

Supplementary Material

Ecological safety thresholds for phenanthrene in Chinese soils: implications for assessing ecological risk to vegetation and for land use

Jiahui Zhu,¹ Qian Yang,¹ Jiawei Wang, Xuke Wang, Shuilin Zhu, Xinhua Zhan*

College of Resources and Environmental Sciences, Nanjing Agricultural University,
Nanjing, Jiangsu Province, 210095, People's Republic of China

¹The two authors contributed equally in this paper.

* Corresponding author: Dr. Xinhua Zhan

Tel: +86-25-84395210

E-mail: xhzhan@njau.edu.cn

The supporting information contains:

-40 pages (S1-S40), -1 text (Text S1) -7 tables (Tables S1-S7), -22 figures (Fig. S1-S23)

Text S1 Toxicity data screening principle

Literature collection was carried out based on the following requirements:

- (1) The phenanthrene contamination resulted from exogenous addition, and the relevant soil properties were documented.
- (2) The exposure time of tested organisms to soil contaminants and the toxicity endpoints should be determined based on literature data. Toxicity effect data such as EC_x (e.g., EC_{10}) should be estimated from dose-response relationships.
- (3) The literature should document the conditions under which the toxicity experiments were conducted, including factors such as soil pH, organic matter content, clay content, temperature, and aging time.
- (4) Toxicity effect data (EC_x) should be obtained through appropriate statistical analysis methods.
- (5) Experimental data that examine the effects of environmental conditions (such as soil temperature changes) on the ecotoxicity of soil contaminants can be used.
- (6) The observed toxicity effects of contaminants in the experiments should be attributed to the target pollutants, avoiding significant interference from non-target contaminants.
- (7) Standardized analytical testing methods must be employed in experimental studies.
- (8) When field experiment data are used to establish benchmarks, in addition to meeting the above criteria, the following conditions should also be satisfied:
 - a) Effect data must originate from the same region and within the same experimental period, with documented data on the physicochemical properties of the test soil.

b) Sample collection, processing, and storage should adhere to standard methods or acceptable procedures.

c) Other relevant conditions of the field experiment, such as the scientific design of sampling, should be evaluated according to the specific experiment.

(9) When establishing ecological safety thresholds for soil pollutants, priority should be given to chronic toxicity or sublethal toxicity indicators that are likely to affect individual or population characteristics of the ecological receptors of concern.

a) For terrestrial plants, endpoints such as biomass and root elongation are selected.

b) For soil invertebrates, endpoints like reproduction rate, population size, and growth rate are focused on.

c) For soil microbes and microbial-driven soil ecological processes, endpoints such as soil nitrification, microbial enzyme activity, and soil respiration are prioritized.

(10) When establishing ecological soil safety benchmarks, the selection of toxicity effect data should follow these principles:

a) When multiple toxicity endpoints are available for the same species, the parameter associated with the most sensitive endpoint is selected.

b) When multiple effect concentrations (e.g., EC_{10} , EC_{20} , EC_{50}) are available for the same toxicity endpoint, priority should be given to EC_{10} , or other concentrations should be converted to EC_{10} using a regression model established between EC_{10} and EC_{20} or EC_{50} .

c) When multiple EC_{10} values are available for different varieties of the same species, their geometric mean is used.

d) NOEC may only be used when the species diversity and trophic levels are limited, and the ecotoxicity dataset contains fewer than 10 data points.

Tables

Table S1 Toxicity data retrieval requirements.

Data type	Index
Chemical compound	Phenanthrene
Species type	Terrestrial plants, terrestrial invertebrates, microorganisms
Species names	Latin name
Exposure type	Direct contact
Exposure time	Day
Toxic Endpoint	EC ₁₀ (primary), NOEC
Toxicity effect	Biotoxicity effect, biological activity inhibition effect
Text method	Experiments used standardized experimental methods and procedures

Table S2 Reliability data relating to species distribution.

Species type	Species name	Number of species
Terrestrial plant	Wheat, rice, soybean, tomato, onion, lettuce, rapeseed, cucumber, brilliant champion, morning glory, clover, motherwort, poppy, mung bean, bok choy, Chinese little greens	16
Terrestrial invertebrates	Azoxystrobin on earthworm, Venice earthworm, springtails, Caenorhabditis elegans, white springtail, striped earthworm, and snail	7
Soil microorganisms and microbial-driven soil ecological processes	Nitrification, dehydrogenase, catalase	3

Table S3 Toxicity data of phenanthrene to terrestrial plants.

Species	EC ₁₀ /NOEC (mg kg ⁻¹)	Test end point (Day)	pH	SOM (g kg ⁻¹)	Reference
Mung bean (<i>Vigna radiata</i> L.)	278	Stem length (14 d)	6.0	100	[12]
Mung bean (<i>Vigna radiata</i> L.)	358	Fresh weight (14 d)	6.0	100	[12]
Bok choy (<i>Brassica campestris</i> L. ssp.)	202	Stem length (14 d)	6.0	100	[12]
Bok choy (<i>Brassica campestris</i> L. ssp.)	306	Fresh weight (14 d)	6.0	100	[12]
Rice (<i>Oryza sativa</i>)	224	Stem length (14 d)	6.0	100	[12]
Rice (<i>Oryza sativa</i>)	364	Fresh weight (14 d)	6.0	100	[12]
Lettuce (<i>Lactuca sativa</i>)	1.4/25.3/41.7	Germination rate (7 d)	8.0	9.0/9.2/44.2	[13]
Lettuce (<i>Lactuca sativa</i>)	1.2/87.4/95.1	Fresh weight (14 d)	8.0	9.0/9.2/44.2	[13]
Chinese little greens (<i>Pterocladia tenui</i>)	202.35	Stem length (14 d)	7.0	100	[2]
Chinese little greens (<i>Pterocladia tenui</i>)	306.13	Fresh weight (14 d)	7.0	100	[2]
Wheat (<i>Triticum aestivum</i>)	6.22-170.72	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Wheat (<i>Triticum aestivum</i>)	213.78-859.78	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result

Rice (<i>Oryza sativa</i>)	5.04-111.87	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Rice (<i>Oryza sativa</i>)	49.00-440.57	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Onion (<i>Allium cepa</i>)	7.70-98.39	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Onion (<i>Allium cepa</i>)	0.94-194.28	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Soybean (<i>Glycine max</i>)	8.81-36.29	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Soybean (<i>Glycine max</i>)	45.29-316.75	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Tomato (<i>Solanum lycopersicum</i>)	0.64-72.99	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Tomato (<i>Solanum lycopersicum</i>)	11.73-31.66	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Lettuce (<i>Lactuca sativa</i>)	0.62-383.48	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Lettuce (<i>Lactuca sativa</i>)	9.24-578.14	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Oilseed rape (<i>Brassica napus</i> L.)	5.31-406.47	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Oilseed rape (<i>Brassica napus</i> L.)	5.07-255.35	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Cucumber (<i>Cucumis sativus</i>)	39.30-330.97	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Cucumber (<i>Cucumis sativus</i>)	49.90-171.15	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Brilliant champion (<i>Lychnis fulgens</i> Fisch.)	1.20-92.38	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Brilliant champion (<i>Lychnis fulgens</i> Fisch.)	0.018-444.73	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result

Morning glory (<i>Catharanthus roseus</i>)	19.75-101.55	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Morning glory (<i>Catharanthus roseus</i>)	5.91-289.44	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Clover (<i>Trifolium repens</i>)	0.97-23.77	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Clover (<i>Trifolium repens</i>)	11.13-ND	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Motherwort (<i>Leonurus japonicus</i>)	3.44-72.03	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Motherwort (<i>Leonurus japonicus</i>)	2.65-49.19	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result
Poppy (<i>Papaver rhoeas</i>)	10.00-39.05	Stem length (7 d)	4.55-5.40	15.48-79.40	Experiment result
Poppy (<i>Papaver rhoeas</i>)	4.52-16.88	Fresh weight (7 d)	4.55-5.40	15.48-79.40	Experiment result

Note: NOEC, no observed effect concentration; SOM, soil organic matter.

Table S4 Toxicity data of phenanthrene to terrestrial invertebrates.

Species	EC ₁₀ /NOEC (mg kg ⁻¹)	Test end point (Day)	pH	SOM (g kg ⁻¹)	Reference
<i>Enchytraeus crypticus</i>	37	Reproduction (21 d)	5.5	40	[3]
<i>Enchytraeus crypticus</i>	370	Reproduction (28 d)	5.6	23	[10]
<i>Enchytraeus crypticus</i>	40	Reproduction (21 d)	6.2	16	[11]
<i>Enchytraeus crypticus</i>	37	Reproduction (21 d)	5.5	40	[16]
<i>Eisenia veneta</i>	23	Reproduction (28 d)	6.2	16	[4]
<i>Folsomia fimetaria</i>	25	Reproduction (28 d)	6.2	16	[5]
<i>Folsomia fimetaria</i>	24.95	Reproduction (28 d)	6.2	16	[7]
<i>Folsomia fimetaria</i>	23	Reproduction (28 d)	6.2	11	[8]
<i>Enchytraeus albidus</i>	11	Reproduction (28 d)	5.8	44	[6]
<i>Folsomia candida</i>	140	Reproduction (28 d)	5.6	23	[10]
<i>Folsomia candida</i>	1.9/5.2/22.8	Reproduction (14 d)	8.0	9.0/5.7/9.2	[13]
<i>Folsomia candida</i>	24.8	Reproduction (28 d)	5.6	23	[15]
<i>Lumbriculus variegatus</i>	187	Reproduction (28 d)	8.2	120	[14]

<i>Helix aspersa</i>	2800	Reproduction (28 d)	6.2	16	[17]
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Note: NOEC, no observed effect concentration; SOM, soil organic matter.

Table S5 Toxicity data of phenanthrene on soil microorganisms and microbial-driven soil ecological processes.

Ecological process	EC ₁₀ /NOEC (mg kg ⁻¹)	Test end point (Day)	pH	SOM (g kg ⁻¹)	Reference
Nitrification	42	Nitrification (28 d)	6.2	16	[9]
Dehydrogenase	299.89	Dehydrogenase (10 d)	7.0	4.3	[1]
Catalase	113.63	Catalase (10 d)	7.0	4.3	[1]

Note: NOEC, no observed effect concentration; SOM, soil organic matter.

Table S6 Equation.

Name	Equation
Normal	$y = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$
Log-normal	$y = \frac{1}{\sigma} \left(\frac{\ln x - \mu}{\sigma} \right)$
Logistic	$y = \frac{1}{1 + e^{-x}}$

Note: y represents the cumulative probability (%); x is the toxicity value (mg kg^{-1}); μ , σ , α , and β are the parameters of the equation.

Table S7 The goodness of fit of three equations.

Equation	Goodness of fit
$y = \frac{1}{\sqrt{2\pi}\sigma} e^{(-\frac{(x-\mu)^2}{2\sigma^2})}$	0.05
$y = \phi(\frac{\ln x - \mu'}{\sigma})$	/
$y = \frac{1}{1 + e^{-x}}$	0.03

Note: y represents the cumulative probability (%); x is the toxicity value (mg kg^{-1}); μ ,

σ , α , and β are the parameters of the equation.

