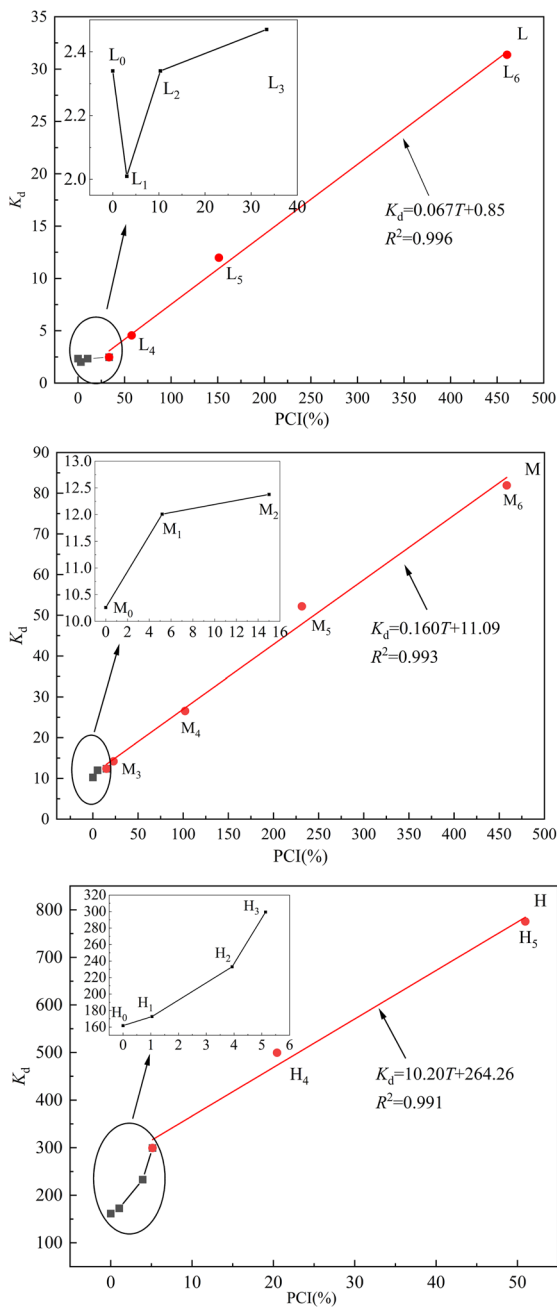


Fig. 1 The DCB sorption coefficient (K_d) versus the PCI (T) in soil. L, M, and H represent soils with three different organic matter contents from low to high. PCI represents petroleum-contaminated intensity (petroleum content as a percentage of soil organic matter content). T represents the PCI value

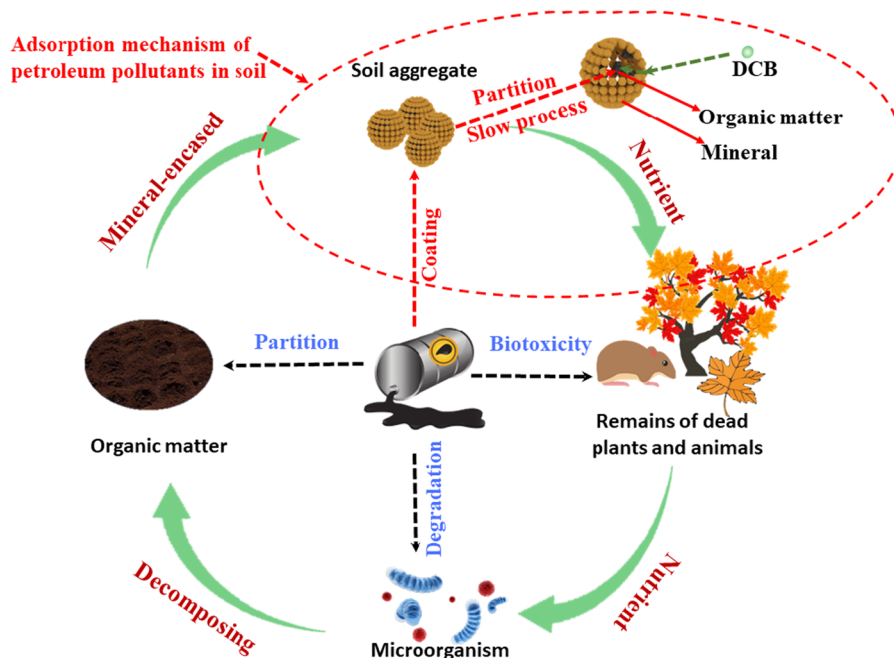
(H_3), the K_d increased by 6.77%, 44.18%, and 85.3%, respectively. With the increase of petroleum content, the increasing rate of K_d increases gradually, but

