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Identification of the estrogen-active compounds via integrating effect-directed analysis and non-target screening in soils of the northeastern China

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Abstract

Background The gaps between estrogenic effect and its effect-active compounds exist frequently due to a large number of compounds that have been reported to induce this effect and the occurrence of pollutants in environments as mixtures. Therefore, identifying the estrogen-active compounds is of importance for environmental management and pollution treatment. In the current study, the effect-directed analysis (EDA) and non-targeted screening (NTS) were integrated to identify the estrogen-active compounds in soils of the rural area with different socioeconomic types (industrial, farming and plantation village) in Northeast China.

Results The cytotoxicity results indicated that the industrial and farming villages showed cytotoxic effects. The detection rates of estrogenic effects for samples of winter and summer were 100% and 87%, respectively. Of which, the effects were found to be stronger in summer than in winter, with significant difference observed from the farming village (0.1–11.3 EEQ μg/kg dry weight). A total of 159 chemicals were detected by NTS. By integrating EDA, triphenyl phosphate (TPhP) and indole were successfully identified from a raw sample and its fraction, explaining up to 19.31% of the estrogen activity.

Conclusions The present study demonstrates that the successful identification of seven estrogen-active compounds in rural areas of northeastern China can be achieved through the combination of effect-directed analysis (EDA) and non-targeted screening (NTS). This finding is beneficial for risk monitoring and pollution management.

Keywords Soil, Risk assessment, Estrogen-active compounds, Effect-directed analysis, Non-target screening

Background

Estrogens are biologically active hormones that are derived from cholesterol and released by the adrenal cortex, testes, ovary and placenta in humans and animals [1]. Natural estrogens including estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-estradiol (17α) [2], are mainly derived from human and livestock excretion [3]. Many synthetic chemicals have estrogen-like effects (e.g., diethylstilbestrol (DES), β-hexachlorocyclohexane (β-HCH), polychlorinated biphenyls (PCBs), 4-nonylphenol (NP), isoflavones and lignans), which can interfere with the normal synthesis,

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secretion, transport and metabolism of natural estrogens in living organisms. In this study, natural estrogens and synthetic chemicals with estrogen-like effects are collectively referred to as "estrogen-active compounds". Estrogen-active compounds have high estrogenic activity, thus affecting the normal physiological functions of living organisms or humans, even at nanomolar concentrations [4, 5].

Identification of the accurate estrogen-active compounds is a prerequisite for effective control. To date, many methods have been developed for detecting the estrogens, including targeted and non-targeted detections. Both of which employ chromatographic techniques coupled with mass spectrometry, such as liquid chromatography and mass spectrometry (LC–MS) [6–8] and gas chromatography and mass spectrometry (GC–MS) [9–11]. Targeted detection can identify specific estrogens with high sensitivity. In turn, non-target screening (NTS) is developed, for detecting both known and unknown compounds in samples that rely on mass spectrometry data acquired from the sample, without references [12]. However, only targeted detection or NTS cannot identify sufficiently which substances contributed the major proportion for the estrogenic effects in environment. Hence, bioanalytical tests, including both in vitro and in vivo assays, are used for compensating this limitation and are more accurately represent the integrated biological effects of estrogen [13], of which, yeast estrogen assay (YES) is widely used [14]. The integration of chemical and biological analyses has demonstrated promising applications in the detection of estrogen [15–17].

Environmental samples are complex mixtures that encompass a variety of compounds. Neglecting mixture effects can lead to an underestimation of chemical risks, as mixtures in complex environments often contain estrogen-active compounds. Therefore, the risks associated with mixtures should not be overlooked during detection. The quantity and composition of contaminants change over time, and certain compounds may display toxicity even below their individual effect thresholds or analytical detection limits [18]. For, example, Carina et al. discovered significant temporal variations in the distribution of E2 in the northern South China Sea [19], and E2 was also found to undergo transformation into E1 in soil [20]. Effect-directed analysis (EDA) serves to identify risk factors within intricate mixtures and to separate bioactive chemicals that might otherwise be concealed by matrix effects [21]. EDA is an effective method for analyzing complex environmental samples that combines biological testing and chemical analysis [22, 23]. Simplifying the sample complexity and narrowing down the array of potentially toxic substances is a fundamental principle of EDA, essential for the successful identification of harmful substances [24]. The objective can be accomplished through the application of fractionation. After biological testing, fractionation can be employed to reduce the complexity of the sample by isolating the portion with significant effects [21]. If necessary, multiple fractionation steps can be performed until the separated fractions are identified through targeted and non-targeted chemical analyses [23]. Estrogenic compounds, assessed based on their effects, can be more accurately reflected for their environmental impact through the application of EDA. The concurrent application of EDA and NTS overcomes the constraints associated with solely conducting NTS, because it fills the information gap regarding the potential toxicity of compounds in environmental samples [25]. The successful application of a combination of EDA and NTS has been demonstrated in the identification of toxic chemicals in dust, wastewater, sewage sludge, and other environmental samples [26–28].