Figures

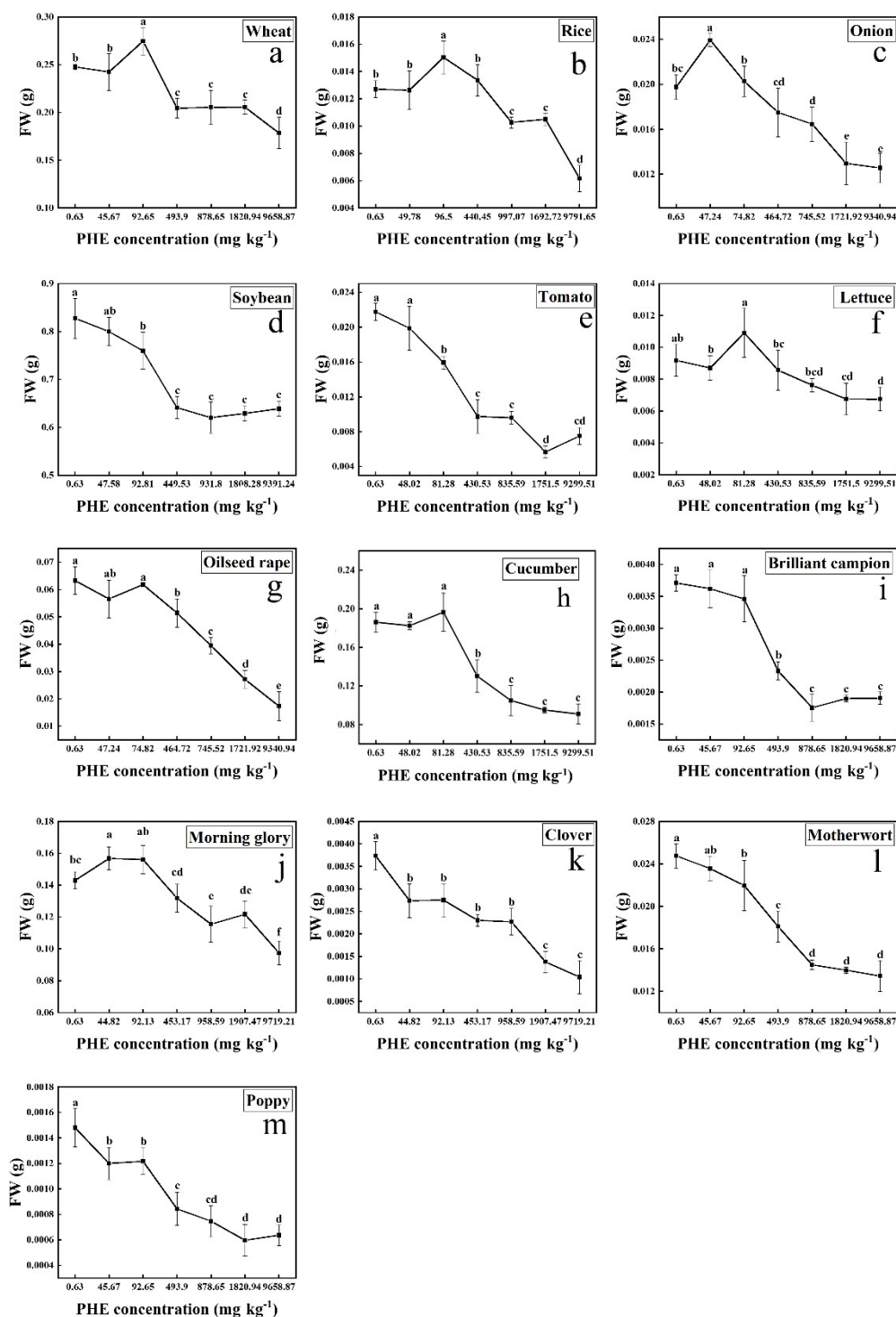


Fig. S1 Effects of different phenanthrene concentrations on plant fresh weight in Ningbo paddy soil. FW, fresh weight; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

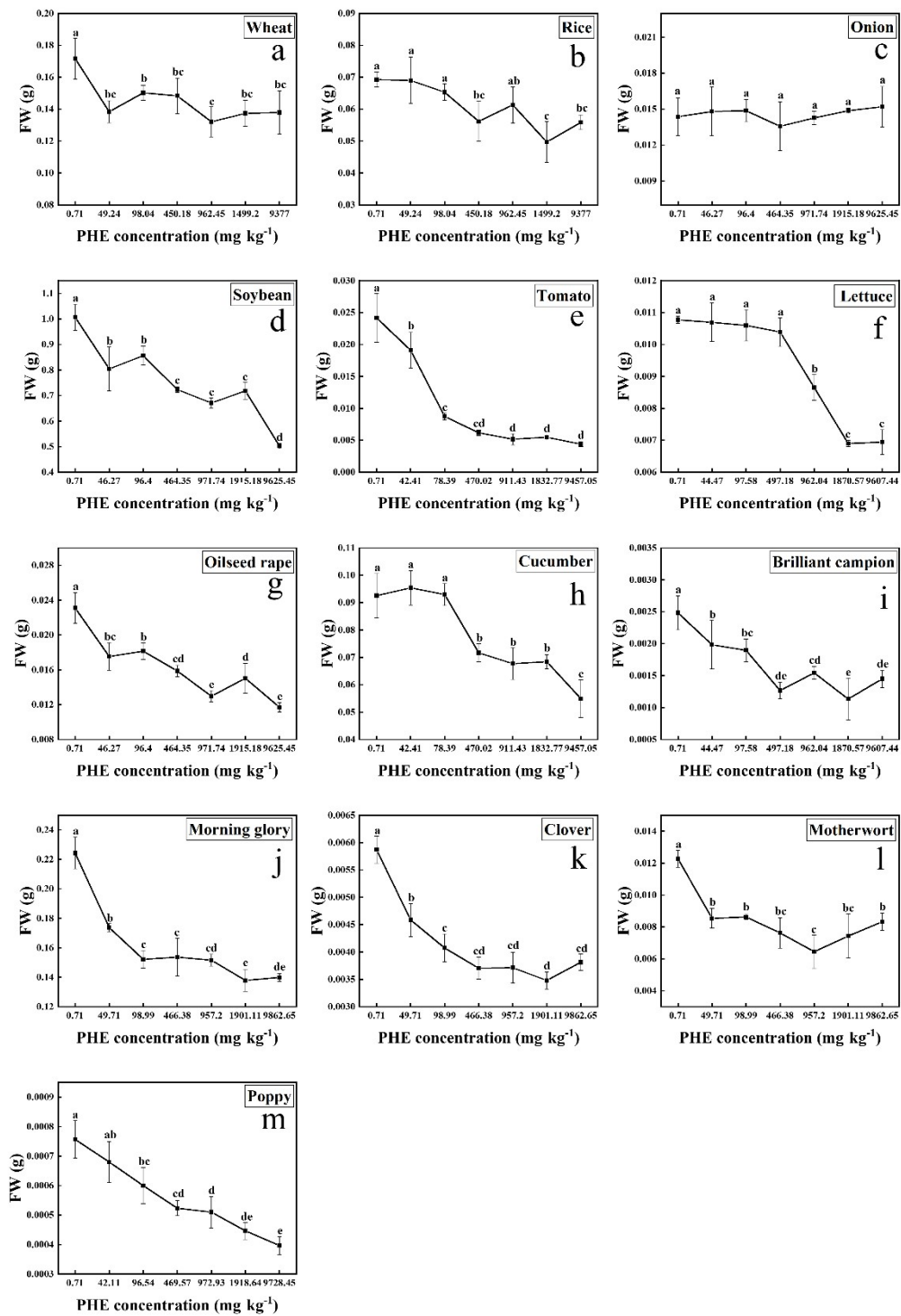


Fig. S2 Effects of different phenanthrene concentrations on plant fresh weight in Ningbo upland soil. FW, fresh weight; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

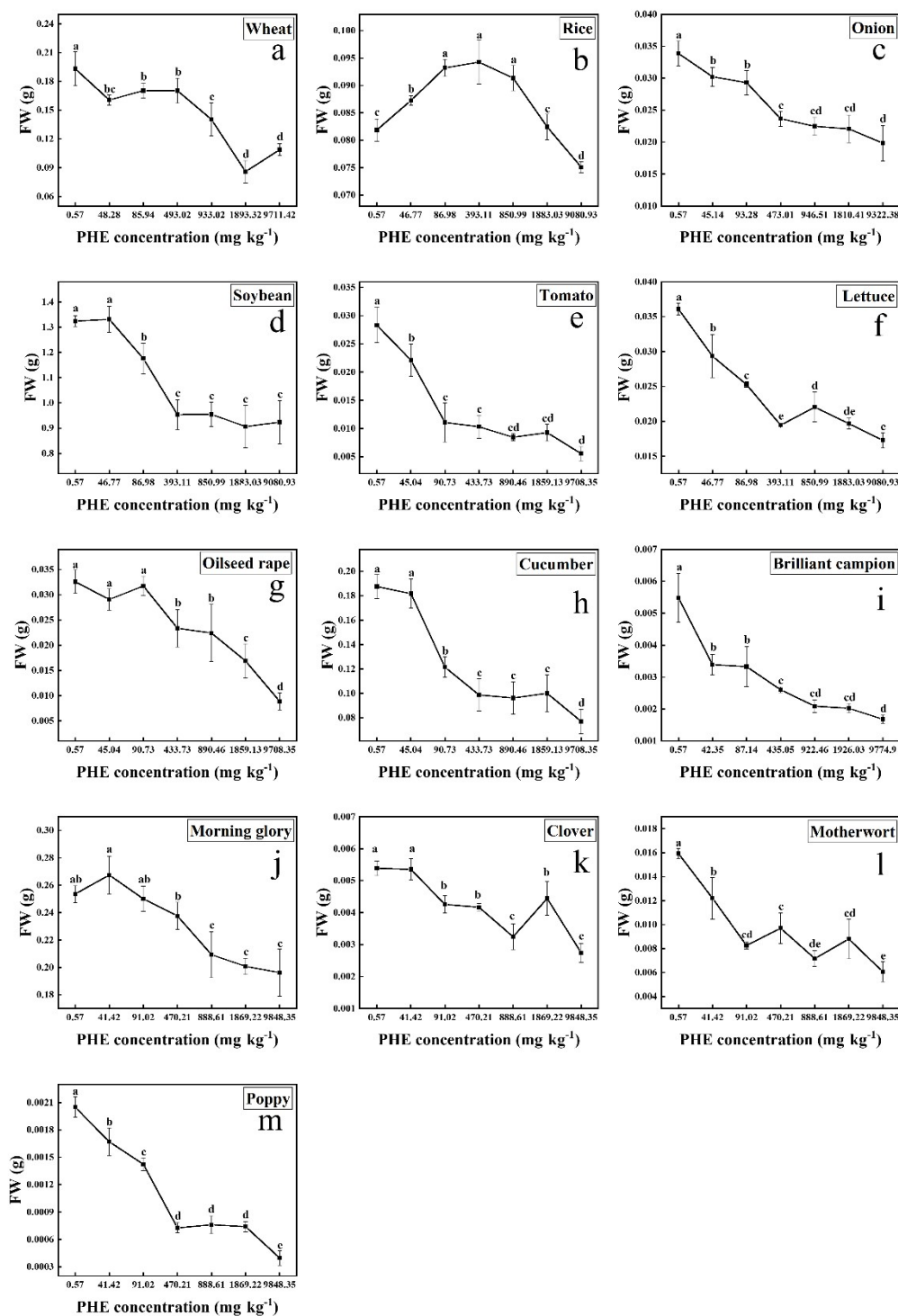


Fig. S3 Effects of different phenanthrene concentrations on plant fresh weight in Yingtan paddy soil. FW, fresh weight; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

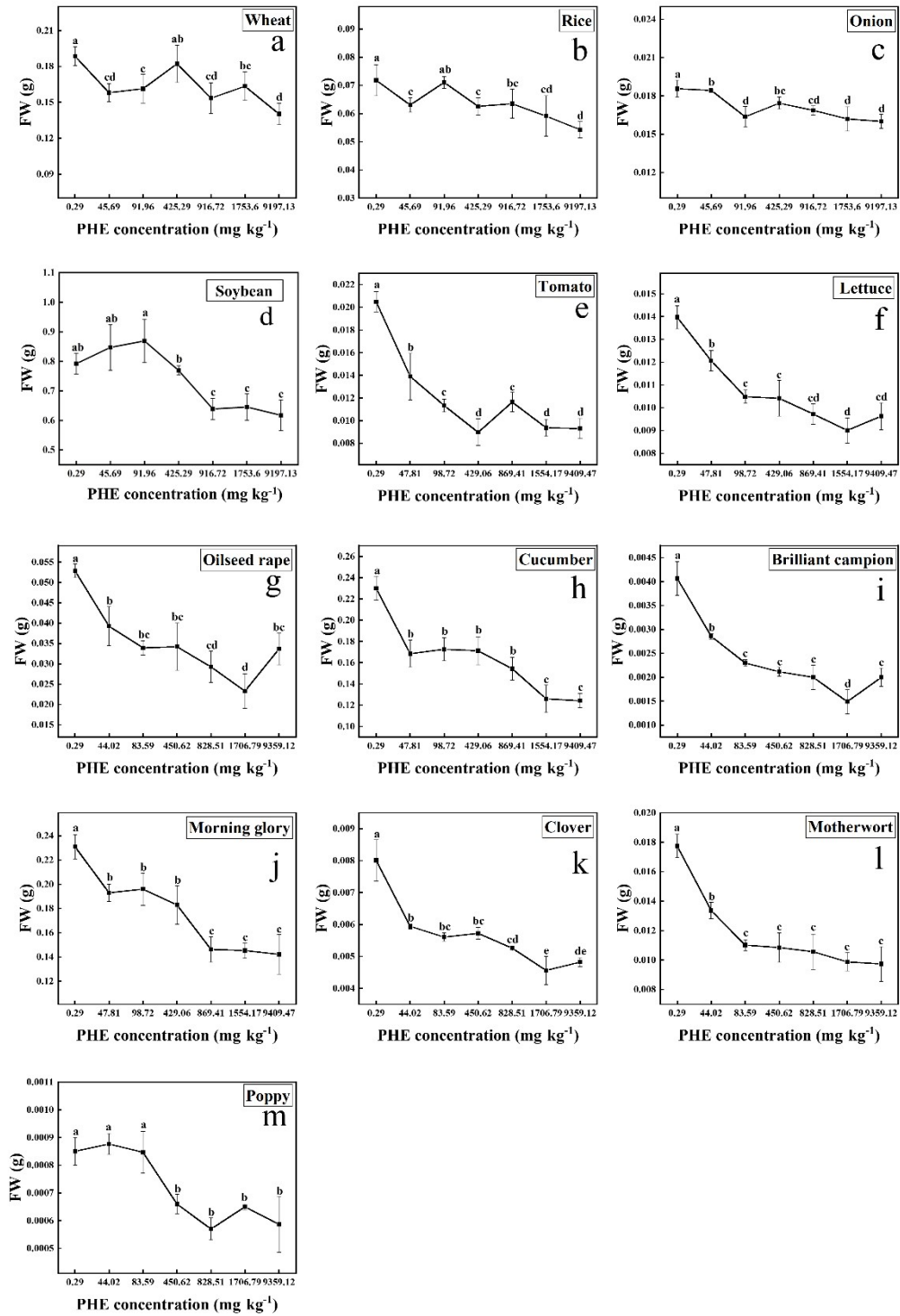


Fig. S4 Effects of different phenanthrene concentrations on plant fresh weight in Yingtan upland soil. FW, fresh weight; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