the contribution of organic matter to K_d gradually decreased, while the contribution of petroleum partition into SOM to K_d increased significantly (built-in Fig. 1-H), which indicated again that petroleum in the organic matter has significant sorption capacity for nonpolar DCB.

The sorption behavior of petroleum in soil

In the soil with low PCI, the partition law of DCB was mainly related to the sorption mechanism of SOM to coexisting organic compounds. The fate of soil organic matter and the sorption mechanism of the pollutants to soil organic matter is shown in Fig. 2. Organic matter that exists in soil for a long time is oxidized and decomposed by microorganisms into more stable organic matter (Aber et al., 1990; Hedges et al., 2001). Organic matter is mainly composed of aromatic compounds, aliphatic carbon, and some oxygen-containing functional groups, which polymerize through hydrogen bonds to form micro-polymers with a three-dimensional structure (Mccarthy et al., 1989; Mott, 2002). However, the remains of plants and animals can be decomposed by soil microorganisms into thousands of organic matter molecules, which were then adsorbed or encased in soil aggregates to prevent further degradation by microorganisms (Ma et al., 2018; Mikutta et al., 2006; Six et al., 2002). Organic matter adsorbed in soil aggregates can further adsorb petroleum coated on the surface of mineral particles, and this process may last for more than a few months before the sorption of petroleum in the SOM is saturated (Xing et al., 1996; Weber & Huang, 1996). In soil aggregate-water environments, oxygen-containing functional groups in organic matter are arranged outside the micropolymer to form a hydrophilic layer, while the inner space formed a hydrophobic layer (Wang et al., 2011). The hydrophobic layer provided a favorable environment for the partition of some hydrophobic organic compounds. When petroleum molecules entered the inner space of micropolymer and were sorbed on the sorption site, the partition of DCB in organic matter was reduced (Fei et al., 2017). Petroleum sorbed into the inner space of micropolymer can also adsorb other low molecular organic compounds (Gong et al., 2017; Jonker et al., 2003). With the increase of the petroleum content, more petroleum entered the micropolymer, and the sorption site of organic matter is occupied by petroleum, which

Fig. 2 Fate of soil organic matter and the sorption mechanism of the pollutants to soil organic matter



limited the partition of DCB in organic matter (L_2 , M_1 and H_3). At this time, SOM has reached petroleum tolerance limit, and the sorption of DCB in the petroleum partitioned into the organic matter reached the maximum. Since SOM and petroleum adsorbed by SOM can adsorb jointly DCB, the sorption rate of DCB increased significantly in the L_1 – L_2 and M_1 – M_2 . Therefore, effective measures can be taken to improve the fixation of oil pollutants by increasing the content of soil organic matter in some areas easily polluted by petroleum, which can increase the sorption and fixation of non-petroleum-saturated SOM to other pollutants, and weaken the diffusion of organic pollutants. Some studies have found that with the increase of SOM content, SOM can fix more petroleum pollutants to reduce the toxic effect of petroleum on soil microorganisms, which also increases the degradation half-life of soil petroleum (Agamuthu et al., 2013; Chen et al., 2019; Yang et al., 2011).

The sorption of DCB in SPP

With the continued increase of petroleum content, after the organic matter was saturated with petroleum (L_2 , M_1 and H_3), additional petroleum can be coated on the soil mineral particles to form a layer of petroleum thin film (Hakim et al., 2017). Therefore,

when a small amount of petroleum was coated on the mineral surface to form a dense petroleum film and weakened the sorption of DCB, which caused the increase rate of K_d value to slow down at L_2 – L_3 and M_1 – M_2 .