In the rural regions of northeastern China, a multitude of traditional small-scale farms operate without adequate regulations and disposal practices. Consequently, wastewater and manure from livestock farming are released and accumulate haphazardly in fields or near these farms. The existence of estrogenic compounds in these discharges poses substantial risks to both the local environment and the health of residents. The objective of this study was to employ the EDA for the identification of estrogen-active compounds in soil. For this purpose, soil samples were collected from farming, industrial, and plantation villages in Northeast China based on the local socioeconomic types. The farming village is the primary area for livestock and poultry farming, the planting village is the main region for crop cultivation, and the industrial village is the primary area for mineral extraction activities. Soil estrogenic activity were evaluated via YES, and were compared between summer and winter for the three village types. The study is helpful to improve the environmental monitoring and pollution treatment by estrogenic contamination risk.

Materials and methods

Chemicals

E2 (98% of purity) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, 98% of purity) were purchased from Aladdin (Shanghai, China). Dimethyl sulfoxide (DMSO, purity > 99.5%) purchased from Sigma-Aldrich. H4IIE rat hepatoma cells were kindly provided by the College of Environment & Resource Sciences of Zhejiang University. The recombinant yeast cells were provided by the Research Center for Eco-Environmental Science, Chinese Academy

of Sciences. All solvents used for sample processing and analyses (n-hexane, acetone, acetonitrile and methanol) were HPLC grade.

Study area and field sampling

Field sampling was conducted in Anshan City, Liaoning Province of China during August 2020 (summer) and January 2021 (winter), respectively. The sampling sites are situated in Northeast China, in the middle latitude zone, significantly influenced by seasonal freezing and thawing processes. The area falls within the temperate continental monsoon climate zone, characterized by well-developed agriculture, animal husbandry, and abundant mineral resources. Activities such as village life, livestock farming, crop cultivation, and mining have resulted in severe organic pollution in the rural areas and towns. The sampling sites were located in Fengyuan village (FY, Pianling town), Goumen village (GM, Chaoyang town) and Daling village (DL, Chaoyang town) (Fig. 1), which can be classed as industrial, farming and plantation village based on the production and lifestyle characteristics. All sampling sites were located in cultivated areas near residential areas. A total of 22 soil samples were collected in aluminum foil bags (26×36 cm), transported to the laboratory under cold conditions (4 °C), and then stored in a refrigerator (− 80 ℃) for use (see Table 1).

Sample extraction

Soil samples were pre-treated according to the method described by Cha [29], with slight modifications. All samples were freeze-dried, homogenized and ground to pass through a 60 mesh (250 μm) sieve. Twenty grams of freeze-dried soils were extracted for 18 h with hexane: acetone (1:1, v/v) solvent mixture using a Soxhlet extractor (JPSXT-06, Shanghai, China). Afterwards, extracts were rotary-evaporated closely to dryness and re-dissolved in 1 mL of methanol.

Quality assurance (QA) and quality control (QC)

To avoid introducing plastic contamination during the experimental process, plastic products are not used in sampling, sample preparation, and sample analysis. All glassware and instruments used throughout the experiment are washed three times with ethanol and Milli-Q water, then dried at 105 °C for 24 h. During the instrument analysis process, blank samples are prepared using deionized water and acetonitrile following the sample procedure to assess potential instrument residual