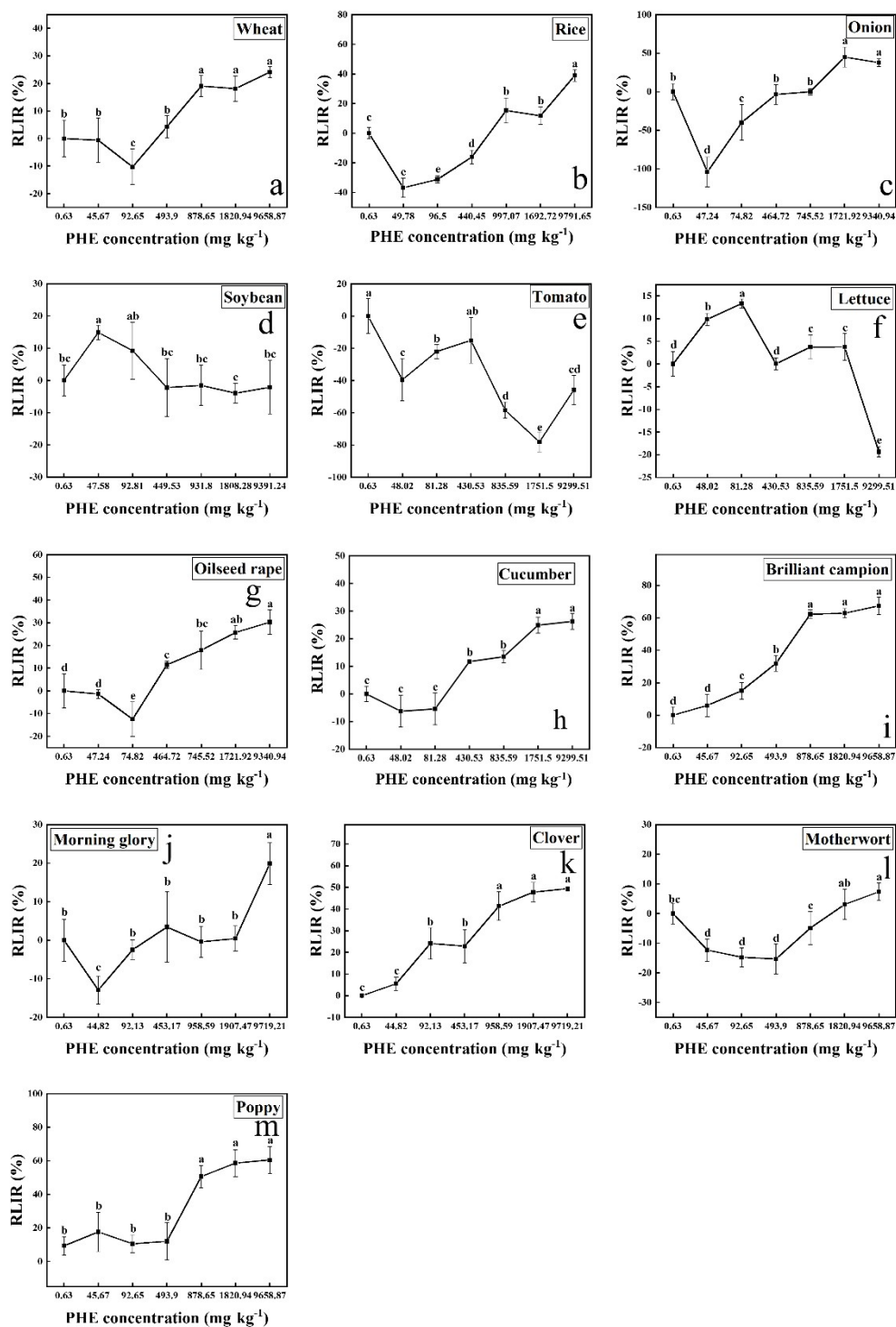


Fig. S5 Effects of different phenanthrene concentrations on plant root length inhibition rate in Ningbo paddy soil. RLIR, root length inhibition rate; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

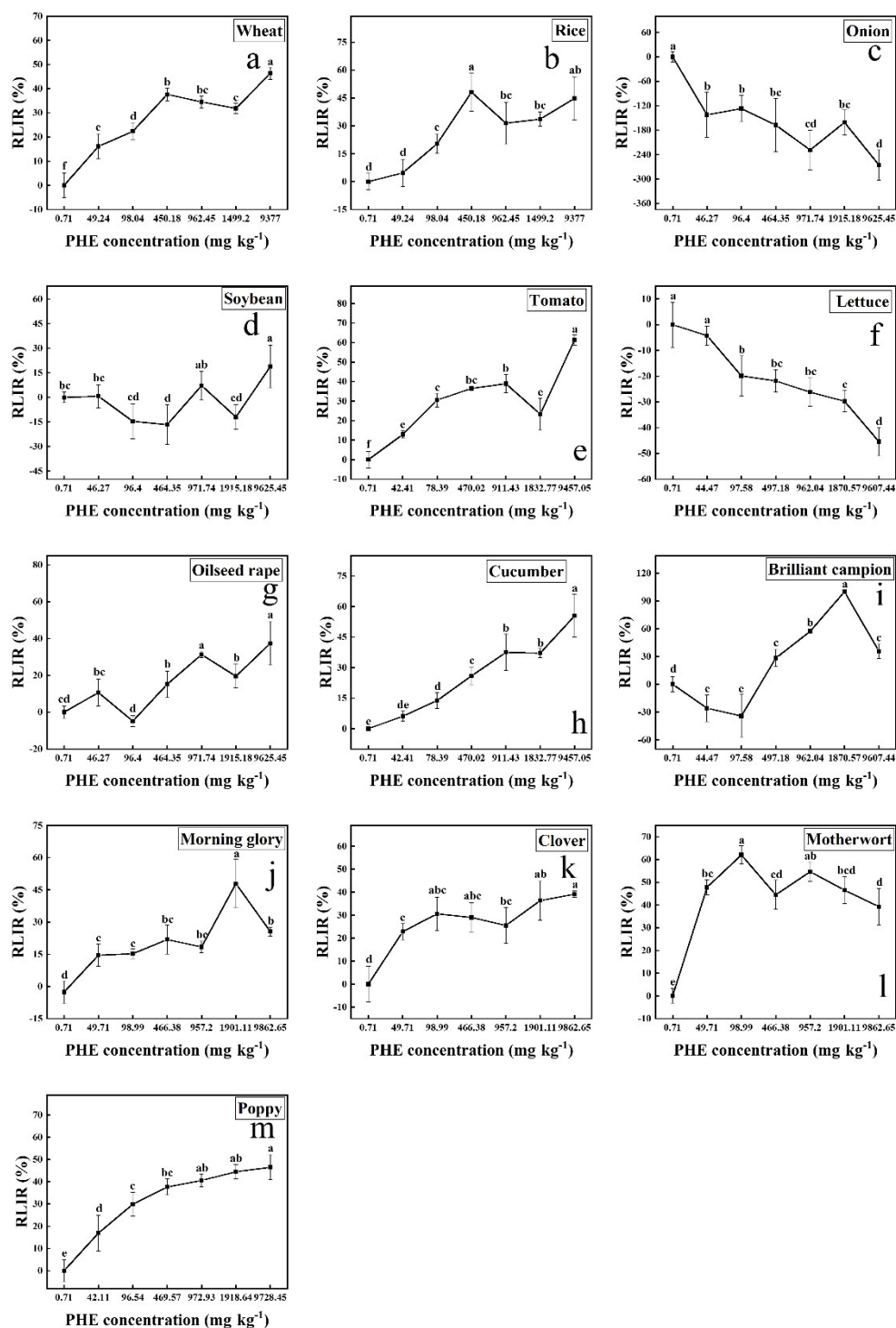


Fig. S6 Effects of different phenanthrene concentrations on plant root length inhibition rate in Ningbo upland soil. RLIR, root length inhibition rate; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

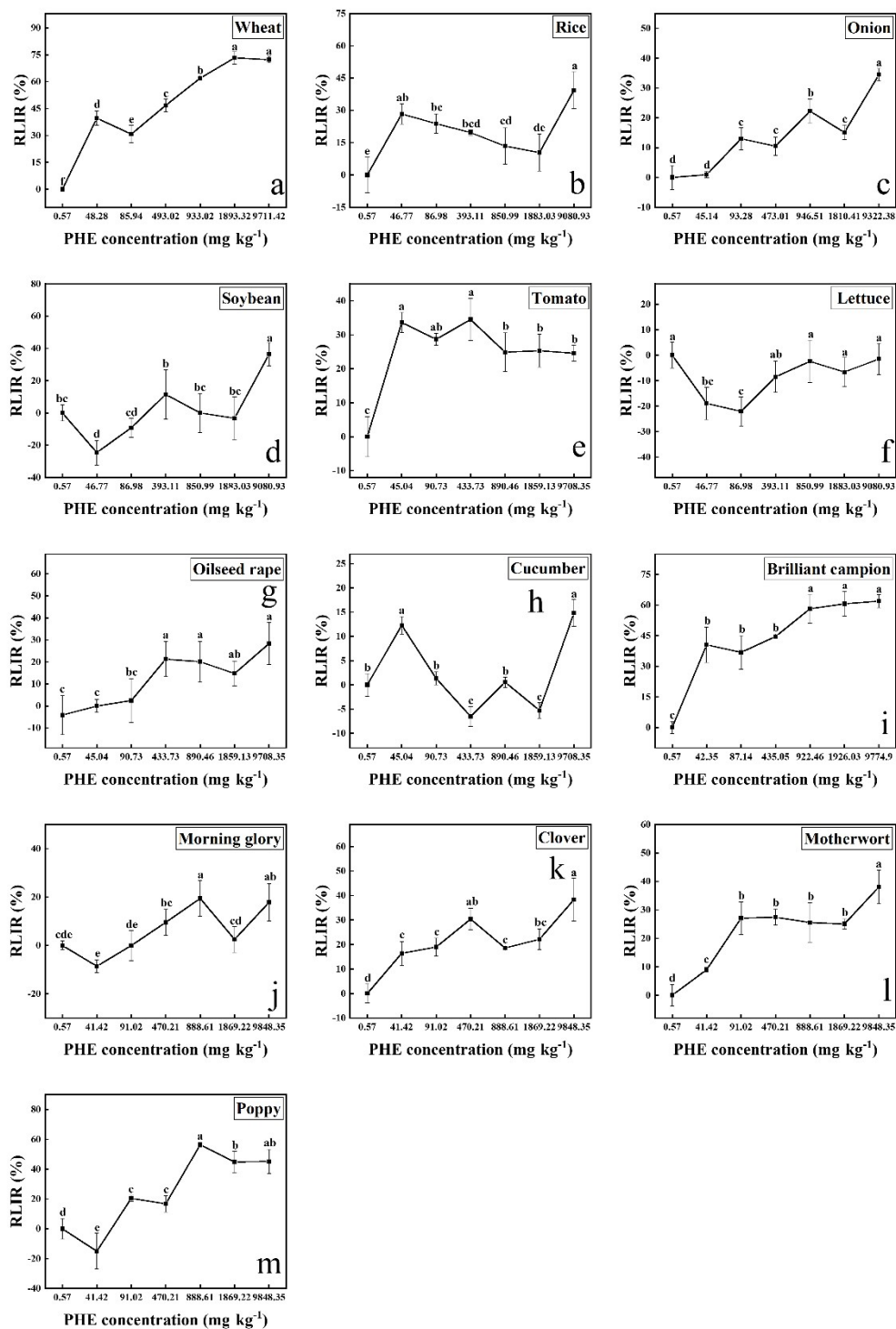


Fig. S7 Effects of different phenanthrene concentrations on plant root length inhibition rate in Yingtan paddy soil. RLIR, root length inhibition rate; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