With the continuous increase of petroleum content in the soil, the petroleum film coated on the soil mineral particles gradually developed from a single layer to a multi-layer. With the increase of petroleum film thickness, the sorption effect of mineral particles on the outer petroleum film was weakened. In L_3 – L_6 , the K_d value of DCB was linearly correlated with PCI, with a slope of 0.067 and a correlation coefficient greater than 0.996. A similar linear relationship was found between PCI and soil K_d values between groups L, M, and H (Fig. 1). The slopes of the correlation equations in group M and group H were 0.160 and 10.20, and the correlation coefficients were 0.993 and 0.991, respectively. It was inferred that the sorption of DCB in petroleum coated on the surface of soil particles was stable. This may be because the sorption equation of petroleum on the mineral surface is also linear (Shen & Jaffe, 2000), and the dissolution of DCB in petroleum was the same as the dissolution effect between non-polar compounds. The sorption isotherms of many compounds (biphenyls, toluene, PAHs, and chlorobenzenes) on soil minerals are also

linear (Li et al., 2020; Mader et al., 1997; Rheinländer et al., 1998). Therefore, when the content of one organic contaminant is high in the soil, it may have a good solubilization effect on foreign organic contaminants, even if there are competitive effects between them in SOM. The particle size of soil minerals also affected the adsorption of organic compounds on the mineral surface, and there were also density differences in the sorption layer formed by the same organic pollutants on the surface of different minerals (Pozhilenkova et al., 2004), which also affected the degradation of petroleum coated mineral surface by microorganisms (Ugochukwu et al., 2014).

Analysis of petroleum tolerance limits in SOM

The formation of SPP increased the exposure of soil organisms in petroleum, which can increase the ecological risk of the soil. The group L soil can form SPP at very low petroleum content (Table 4 and Fig. 1-L). However, in the case of the same petroleum content, the SOM could not be saturated by the petroleum in group M and group H. Therefore, compared with the petroleum content, the PCI was a better index to evaluate the occurrence of SPP.

Based on the above results and discussion, the existing states of petroleum can be decided by the change of DCB sorption coefficient (K_d) in soil with PCI (Fig. 1). The relationship of DCB K_d with PCI in the soil–water system was divided into three stages. The first stage was for the DCB partition to the SOM, which included L_1 – L_2 , M_0 – M_1 and H_0 – H_3 systems. The second stage was a transition state, in which DCB was partitioned to the SOM–petroleum mixed (L_2 – L_3 , M_1 – M_2). In the third stage, DCB was partitioned predominantly to the excess SPP phase (L_3 – L_6 , M_2 – M_6 , and H_3 – H_5), in which the K_d exhibited a good linear relation to the PCI.

When the PCI of soil ranged from 10.03% (L_2) to 33.33% (L_3) in group L, the sorption mechanism of DCB was the dissolution of DCB into SPP thin film coated on mineral particles (Fig. 1-L). The same phenomenon was also observed in group M with PCI of 5.18% (M_1) to 15% (M_2) (the embedment graph of Fig. 1-M). However, in group H with low mineral content, when SOM reached the petroleum tolerance limit, the effect of SPP thin film coated on mineral particles on DCB sorption was much less than that of DCB sorption in oily organic matter, which can more

accurately judge the petroleum tolerance limit state of organic matter. It was calculated that the petroleum tolerance limit content of organic matter was about 5% of the SOM content in group H (PCI=5.14%). When SOM reached petroleum tolerance limit (L_2) in group L, the PCI of soil was about 10% (10.3%). When SOM reached petroleum tolerance limit (M_1) in group M, the PCI of soil was about 5% (5.18%). Chen et al. (2008) found that when the petroleum content was 1 g/kg in the soil, the separate petroleum phases that appeared on the surface of mineral particles could be detected by FTIR. Jonker and Barendregt (2006) found that when the petroleum content was 1–3 g/kg in the soil, the separate petroleum phases formed on the surface of mineral particles could be observed by microscope. The organic matter content of these two soils was 1.56% and 1.41% respectively under uncontaminated conditions. When SPP was present, the sorption coefficient (K_d) of foreign organic pollutants also showed a linear relationship with petroleum content. Through the calculation of their literature data, it was found that the PCI values were 6% and 7% respectively, which were close to the calculated result (5%) of this investigation. In general, petroleum adsorbed on mineral particles loses more than petroleum partition in organic matter in the environment (Hunt, 1991). After the difference value of organic carbon content in three experimental groups (L_1 and L_2), (H_1 and H_2) and (H_2 and H_3) to calculate the petroleum content in organic matter (organic carbon content of petroleum is 81.2%), the loss rate of petroleum in organic matter is -2.6%, 39% and 7.8%, respectively. The petroleum content calculated based on the difference value of soil organic carbon content (H_1 and H_2) is significantly different from the actual petroleum content and higher than the petroleum loss rate on soil particles. It may be caused by the large difference in soil petroleum content and organic matter content, and the calculated petroleum loss rate is smaller (7.8%) with the increase of petroleum content. It also indicates that the petroleum adsorbed in soil organic matter in this experiment is less lost during the aging process.