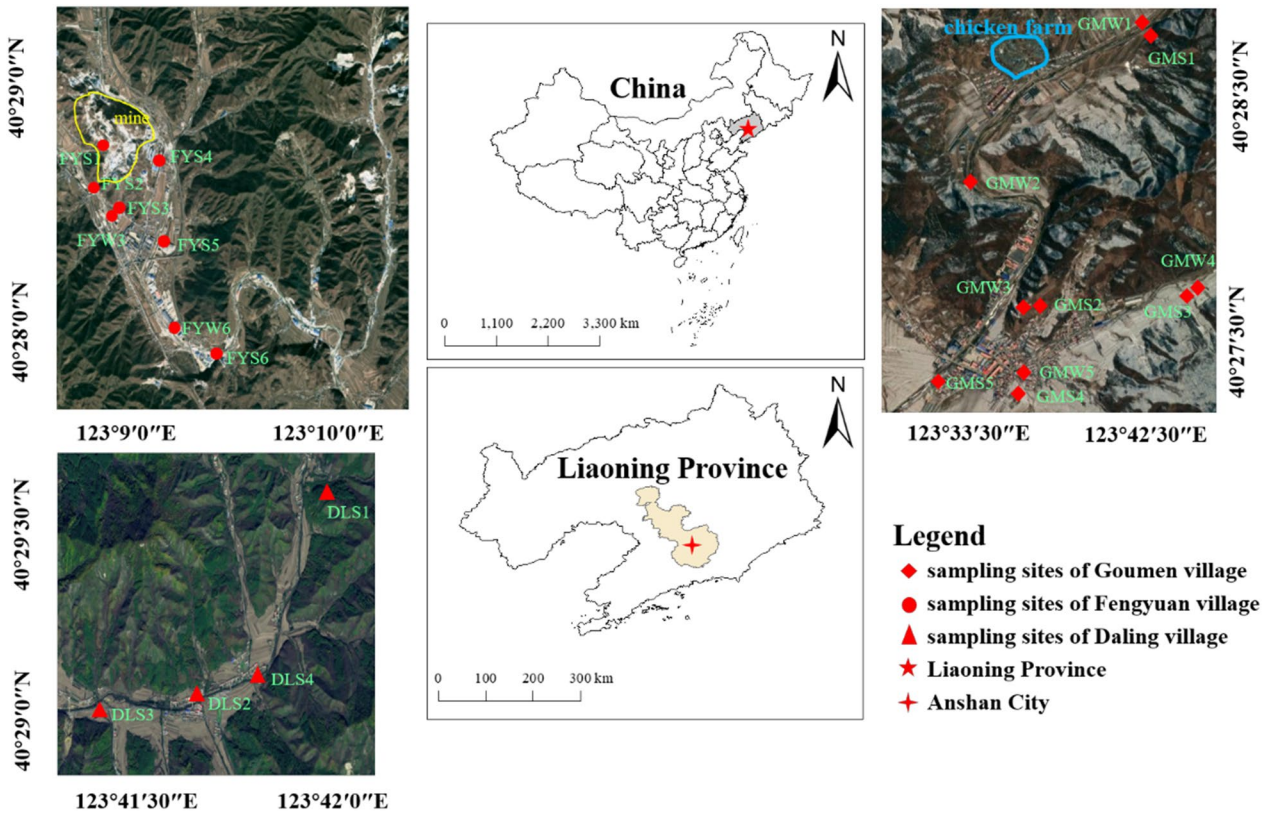

Fig. 1 The location of the study area and sampling sites

Table 1 Sampling sites and descriptive information

Sample code	Sampling date	Longitude	Latitude	Land use
GMS1	August 2020	123.567375	40.477047	Corn cropland
GMS2	August 2020	123.557890	40.460311	Vegetable arable land
GMS3	August 2020	123.569710	40.462388	Corn cropland
GMS4	August 2020	123.560631	40.457163	Corn cropland
GMS5	August 2020	123.550928	40.455371	Corn cropland
GMW1	January 2021	123.561803	40.475719	Riparian
GMW2	January 2021	123.551254	40.468572	Riparian
GMW3	January 2021	123.552300	40.458974	Riparian
GMW4	January 2021	123.564145	40.461070	Cultivated land
GMW5	January 2021	123.555048	40.455832	Cultivated land
FYS1	August 2020	123.154179	40.478143	Cultivated land
FYS2	August 2020	123.150755	40.486239	Arable land
FYS3	August 2020	123.159914	40.479745	Corn cropland
FYS4	August 2020	123.169742	40.489276	Corn cropland
FYS5	August 2020	123.168474	40.477697	Grassland
FYS6	August 2020	123.179189	40.458461	Corn cropland
FYW3	January 2021	123.154179	40.478143	Cultivated land
FYW6	January 2021	123.160951	40.467974	Cultivated land
DLS1	August 2020	123.709129	40.494304	Grassland
DLS2	August 2020	123.693161	40.479381	Cultivated land
DLS3	August 2020	123.678729	40.480573	Corn cropland
DLS4	August 2020	123.698699	40.481174	Grassland

contamination, and blanks are subtracted in subsequent analyses.

Cytotoxicity assay

Micro-level cytotoxicity studies focus on the direct impact of compounds or factors on individual cells, whereas the macro-level distribution of estrogen involves widespread effects within the organism. Estrogens exert their actions by binding to specific receptors, the estrogen receptors (ERs), which in turn activate transcriptional processes and/or signaling events that result in the control of gene expression [30]. Micro-level cytotoxicity studies can reveal the direct effects of estrogen on individual cells.

In order to avoid cytotoxic effects in the estrogenic effects test, the MTT assay was carried out with H4IIE rat hepatoma cells according to the method described by Shao et al. [31], with slight modifications. Cells were cultured at 37 °C with 5% CO_2, and 95% humidity in Dulbecco's modified Eagle's medium (DEME, BI) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin–streptomycin solution (PS, BI). The maximum concentration of the sample extract is 20 mg/mL, successively halved to 7.81E−02 mg/mL. Before exposure, a volume of 100 µL/well of the cell suspension at a density of 2×10^4 cells/mL was seeded in 96-well plates for 24 h at 37 °C. After 24 h incubation, the cells were exposed to a 1:2 dilution series of each sample in triplicate [32]. After 48 h exposure, 100 µL of MTT (0.5 mg/mL) solution was added to each well and incubated for 30 min. Then the MTT media was exchanged with 200 µL/well DMSO. Cell viability estimation was performed with the absorbance at 492 nm using a microplate reader (Synergy LX, BioTek, USA), which was expressed as the relative survival of exposed cells compared to untreated control cells, and results were given as percentage of control.