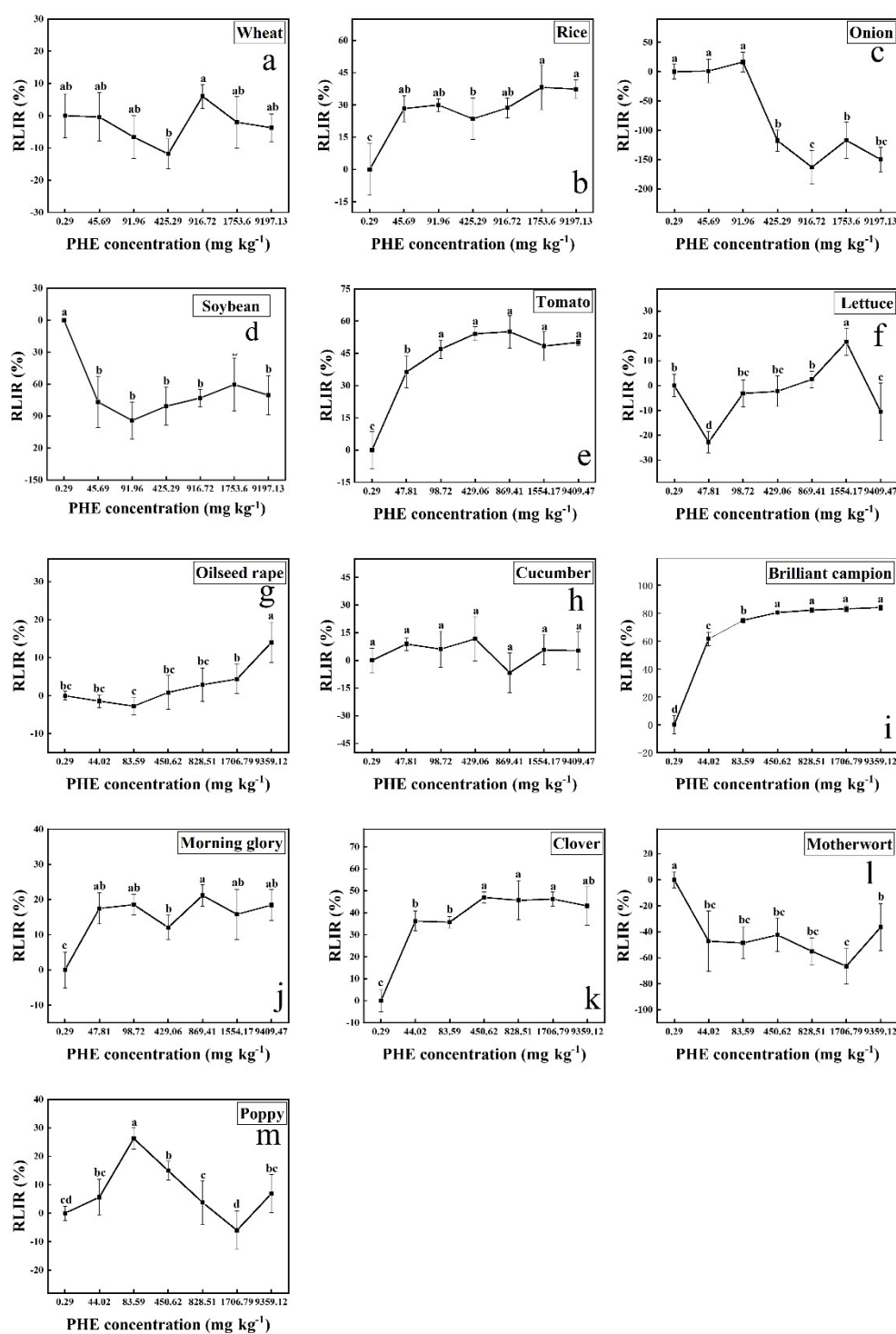


Fig. S8 Effects of different phenanthrene concentrations on plant root length inhibition rate in Yingtan upland soil. RLIR, root length inhibition rate; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

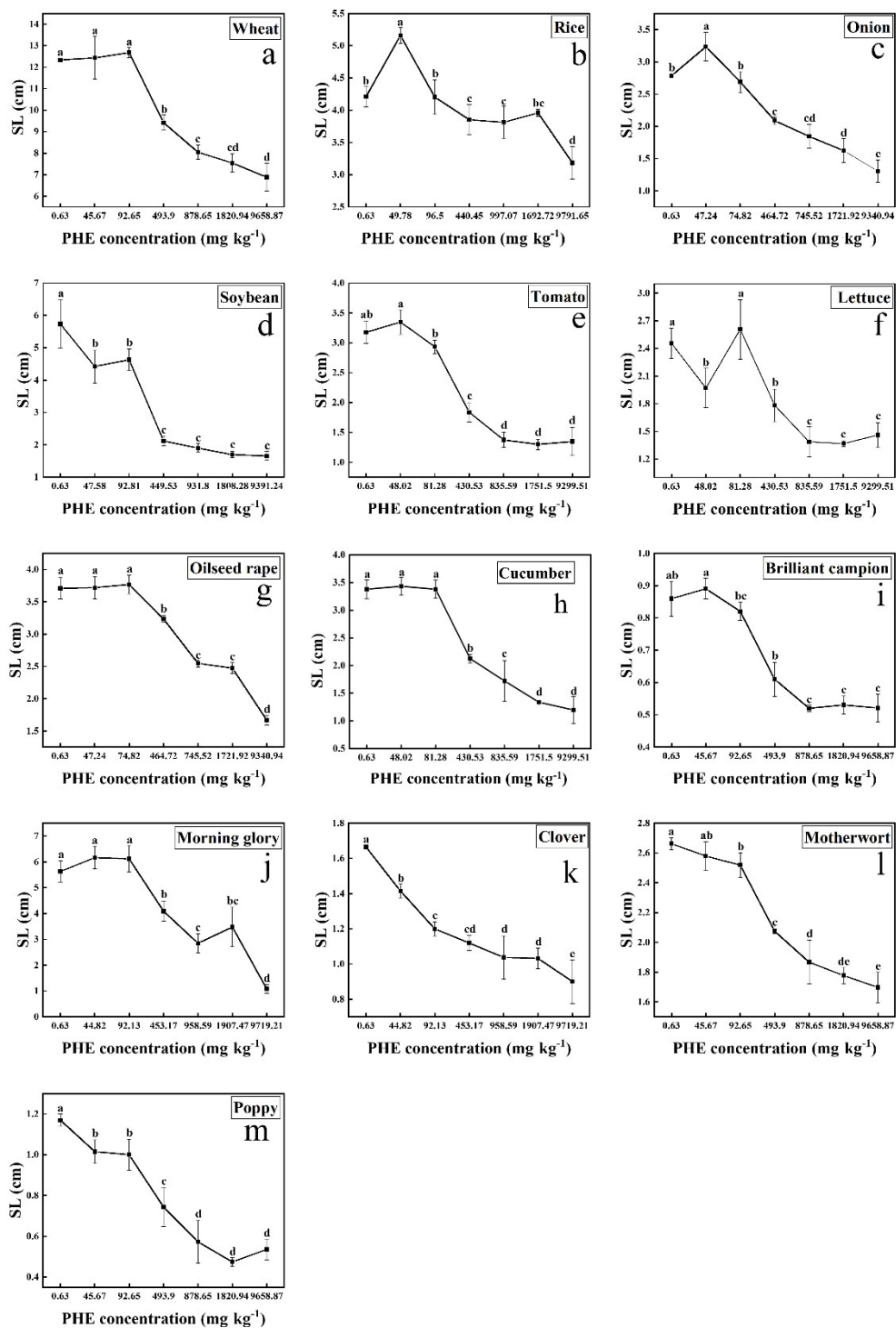


Fig. S9 Effects of different phenanthrene concentrations on plant stem length in Ningbo paddy soil. SL, stem length; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

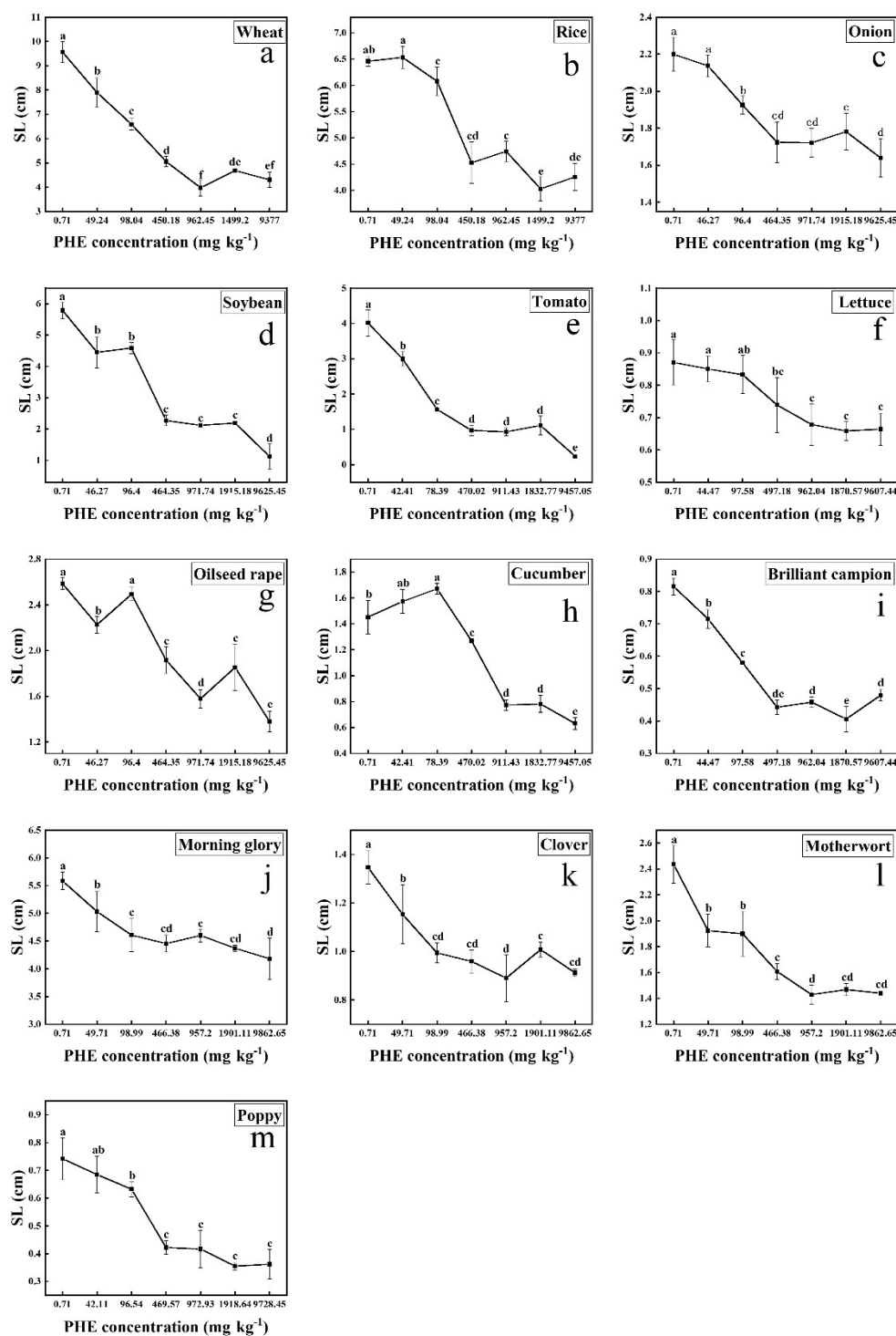


Fig. S10 Effects of different phenanthrene concentrations on plant stem length in Ningbo upland soil. SL, stem length; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