However, when SOM reached petroleum tolerance limit, PCI was 10.3% (L_2) in group L, which was significantly different from the experimental results. This may be caused by the loss of SOM, which also indicates that the composition of SOM in group L is unstable (Table 2). It is because there

were no significant differences between the K_{oc} values of residual SOM in stable natural soil environments (Kile et al., 1995a, 1995b; Lu & Pignatello, 2004). Combined with this study, it is indicated that organic matter in a long-term stable (no-till) environment does not have much difference in the sorption of organic pollutants despite its complex composition. On the other hand, the SOM content of group L was low, which made the change of SOM have a great influence on the judgment of the organic matter tolerance limit. When the content of soil organic matter was less than 0.1%, the adsorption of foreign pollutants to soil organic matter was affected significantly by mineral particles, which might make it difficult to judge accurately the tolerance limit of organic matter (Schwarzenbach & Westall, 1981). Therefore, in some unstable natural environments, the variation and low content of organic matter may affect the judgment of the petroleum tolerance limit of organic matter.

The petroleum pollutants entered the soils and volatilized the low components petroleum to reach the stable initial pollution concentration under the action of the environment. According to the GC–MS diagram (Fig. 3) of petroleum (N) and petroleum in aged soils (O), It can be found that while most petroleum components are largely present, they all have losses. (Fig. 3-O). This paper analyzed the loss of soil petroleum through the difference between petroleum content (ΔC_p) and organic carbon content (Δf_{pc}) of petroleum-contaminated

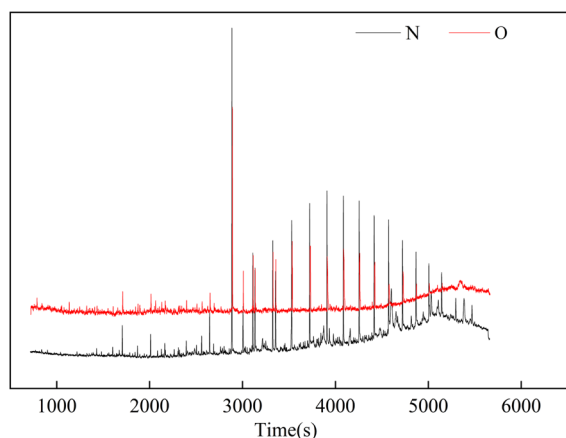


Fig. 3 GC–MS diagram of unaged petroleum (N) and petroleum (O) in aged soil

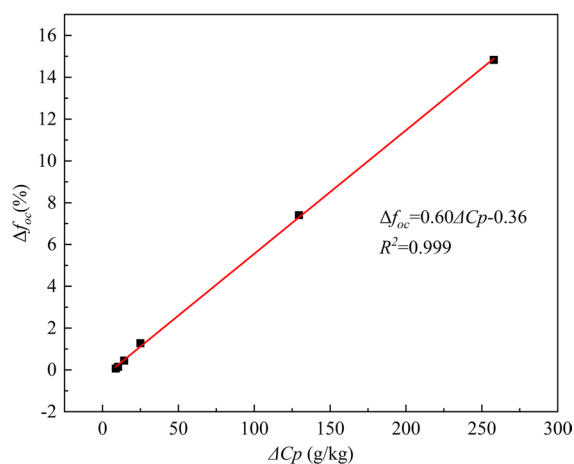


Fig. 4 Relation between gradient difference of petroleum content and gradient difference of organic matter content in petroleum-contaminated soils