Estrogenicity evaluation

The two-hybrid yeast screen systems were used to assess the estrogen receptor-mediated endocrine disruptive effects of environmental materials, and the estrogen agonist activities of the samples were determined by measuring-galactosidase activity [33]. The yeast assay was conducted as described by Ma [34], with slight modifications. Briefly, yeast was incubated overnight at 30 °C until the logarithmic growth phase, the final culture was adjusted to an optical density (OD_{600}) of 0.75, and then exposed to a 1:2 dilution series of samples in 96-well plates for 4 h. The highest concentration of the tested samples was defined based on the MTT assay. A dilution series of E2 (4, 8, 20, 40, 80, 200, 400, 800, 2000 pM) and a DMSO solvent control were included in each

experiment. Cell density estimation was performed at 600 nm and the exposure was terminated by addition of sodium carbonate (Na_2CO_3). β-Galactosidase activities were determined by the absorbance at 420 nm, and calculated according to the following equations [35]:

$$U = \frac{OD_{420} - OD^{'}{}_{420}}{t \times V \times OD_{600}} \times D,$$

where U is the β-galactosidase activity. t, V and D are enzyme reaction time, volume and diluting factor. OD_{600} is the absorbance measured at 600 nm, OD_{420} and OD'_{420} are absorbance at 420 nm for sample exposure group and negative control, respectively. Each sample was tested at nine 1:2 serial dilutions. All samples were analyzed with three replications, of which each with three internal replicates.

Fractionation

The samples were fractionated using an HPLC-fractionation collector with an Agilent HC-C18 column (4.6 × 150 mm, 5 µm, Thermo Fisher Scientific, USA) and acetonitrile as the mobile phase. The column temperature was 35 °C, the injection volume was 100 µL, the flow rate was 500 µL/min, and the total fractionation duration was 18 min. Fractions were collected in a 24-well plate, separating by every 3 min. Fractions were transferred to a 2 mL vial and blown dry with nitrogen. The fractionation process was done with twice for each sample, of which one fraction was fixed to 100 µL with DMSO for estrogenic activity assay, and the other one was fixed to 100 µL with methanol for NTS.

Chemical analysis

Chemicals identification analysis was conducted on an UPLC-QExactive Plus orbitrap-HRMS (Thermo Fisher Scientific, USA) with an electrospray ionization (ESI) source. The liquid chromatograph column was a Hypersil Gold C18 (2.1 × 100 mm, 3.0 µm, Thermo Fisher Scientific, USA) with the column temperature of 40 °C during the measurement. The injection volume was 10 µL, and the binary mobile phases were water (A) and acetonitrile (B) and both containing 0.1% formic acid, the flow rate was 400 µL/min. The gradient elution model was carried out according to the following conditions: 0–0.5 min (95% A phase, 5% B phase), 0.5–20 min (0% A phase, 100% B phase), 20–21 min (0% A phase, 100% B phase), 21–21.1 min (95% A phase, 5% B phase), 21.1–25 min (95% A phase, 5% B phase). The electron spray voltage was 40 V, the electrospray flow rate was 50 L/h, the ion source temperature was 120 °C, the desolvent temperature was 350 °C, the scanning frequency was 5 Hz, and the excitation voltage was 10/30/50 V. NTS was performed using Compound Discoverer 3.2 (Thermo Fisher Scientific, USA) software. These chemicals were screened in the mzCloud database using the following criteria: deviation of primary parent ion mass number less than 5×10^{-6}, deviation of retention time less than 0.2 min, and isotope matching threshold within 30%. Finally, the compounds were screened according to the requirements of peak area > 1×10^5 and pairing fraction > 60.

The "Environmental w Stats Unknown ID w Online and Local Database Searches" analysis process in Compound Discoverer 3.2 software was used to screen for unknowns, with appropriate modifications (Additional file 1: Table S1). The core part of this workflow is to perform mzCloud and Mass Lists searches to detect unknown compounds. To reduce the interference of false positive results, the Mass Tolerance were set to 5 (ppm) and the S/N Threshold to 3 at the Detected Compounds node. After performing the analytical workflow, the results were analyzed and identified with the identification criteria reference. The first step was to subtract the Sample/Blank ratio less than 5, in order to reduce the number of false positive results and the workload of manual identification. The second step was to filter the mzCloud matches with scores less than 60. Lists is a mass spectral information added to the local database by itself. After the previous steps, the secondary fragment matches need to be manually screened to remove compounds that only have parent ion fragment features that can be matched to the library.

Statistics analysis

Microsoft Excel™2019 and OriginPro 2023 (Origin Lab Corporation) were used for data sorting and mapping, respectively. Kruskal–Wallis ANOVA with Dunn's test was used for multiple comparisons when the assumptions of homogeneity of variances and normal distribution were not met. The level of significance was set at $p \leq 0.05$.

To determine the EC_{20} and EC_{50} of E2 and environmental samples in the YES assay, dose–response curves for concentration versus β-galactosidase activity were plotted and fitted using GraphPad Prism (version 8.0, USA). The cytotoxic effect was also curve-fitted using this software. The estrogenic equivalent (EEQ) was calculated using the following equation [36]:

$$EEQ_{bio} = \frac{EC_{20,ref}}{EC_{20,sample}},$$

where $EC_{20,sample}$ is the concentration corresponding to the sample when the estrogenic activity is equal to the estrogenic activity at the EC_{20} concentration of E2.