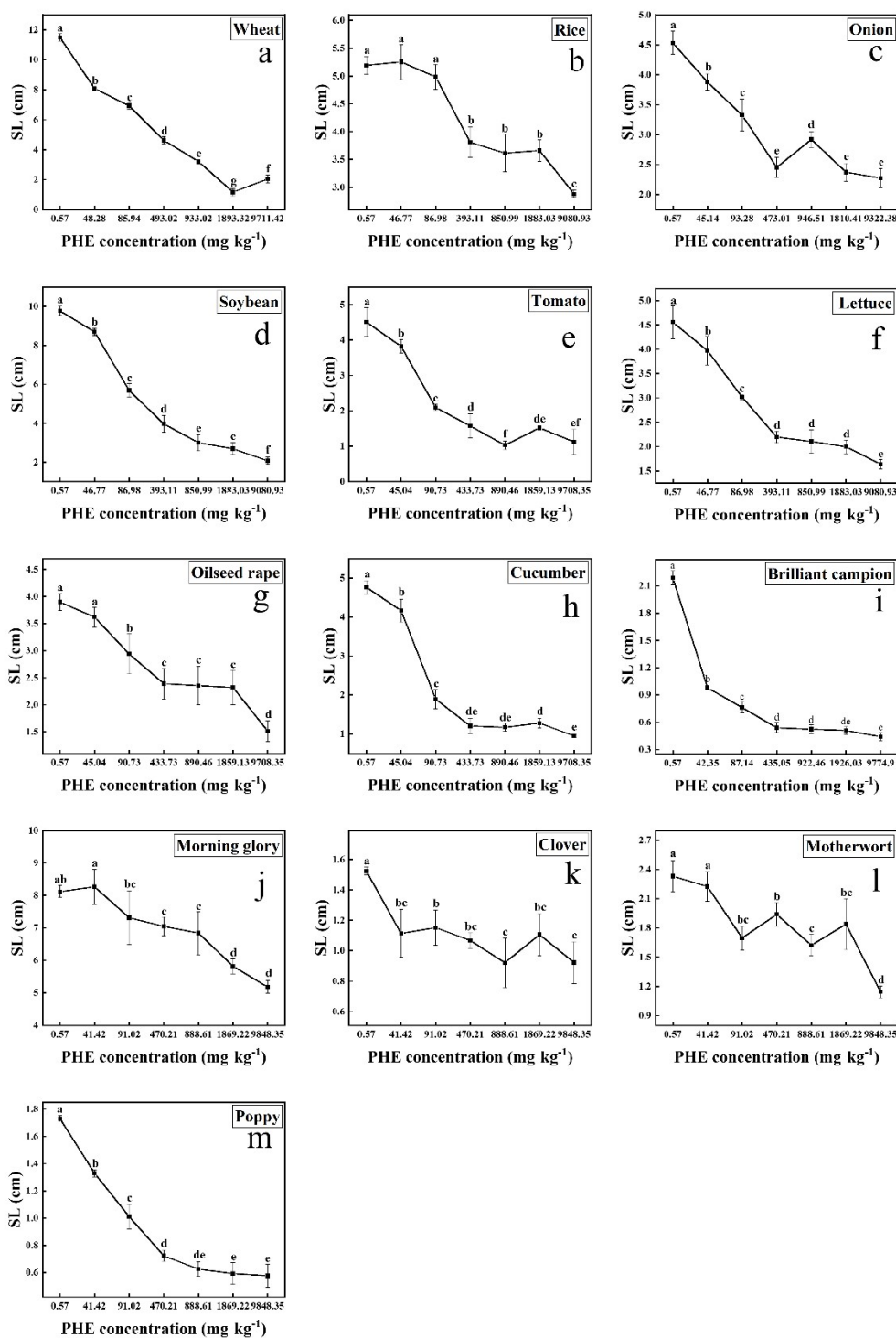


Fig. S11 Effects of different phenanthrene concentrations on plant stem length in Yingtan paddy soil. SL, stem length; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

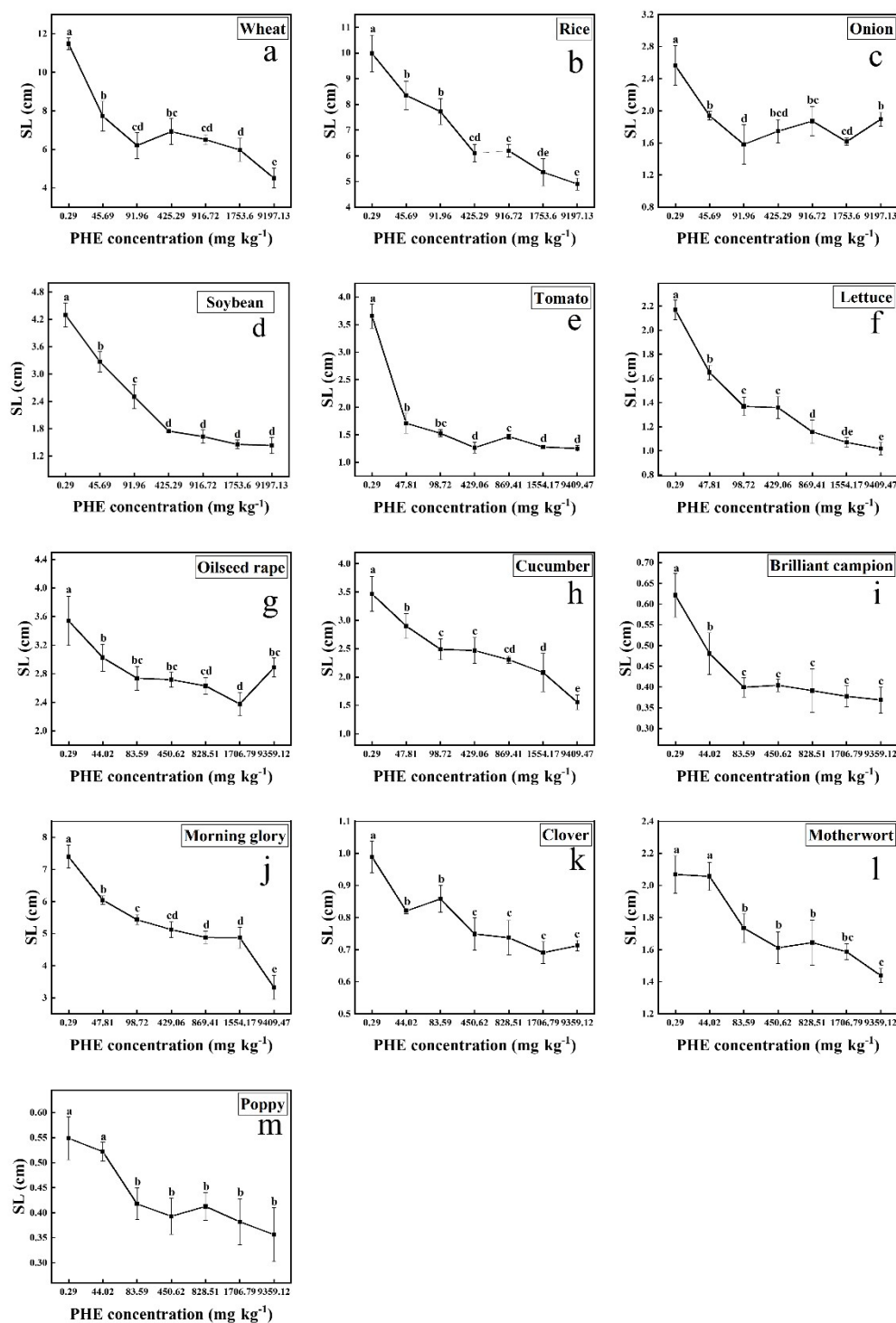


Fig. S12 Effects of different phenanthrene concentrations on plant stem length in Yingtan upland soil. SL, stem length; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to Duncan's test.

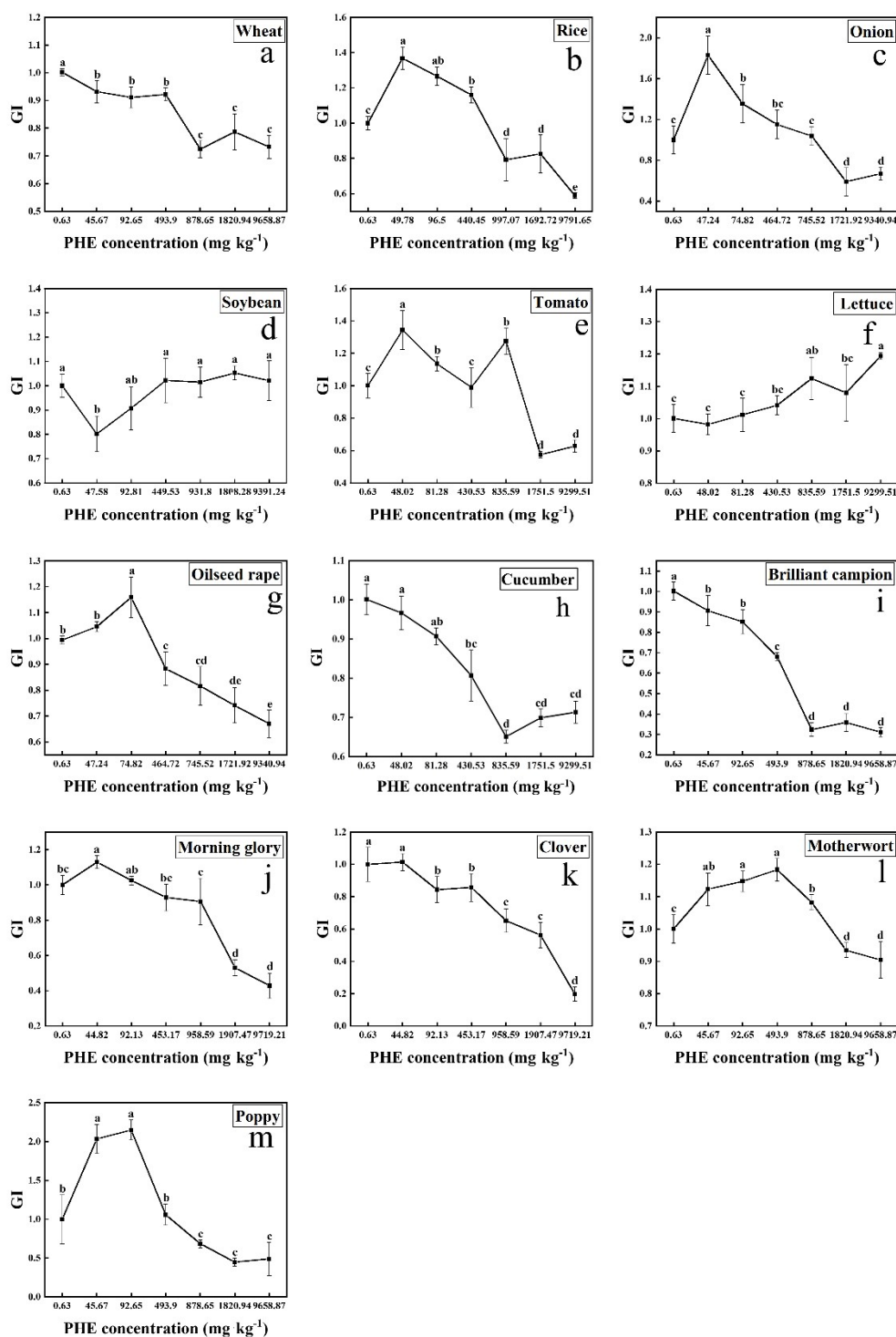


Fig. S13 Effects of different phenanthrene concentrations on plant germination index in Ningbo paddy soil. GI, germination index; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

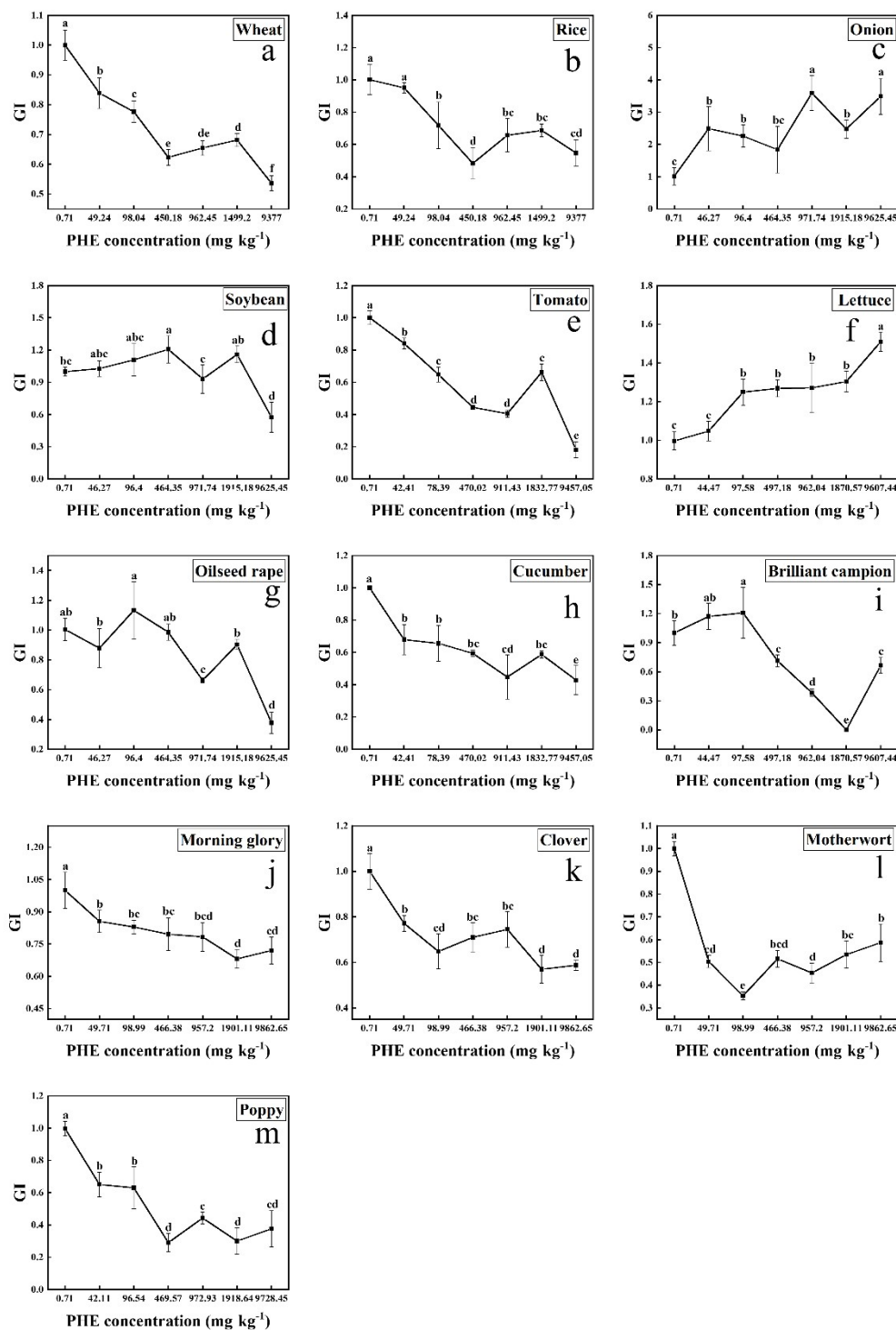


Fig. S14 Effects of different phenanthrene concentrations on plant germination index in Ningbo upland soil. GI, germination index; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