soils. Figure 4 was drawn according to Table 5, and its linear equation was $\Delta f_{pc} = 0.60\Delta C_p - 0.36$, $R^2 = 0.999$. When the soil petroleum content is greater than 2 g/kg, a linear relationship appeared between the measured values of organic carbon and petroleum content in the three soils (Fig. 4). The organic carbon content of petroleum was 81.2%, i.e., $\Delta f_{pc} = 0.812\Delta C_p$. Thus, the petroleum loss on the surface of soil mineral particles was 21.2%. When soil petroleum content was low, petroleum loss (organic carbon content) was easily affected by soil organic matter and mineral particles. It

Table 5 The gradient difference of petroleum content (ΔC_p) and difference of organic matter content (Δf_{oc}) in petroleum-contaminated soils

Samples	C_p , g/kg	f_{oc} , %	ΔC_p , g/kg	Δf_{oc} , %
L ₅	4.99	0.79	–	–
L ₆	15.2	0.94	10.21	0.15
M ₃	2.51	0.77	–	–
M ₄	11.23	0.84	8.72	0.07
M ₅	25.46	1.29	14.23	0.45
M ₆	50.41	2.57	24.95	1.28
H ₃	43.4	51.9	–	–
H ₄	172.8	59.31	129.4	7.41
H ₅	430.6	74.14	257.8	14.83

Δf_{oc} , the difference in organic carbon content between soils with different petroleum content; ΔC_p , the difference in petroleum content between soils with different petroleum content

was because SOM can effectively fix petroleum to reduce loss and microbial degradation of the petroleum. A small amount of petroleum adsorbed on the surface of mineral particles can also be subjected to the force of the mineral surface (Chen et al., 2015; Hassett & Anderson, 1982; Tang et al., 2012).

It is well-known that petroleum has a slow dynamic sorption equilibrium process between the SOM and the surface of mineral particles. When the volatile petroleum components were lost in the organic matter, the petroleum on the mineral surface could be adsorbed by the SOM to make SOM reach constantly petroleum a tolerance limit (Liu et al., 2012). Studies show that organic matter plays a leading role in the adsorption of DCB by the soil when the content of petroleum in the soil is low, which also leads to the nonlinear adsorption of organic matter to petroleum (Vasudevan et al., 2015, 2016c). It also showed that the petroleum is fixed in the organic matter, and finally reached a dynamic adsorption equilibrium. However, some of the light components that enter the organic matter may also volatilize over time, but some residual persistent organic components can re-enter the organic matter (Vasudevan et al., 2016a). Therefore, there is always a dynamic equilibrium relationship between organic matter and petroleum in soil. Therefore, some light components of petroleum adsorbed by SOM were lost due to volatilization, and the petroleum pollutants adsorbed on minerals could also be redistributed to SOM by slow dynamic adsorption in the practical contaminated sites, which did not affect the saturated adsorption of organic matter on soil petroleum. Microorganisms can also degrade some petroleum components on the surface of mineral particles without affecting the petroleum present in the SOM. When petroleum content exceeds 5% of SOM content, SPP can be preferentially degraded by microorganisms while petroleum adsorbed by the SOM cannot be affected by microorganisms. The petroleum tolerance limit of SOM (5%) can be used to analyze the biological toxicity and the biological availability of soil petroleum pollutants, and effective measures can be taken to restore soil ecology in time. When petroleum pollutants exceed 5% of SOM content, some petroleum bacteria can be added to degrade petroleum pollutants and restore soil ecology.