Results

Cytotoxicity

Cytotoxicity is a disturbance that can affect the assessment results of estrogenic activity [37]. As can be seen from Additional file 1: Fig. S1, the samples from the plantation village did not exhibit cytotoxicity to H4IIE cells, with cell survival rates amounted above 80%. In the industrial village, samples FYS1, FYS2, FYS4, and FYW3 induced slightly cytotoxic effects, with cell survival rates of 74.79%, 78.92%, 77.62%, and 72.73%, respectively (Additional file 1: Fig. S2). Among the samples collected from the farming village, only GMS3 and GMW1 showed significantly cytotoxicity (Additional file 1: Fig. S3). The cell viability sharply decreased starting from an exposure concentration of 2.5 g/L and reaching below 50% at an exposure concentration of 20 g/L in GMW1. With GMS3, the cell viability slightly drop below 80% only at the highest exposure concentration (20 g/L). Overall, cytotoxic effects on H4IIE cells were detected at the highest exposure concentration, leading to cell survival rates of 77.05% and 33.87%, respectively.

Estrogenic activities

The estrogenic activity of soil extracts was assessed through the YES test employing two-hybrid yeast screen systems. The concentration of soil extract varied from 0.39 g/L to 100 g/L. Concentration–effect curves were generated, and the samples' estradiol equivalents (EEQ) were computed based on the EC_{20} of E2 [38]. As shown in Fig. 2, estrogenic activity was detected in all samples collected from the plantation village, with EEQ ranging from 0.21 to 2.24 μg/kg dry weight (Additional file 1: Table S2). Sample DLS2 exhibited the highest estrogenic effect, while DLS3 demonstrated the lowest estrogenic effect, with the former's EEQ being ten times more than that of the latter.

Concerning the industrial village, the mean EEQ values were 0.73 μg/kg d.w. in winter samples and 0.52 μg/kg d.w. in summer samples, with no significant difference. FYS6 exhibited the strongest estrogenic effect, while FYW3 and FYW5 did not show significantly estrogenic effects (Fig. 3).

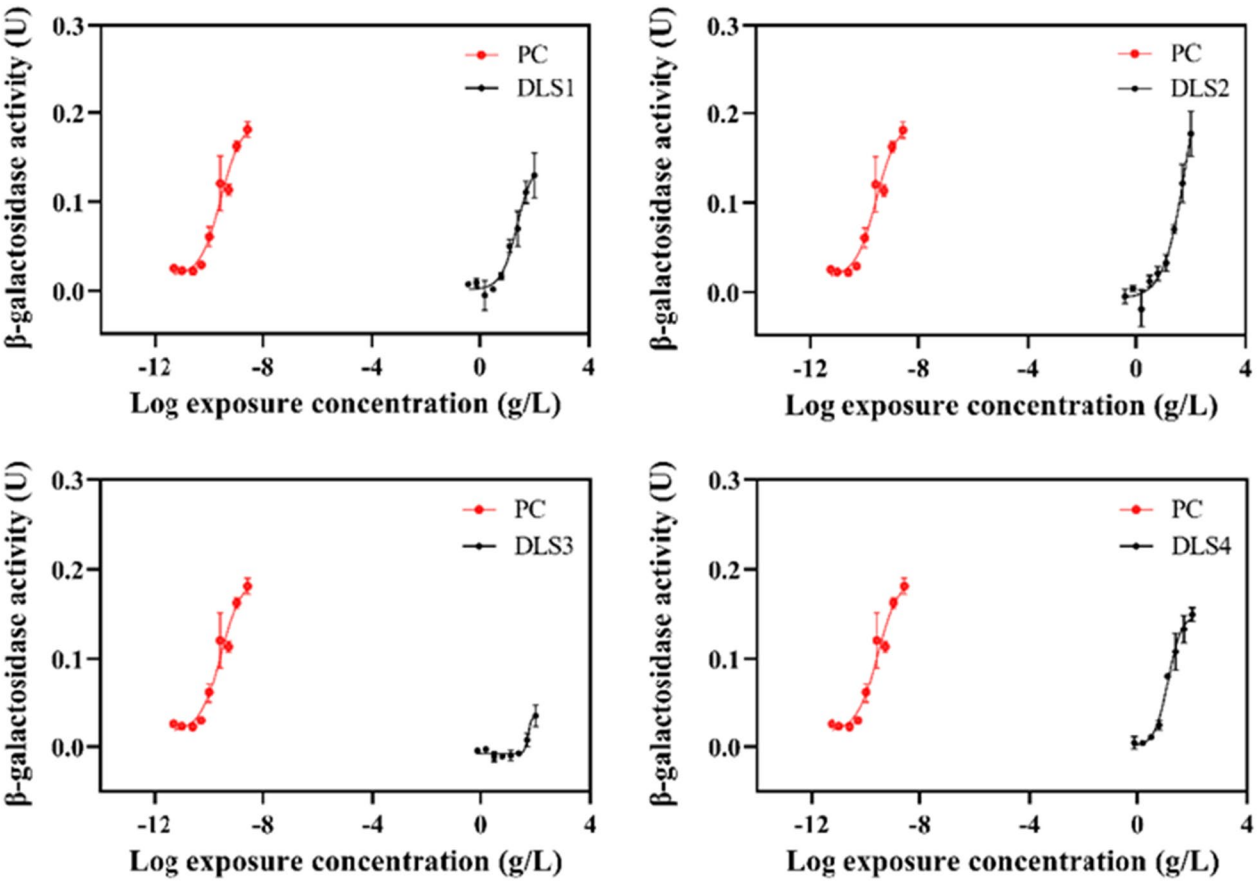

Fig. 2 Concentration–response curves in the YES assay for the soil in the plantation village (black closed circles), and the E2 standard (red closed circles). Concentration values on the x-axis refer to the actual exposure of the sample in the experiment. PC: positive control, DLS: Daling Summer