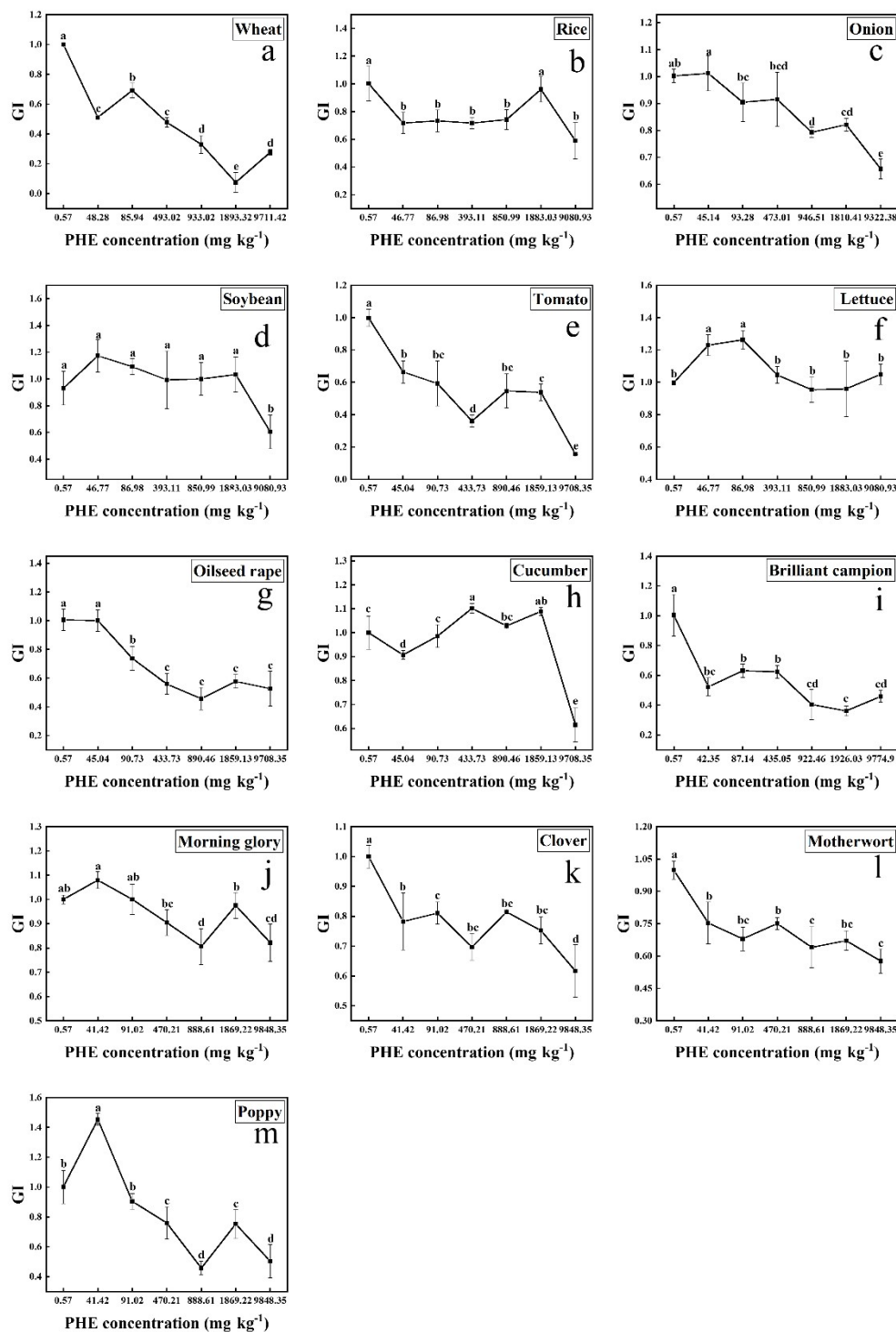


Fig. S15 Effects of different phenanthrene concentrations on plant germination index in Yingtan paddy soil. GI, germination index; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

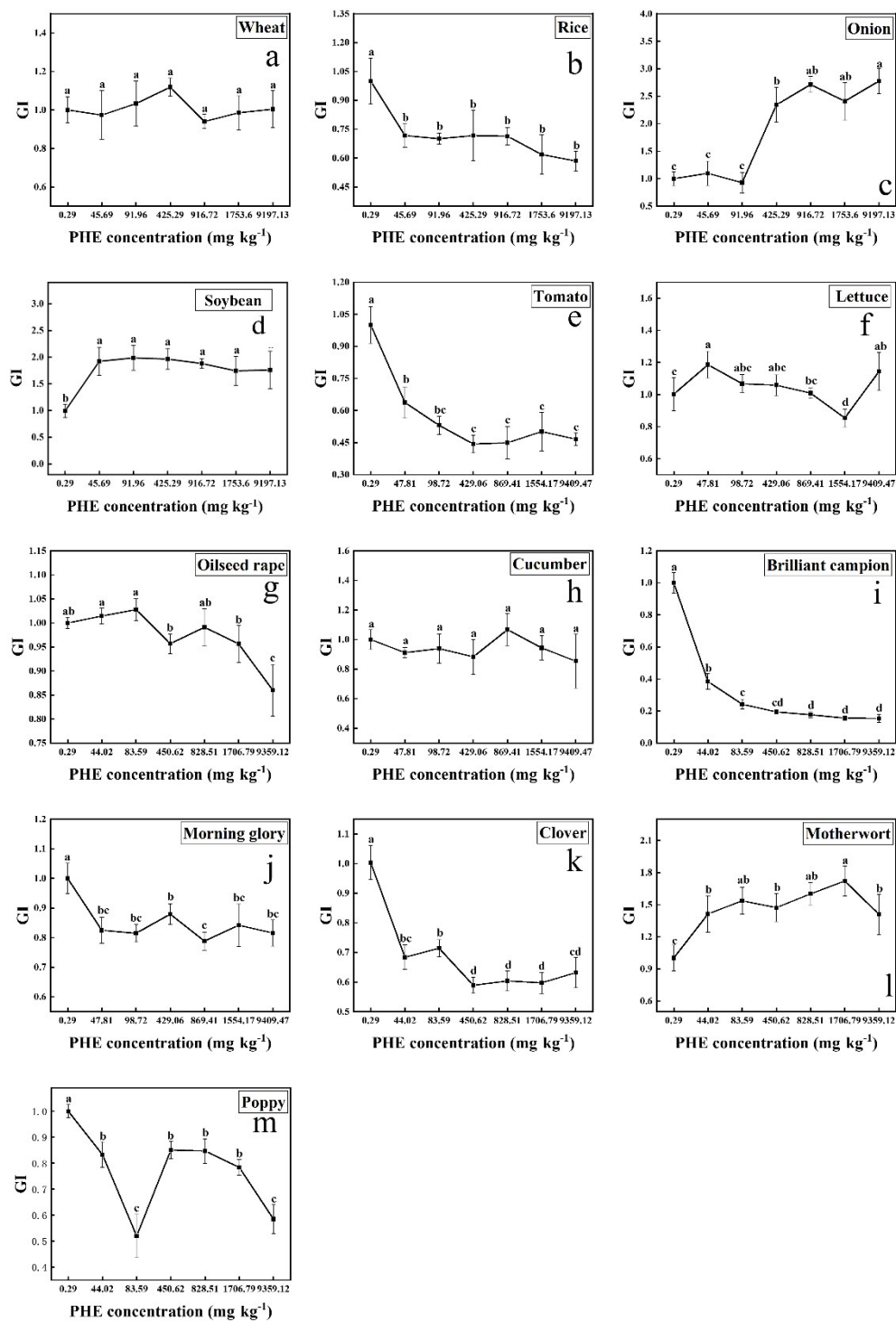


Fig. S16 Effects of different phenanthrene concentrations on plant germination index in Yingtan upland soil. GI, germination index; PHE, phenanthrene. Different letters in the same figure indicate significant difference at $P < 0.05$ according to *Duncan's* test.

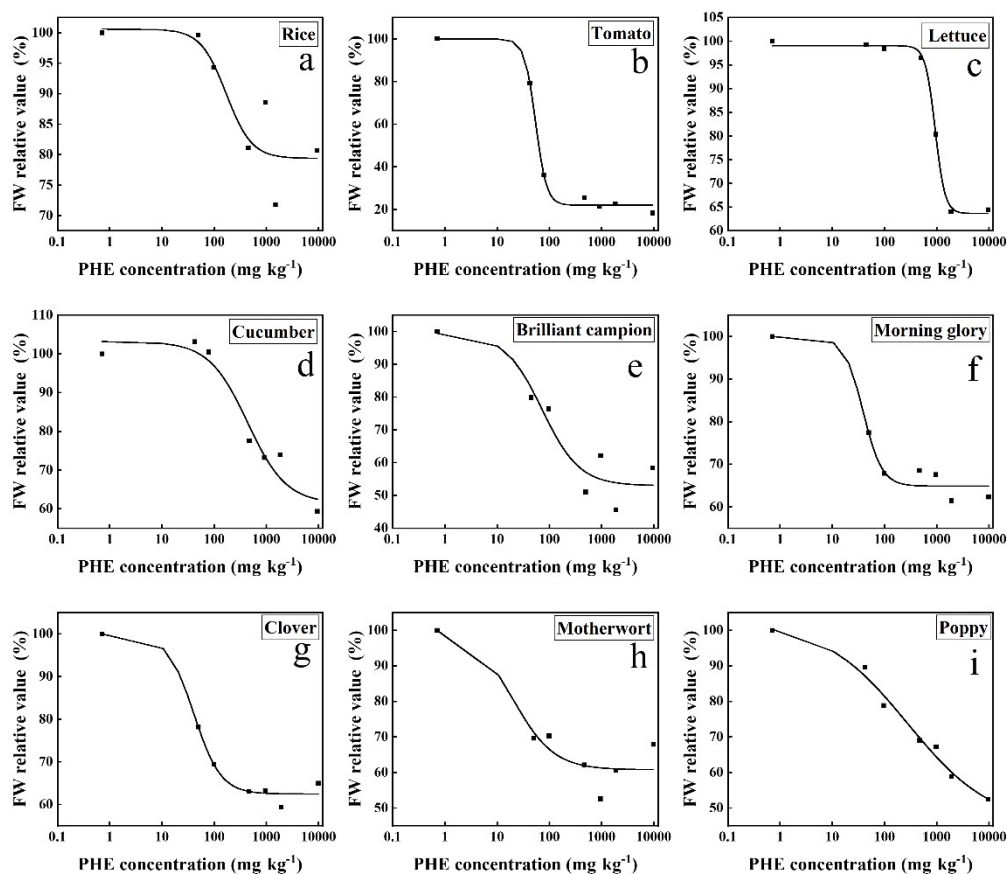


Fig. S17 Dose-response relationship curves between phenanthrene concentration and plant fresh weight in Ningbo upland soil. FW, fresh weight; PHE, phenanthrene.