Effects of PCI on soil plants and microorganisms

Initially, the petroleum entered the hydrated soil by partition into the SOM, which reduced the toxic effect of petroleum on soil organisms (Meki et al., 2022). Therefore, the petroleum content is less accurate than PCI to assess soil toxicity. It was found that when PCI exceeded 5%, a separate SPP in soil was gradually formed. The free petroleum phase can travel into the vadose zone and will reach the groundwater or will be redistributed along the unsaturated zone, which can be toxic to more soil plants and microbes (Dror et al., 2002; Li et al., 2023). In this experiment, rape seed (*Brassica napus*) and Ryegrass seed (*Lolium perenne* L.) were cultured in soil with different PCIs, and their germination rates were measured (Fig. 5). At 4–7% PCIs, the germination rates of rape seed (*Brassica napus*) and Ryegrass seed (*Lolium perenne*

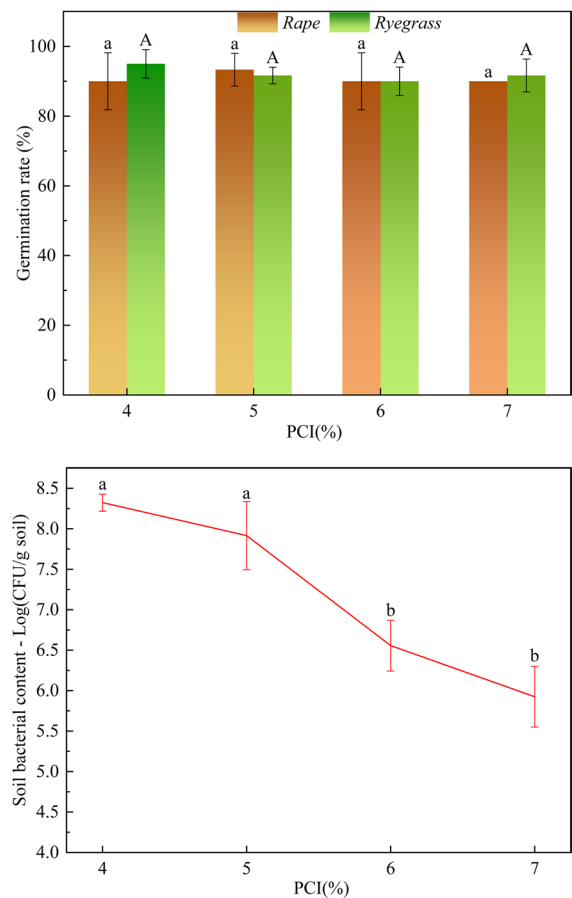


Fig. 5 Changes of bacterial number and plant germination rate at different PCIs

L.) were more than 90%, and no significant difference was noted among the groups ($P > 0.05$), which also indicated that a small amount of petroleum had a weak effect on the growth of plant seeds. Therefore, there may be a large error in judging soil toxicity by plant germination rate. However, there were differences in the number of microorganisms added to soil groups with different PCIs. The detected bacteria were around $\text{Log CFU/dry soil} = 8$ in the soil groups of 4–5% PCI, and $\text{Log CFU/dry soil} = 6.5$ in groups of 6–7% PCI. There was a significant difference in microbial number between the first two soil groups (PCI=4% and PCI=5%) and the last two soil groups (PCI=4% and PCI=5%) ($P < 0.05$). This suggested that the toxicity of soil to microorganisms increased rapidly after $\text{PCI} > 5\%$ when the SPP began to form. Chaineau et al. (2003) showed that when 18000 mg/kg petroleum-contaminated soil (organic carbon content of natural soil was 11%) was repaired for about 90 days, the number of petroleum bacteria in the soil decreased, and the petroleum content in the petroleum-contaminated soil was about 9000 mg/kg (T2). By calculation, the petroleum content is about 8.2% of the soil organic carbon. According to the relationship between soil organic carbon and organic matter content (Paramanathan et al., 2018; Schumacher, 2002): $f_{\text{om}} = f_{\text{oc}} \times 1.724$, we found that the petroleum content was about 5% of the soil organic matter content, which further verified the petroleum tolerance limit of soil organic matter.