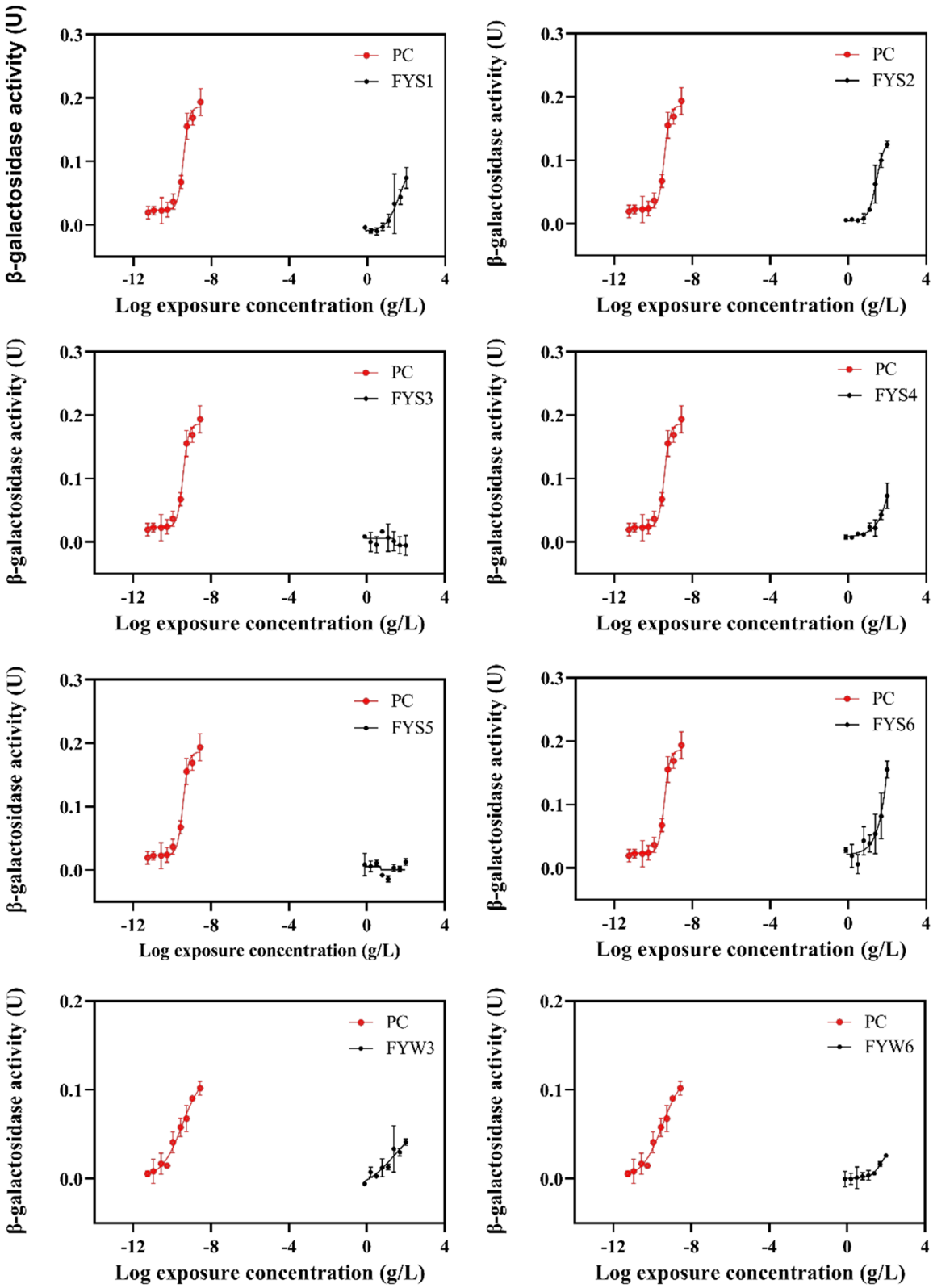

Fig. 3 Concentration–response curves in the YES assay for the soil in the industrial village (black closed circles), and the E2 standard (red closed circles). Concentration values on the x-axis refer to the actual exposure of the sample in the experiment. PC: positive control, FYS: Fengyuan Summer, FYW: Fengyuan Winter

Regarding the farming village samples, GMS1 demonstrated the highest estrogenic activity (EEQ = 11.3 μg/kg dry weight), followed by GMS2, which is closer to the farms. Conversely, samples of GMS3, GMS4, and GMS5 showed mild estrogenic activity and were collected from the riverbank along this village (Fig. 4). The research findings of Song et al. indicate that the concentration of E1 in the soil near a farm in Shenyang, China, reached 15.15 μg/kg d.w., which is similar to the results of this study [39].