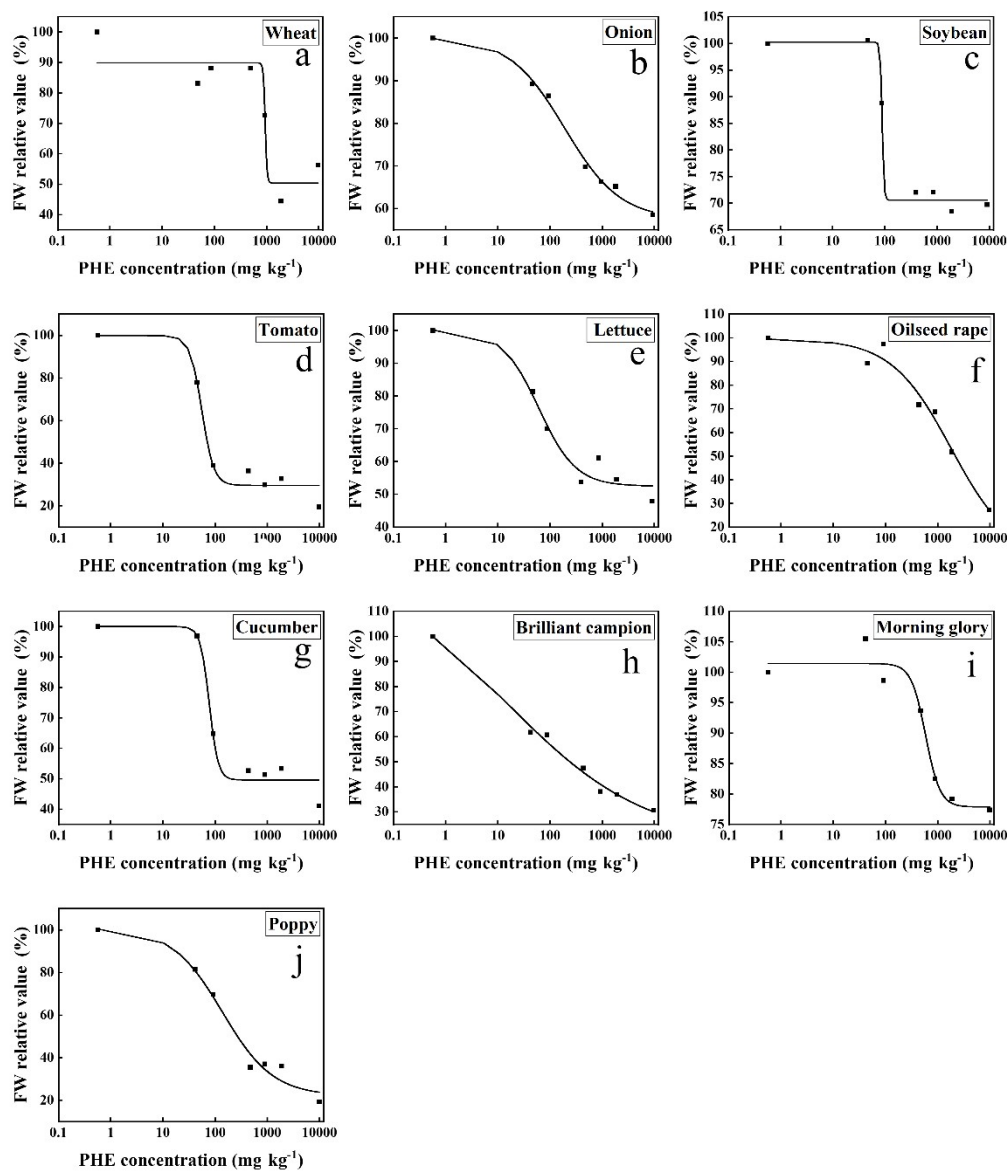


Fig. S18 Dose-response relationship curves between phenanthrene concentration and plant fresh weight in Yingtan paddy soil. FW, fresh weight; PHE, phenanthrene.

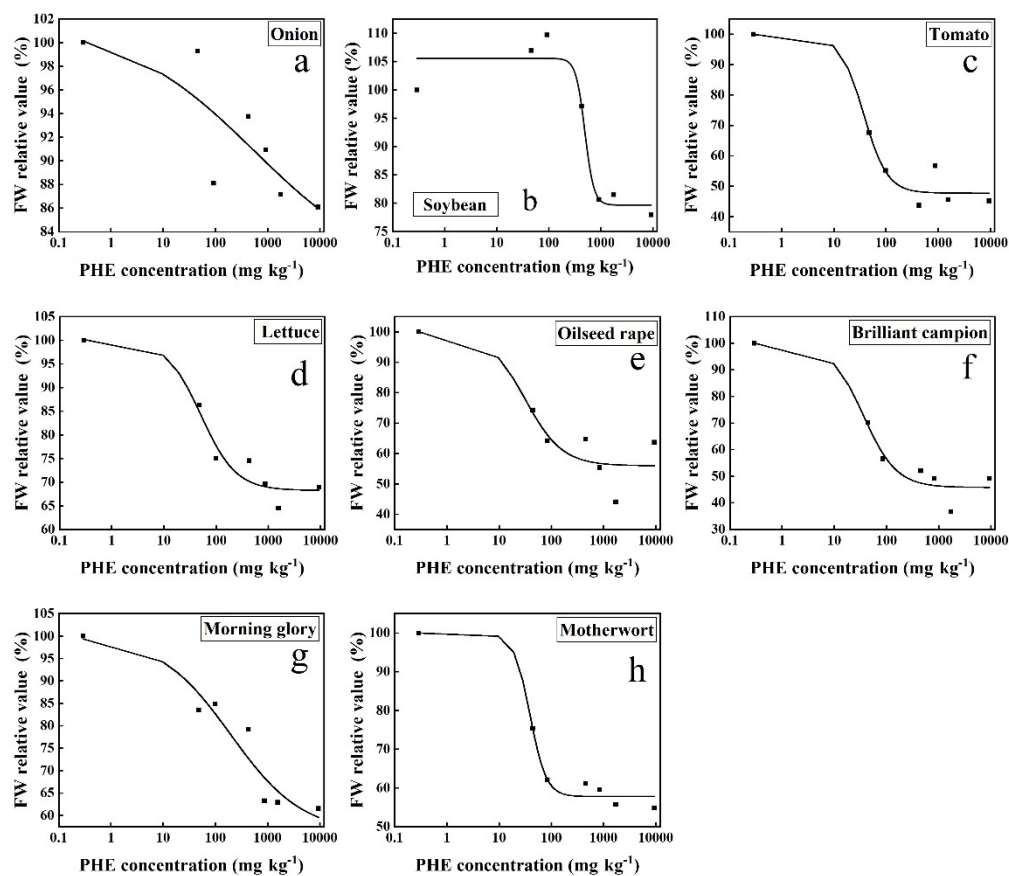


Fig. S19 Dose-response relationship curves between phenanthrene concentration and plant fresh weight in Yingtan upland soil. FW, fresh weight; PHE, phenanthrene.

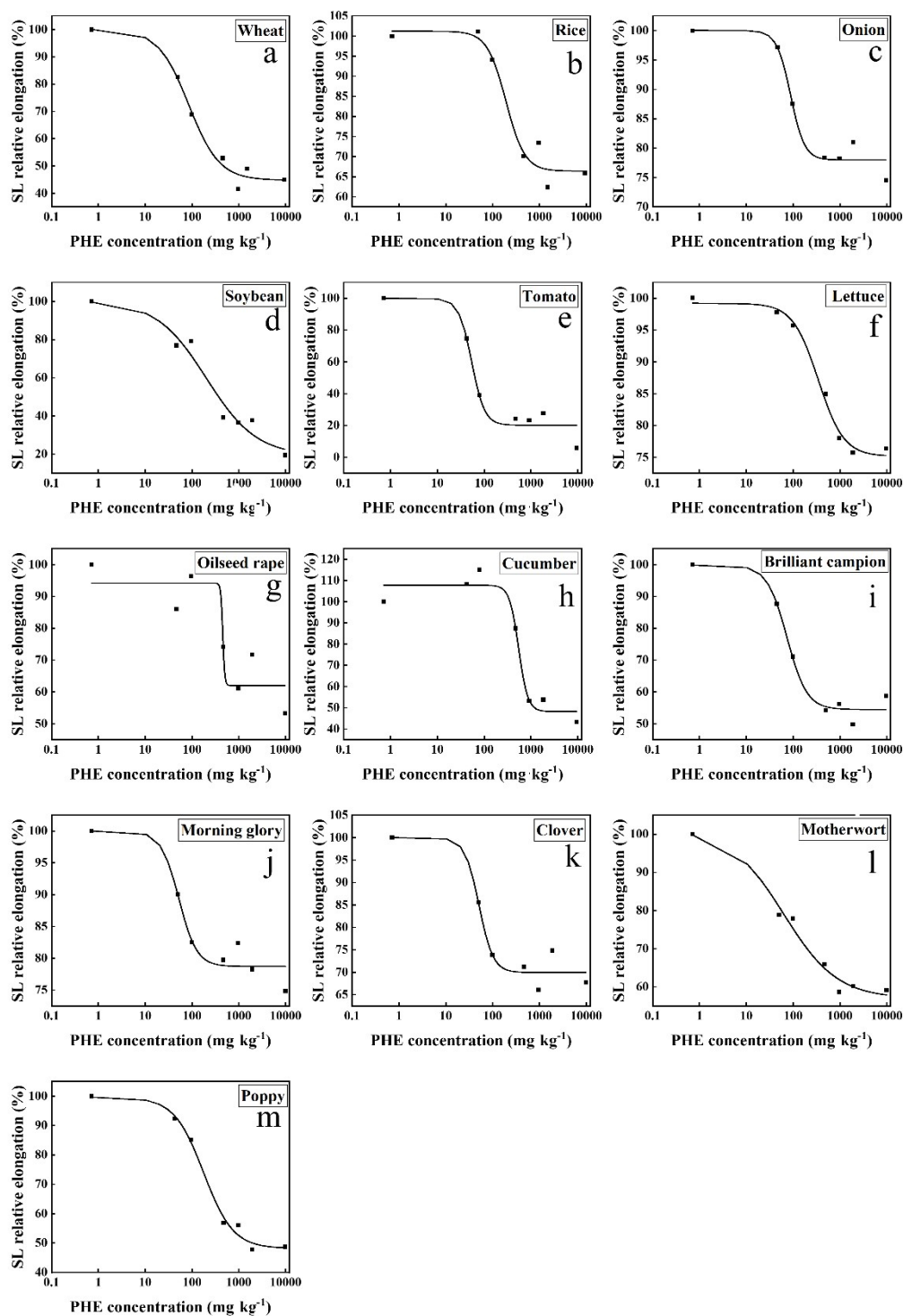


Fig. S20 Dose-response relationship curves between phenanthrene concentration and plant stem length in Ningbo upland soil. SL, stem length; PHE, phenanthrene.

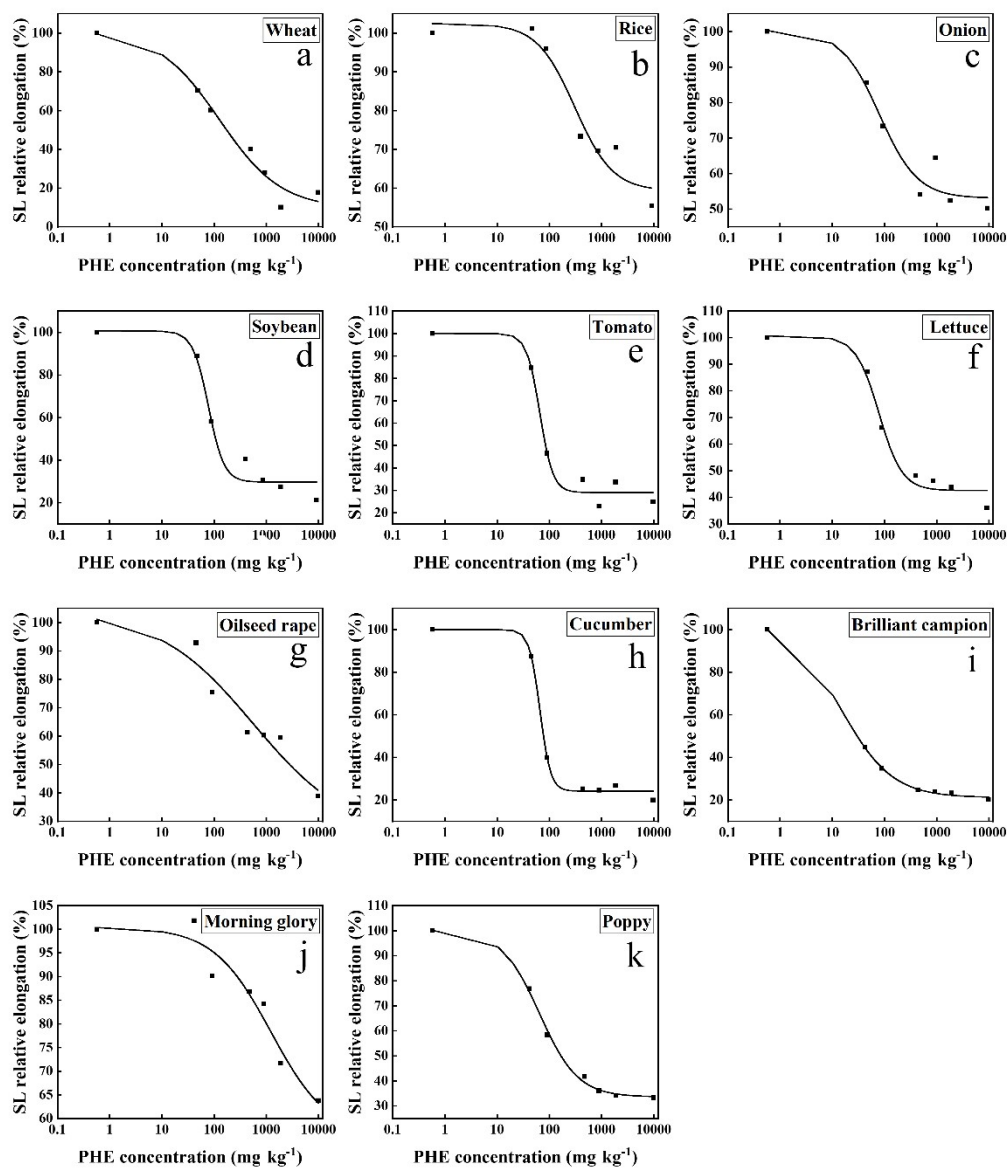


Fig. S21 Dose-response relationship curves between phenanthrene concentration and plant stem length in Yingtan paddy soil. SL, stem length; PHE, phenanthrene.