Nonionic pollutants have been reported to be sorbed to soil organic matter by H-bonding, van der Waals forces and charge-transfer complexes, which makes it difficult for the pollutants sorbed by SOM to be desorbed and come into contact with microorganisms (Novak et al., 1995; Petenello et al., 2014). But when small amounts of pollutants were adsorbed on soil particles, which made pollutants direct contact with microorganisms to kill them. According to the literature, when the soil PCI exceeded 5% (430 mg/kg), the relative inhibition rate of soil to microorganisms exceeded 50%. When the soil petroleum content reached 1 g/kg, the inhibition rate of soil to microorganisms reached 100% (Tang et al., 2011), and the inhibition rate of soil microorganisms increased rapidly from 25 to 65% when the petroleum content increased from 300 to 500 mg/kg, which indicated that the sensitivity of soil bacteria number changes to determine soil toxicity is high. Some

petroleum-degrading bacteria may have a high tolerance to petroleum. At a low petroleum content of 1–3%, many added petroleum-degrading bacteria can also effectively degrade the petroleum in soil (Fan et al., 2013; Xu & Lu, 2010). Therefore, we can add petroleum-degrading bacteria to the soil to reduce the soil PCI below 5% in some areas susceptible to organic pollutants, which can protect effectively the soil microbial diversity.

This paper investigated the sorption of DCB in petroleum-contaminated soil with different properties and relevant literature data, which showed that the petroleum tolerance limit of SOM in long-term stable petroleum-contaminated soil is about 5% of the petroleum content to organic matter. It was of great significance for evaluating the migration of soil petroleum pollutants and the risk of soil pollution. In China, the soil environmental quality risk control standard for developed lands (GB 36600-2018) is determined by assessing the migration of pollutants in soil and the risk of pollutants to human health. According to the standard (GB 36600-2018), the screening value of petroleum hydrocarbons for residential areas and parklands is 826 mg/kg, and for industrial, commercial and plaza lands is 4500 mg/kg. It is considered that the health risk to humans may be ignored if the petroleum hydrocarbon levels in the soil are equal to or below the risk screening values. In this study, petroleum contents in S_1 (100 mg/kg), L_2 (340 mg/kg), and M_1 (570 mg/kg) soil were far below the risk screening values of contaminated soil either for residential and parklands or for industrial lands, falling to the low health risk. In L_2 (1100 mg/kg) and M_2 (1650 mg/kg), a small amount of petroleum coated the mineral particles to form petroleum thin films after SOM was saturated with petroleum. The interaction between mineral particles and petroleum film prevented the petroleum from migrating and spreading in the soil, reducing the health risk of the soil. The presence of SOM can reduce the toxic effect of soil organic compounds, so we can deduct the sorption of petroleum to organic matter. L_3-L_2 (1100–340) mg/kg = 760 mg/kg and M_1-M_2 (1650–570) mg/kg = 1080 mg/kg were very close to the screening value (826 mg/kg), which provided an effective explanation for soil risk control standards. However, the risk assessment of soil quality in some areas is based on the total soil contamination concentration, but Brand et al. (2013) found that risk

assessment based on (measured) total concentrations may lead to an inaccurate assessment of the actual risk, and the impact of the soil bioavailability components on soil ecosystems needed to be considered, and it was recommended to improve the accuracy of soil quality assessments by considering bioavailability in (higher level) risk assessments. Stokes et al. (2005) also found that compared to the total concentration of contaminant present in the soil, the bioavailable fraction is key to evaluating soil quality. Therefore, the petroleum tolerance limit of SOM (PCI \approx 5%) was determined, which can provide a theoretical support for the assessment of bioavailability in the field of petroleum-contaminated soil remediation.

Conclusions

The SOM can adsorb petroleum components and other organic contaminants, and the SOM can reduce significantly the toxic effect of petroleum before its tolerance level in SOM. In this study, the critical effect of the petroleum tolerance limit with SOM was well demonstrated by the DCB sorption data with different soils over a good range of petroleum contamination levels. When the petroleum content exceeded the tolerance limit in SOM (PCI > 5%), the extra petroleum coated on mineral surfaces was in contact with soil microorganisms, which can increase significantly soil toxicity. This index may have errors in judging the petroleum tolerance limit of organic matter in some petroleum-contaminated soil with low organic matter content (SOM contents < 0.1%) and incomplete aging organic matter. When soil PCI was greater than 5%, the number of microorganisms had a significant downward trend, which demonstrated the critical effect of SOM on the petroleum tolerance limit. This investigation can provide a guidance for the formulation of remediation standards for soil petroleum pollution and the evaluation of soil petroleum bioavailability.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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