The EEQ of the summer samples from the farming village is significantly greater than those from the plantation and industrial villages. The average EEQ between the industrial village and the plantation village did not show significant difference (Fig. 5).

After fractionating sample DLS4, six fractions (G1–G6) were obtained. Estrogenic effect testing revealed that G2 exhibited estrogenic activity (EEQ of 0.28 μg/kg d.w.), while the other fractions did not demonstrate estrogenic effects in significant level. The ∑EEQ of the fractions accounted for 19.31% of the original sample's EEQ. Nakada et al. [40] found that estrogenic activity in Fraction 3 of municipal sewage treatment plant (STP) secondary effluent accounted for 10% of the total EEQ of the original sample, which is comparable to the results of this study.

Non-target screening

Hollender et al. [41] defined the general workflow of non-targeted screening as: sampling, analysis, data preprocessing, prioritization, identification. In this study, the Soxhlet extraction method was chosen for sample processing to extract compounds from the soil. Sample analysis employed UPLC-QExactive Plus Orbitrap-HRMS, and data pre-processing utilized Thermo Fisher's Compound Discover 3.2. Estrogen-active compounds were identified as the highest priority, and all identified estrogen-active compounds were classified based on Schymanski et al. [42].

Considering sample DLS4 (EEQ of 1.45 ng/g d.w.) is in the middle of the range, and its environment is more representative of a rural environment, DLS4 was selected as the sample for the NTS. To identify the pinpoint the estrogenic effects induced compounds, the sample of DLS4 was selected and NTA was employed for DLS4 and its six fractionations. A total of 159 chemicals in sample DLS4 were identified, with detailed information available in Additional file 1: Table S3. From Additional file 1: Fig. S4, it can be observed that each fraction was screened for 13–45 organic chemicals, with the highest quantity found in G2, consistent with the estrogenic effect results. Out of these 159 chemicals, 7 estrogenic compounds, including triphenyl phosphate (TPhP) [43], bis (2-ethylhexyl) phthalate (DEHP) [44], indole [45], daidzein [46], genistein, naringenin and glycitein [47] were confirmed. The fractions detection indicated TPhP and indole were found in G2, DEHP in G4, and indole in G6. Conversely, no estrogenic active substances were identified in the remaining fractions (G1, G3, and G5).

Discussion

Potential risk of cytotoxicity

In the current study, GMW3 exhibited the strongest cytotoxicity, while GMW1, GMS1, and GMW2, which are closer to the poultry farm, did not show significant cytotoxic effects. The reason may be due to the pollutants transport along the river the longitudinal gradients. The GMW3 is relatively closer to the chicken farm and may be influenced by the wastewater and feces from the chicken farm [48]. Soil undergoes changes in physical, chemical, and biological properties during the freezing process, slowing down the migration and transformation of organic pollutants in the soil [49], leading to the accumulation of organic pollutants in the soil, consistent with the cytotoxicity results of this study.

Effects of soil environment on estrogenic activities

Chicken and duck manure contain a significant amount of natural and synthetic estrogenic compounds [50, 51], leading to estrogen pollution in farms and the surrounding soil. Hence, the primary origin of estrogenic-active compounds in GMS1 and GMS2 is predominantly livestock farming excreta. The predominant source of estrogens in livestock farming comprises excretions and blends of steroids derived from raw materials or veterinary medicine [52, 53].

The agricultural industry plays a crucial role in Liaoning province of China, encompassing the cultivation of various crops including soybeans, corn, and wheat, etc. The northeastern region of China experiences long and harsh winters [54], where low temperatures and insufficient rainfall during the winter limit the growth of crops. Therefore, this region engages in more frequent agricultural activities during the summer. Studies have indicated that certain pesticides may induce estrogenic activity [55, 56], and extensive pesticide use is involved in summer agricultural practices in this region [57]. Chemical pesticides and fertilizers used in agricultural activities may contain estrogen-active compounds. These compounds can enter water bodies, accumulate in crops, and ultimately enter the human body, posing risks to human health. Therefore, the estrogenic risks associated with pesticides and irrigation wastewater should not be overlooked.

The application of animal manure to agricultural land has been identified as a main source of estrogens in the