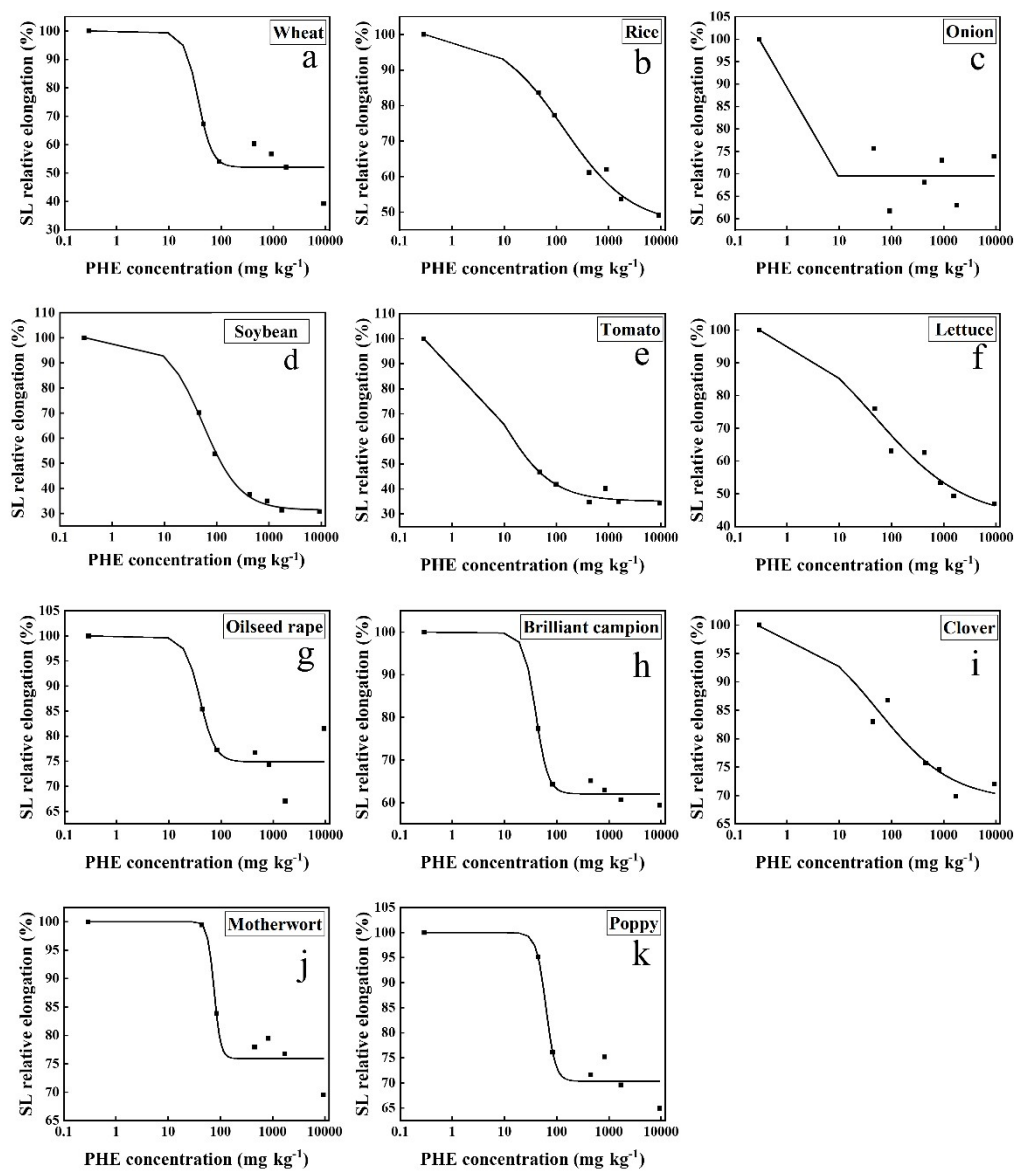


Fig. S22 Dose-response relationship curves between phenanthrene concentration and plant stem length in Yingtan upland soil. SL, stem length; PHE, phenanthrene.

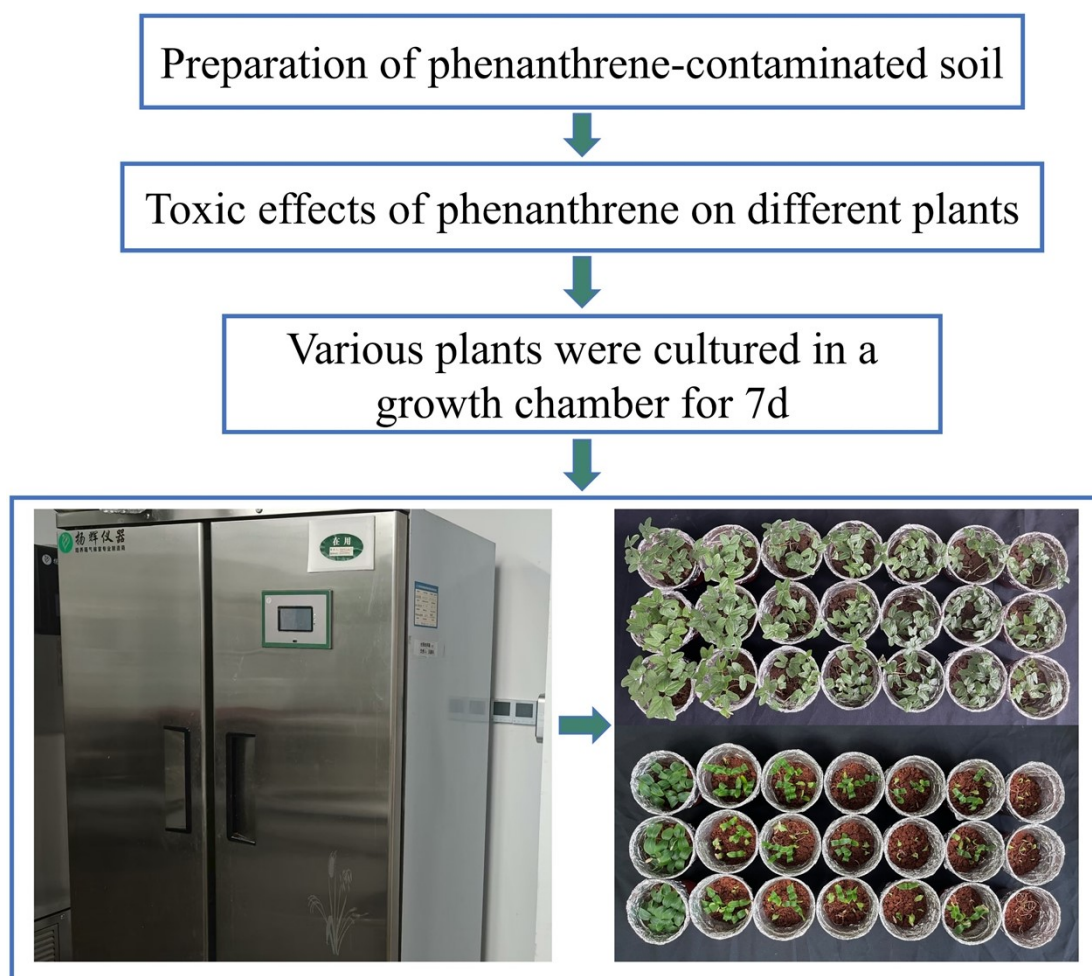


Fig. S23 The experimental setup.

References

- [1] Xing, W., Genxiang, S., Shuangqing, H., Hairong, G., Chunyan, C., Mingyuan, Z., Xiaoxiang, Z Effects of single and joint pollution of chromium (VI) and phenanthrene on microbiological enzyme activities in soil[J]. Journal of Agro– Environment Science, 2016, 35(7): 1300-1307.
- [2] Cui, C. Study on ecotoxicological effects ofchromium(vi) and phenanthrene combinedpollution on soil biont and its hazardassessment methods [D]. Donghua University, 2016.
- [3] Roelofs D, Bicho R C, de Boer T E, et al. Mechanisms of phenanthrene toxicity in the soil invertebrate, *Enchytraeus crypticus*[J]. Environmental toxicology and chemistry, 2016, 35(11): 2713-2720.
- [4] Sverdrup L E, Krogh P H, Nielsen T, et al. Relative sensitivity of three terrestrial invertebrate tests to polycyclic aromatic compounds[J]. Environmental Toxicology and Chemistry: An International Journal, 2002a, 21(9): 1927-1933.
- [5] Sverdrup L E, Nielsen T, Krogh P H. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility [J]. Environmental science & technology, 2002b, 36(11): 2429-2435.
- [6] Amorim M J B, Oliveira E, Teixeira A S, et al. Toxicity and bioaccumulation of phenanthrene in *Enchytraeus albidus* (*Oligochaeta: Enchytraeidae*)[J]. Environmental toxicology and chemistry, 2011, 30(4): 967-972.

- [7] Nota B, Bosse M, Ylstra B, et al. Transcriptomics reveals extensive inducible biotransformation in the soil-dwelling invertebrate *Folsomia candida* exposed to phenanthrene[J]. *Bmc Genomics*, 2009, 10: 1-13.
- [8] Sverdrup L E, Jensen J, Krogh P H, et al. Studies on the effect of soil aging on the toxicity of pyrene and phenanthrene to a soil-dwelling springtail[J]. *Environmental Toxicology and Chemistry: An International Journal*, 2002c, 21(3): 489-492.
- [9] Sverdrup L E, Ekelund F, Krogh P H, et al. Soil microbial toxicity of eight polycyclic aromatic compounds: effects on nitrification, the genetic diversity of bacteria, and the total number of protozoans[J]. *Environmental Toxicology and Chemistry: An International Journal*, 2002d, 21(8): 1644-1650.
- [10] Droge S T J, Paumen M L, Bleeker E A J, et al. Chronic toxicity of polycyclic aromatic compounds to the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*[J]. *Environmental Toxicology and Chemistry: An International Journal*, 2006, 25(9): 2423-2431.
- [11] Sverdrup L E, Jensen J, Kelley A E, et al. Effects of eight polycyclic aromatic compounds on the survival and reproduction of *Enchytraeus crypticus* (Oligochaeta, Clitellata)[J]. *Environmental Toxicology and Chemistry: An International Journal*, 2002e, 21(1): 109-114.
- [12] Hu S, Gu H, Cui C, et al. Toxicity of combined chromium (VI) and phenanthrene pollution on the seed germination, stem lengths, and fresh weights of higher plants[J]. *Environmental Science and Pollution Research*, 2016, 23: 15227-15235.

- [13] Lors C, Ponge J F, Aldaya M M, et al. Comparison of solid-phase bioassays and ecoscores to evaluate the toxicity of contaminated soils[J]. *Environmental Pollution*, 2010, 158(8): 2640-2647.
- [14] Paumen M L, Stol P, Ter Laak T L, et al. Chronic exposure of the oligochaete *Lumbriculus variegatus* to polycyclic aromatic compounds (PACs): bioavailability and effects on reproduction[J]. *Environmental science & technology*, 2008, 42(9): 3434-3440.
- [15] Tourinho P S, Waalewijn-Kool P L, Zantkuijl I, et al. CeO₂ nanoparticles induce no changes in phenanthrene toxicity to the soil organisms *Porcellionides pruinosus* and *Folsomia candida*[J]. *Ecotoxicology and environmental safety*, 2015, 113: 201-206.
- [16] Castro-Ferreira M P, Roelofs D, van Gestel C A M, et al. *Enchytraeus crypticus* as model species in soil ecotoxicology[J]. *Chemosphere*, 2012, 87(11): 1222-1227.
- [17] Sverdrup L E, De Vaufleury A, Hartnik T, et al. Effects and uptake of polycyclic aromatic compounds in snails (*Helix aspersa*)[J]. *Environmental Toxicology and Chemistry: An International Journal*, 2006, 25(7): 1941-1945.