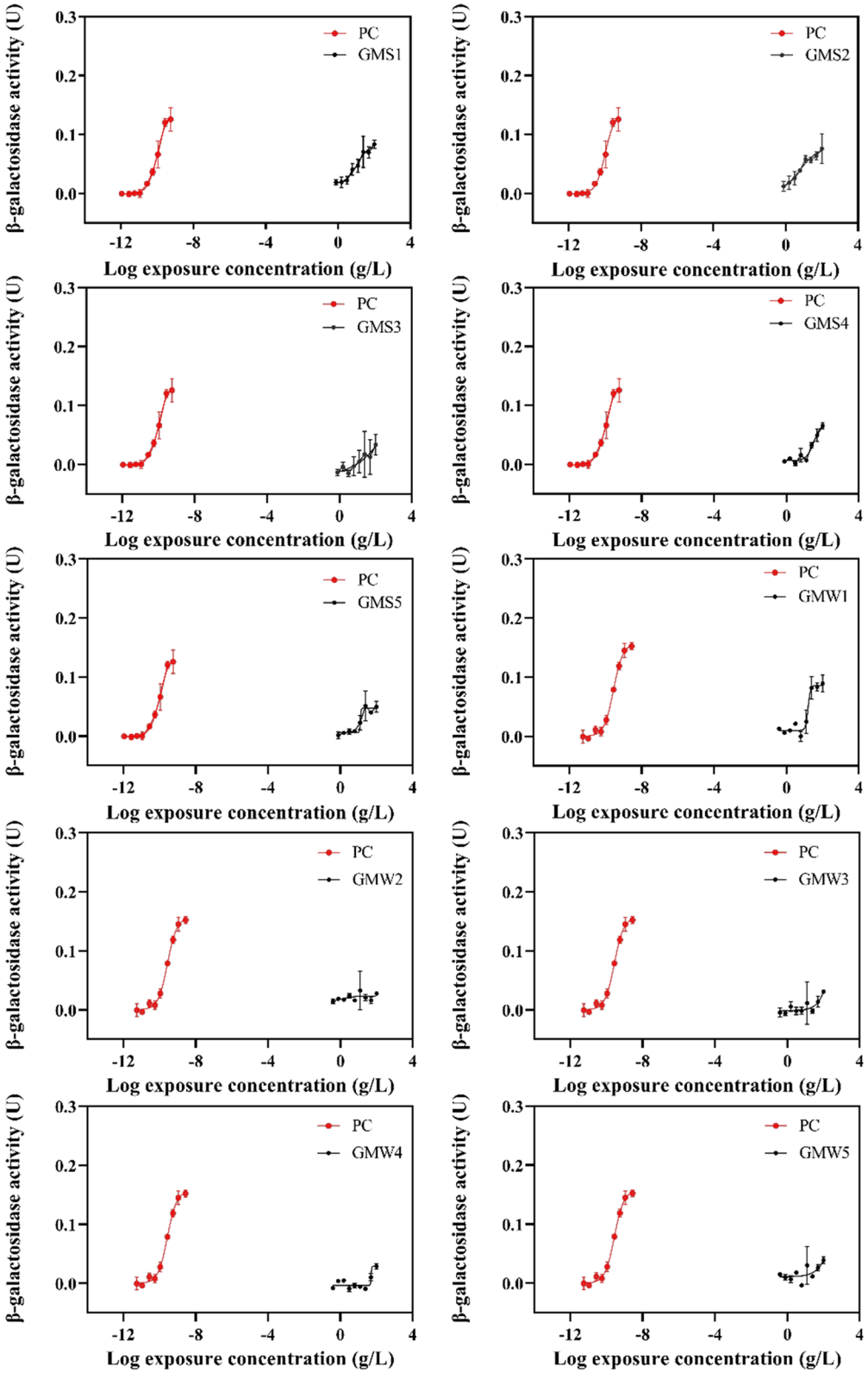

Fig. 4 Concentration–response curves in the YES assay for the soil in the farming village (black closed circles), and the E2 standard (red closed circles). Concentration values on the x-axis refer to the actual exposure of the sample in the experiment. PC: positive control, GMS: Goumen Summer, GMW: Goumen Winter

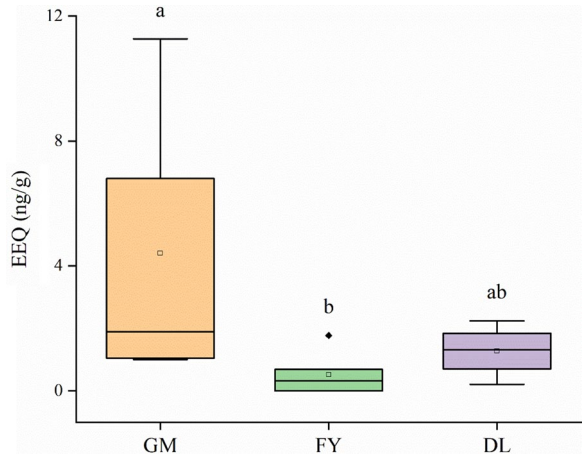

Fig. 5 Box chart of estrogen equivalent (EEQ) comparison of samples from different villages in summer. FY: FengYuan village; GM: Goumen village; DL: Daling village. The different letters above the column indicate significant difference at the $p < 0.05$

environment [58]. In urban areas, livestock and poultry are typically raised on a large scale, and waste generated undergoes centralized management. In rural areas, however, most farming is done by individual households, and the waste is often disposed of openly or directly released into fields. While the former generates a larger quantity of waste, it generally causes minimal or no pollution to the environment after proper treatment. The latter, on the other hand, can result in more significant pollution. Rural areas predominantly consist of open soil, leading to faster migration of estrogen-active compounds between soil, surface water, and groundwater. In contrast, the presence of hardened roads in urban areas slows down the migration process.

Currently, numerous studies have confirmed that plasticizers exhibit estrogenic effects [59–61]. Microplastics can adsorb estrogenic compounds. The higher the crystallinity, the lower the adsorption capacity [62], thereby affecting the migration of estrogenic compounds in the environment. Agricultural cultivation involves the extensive use of plastic films. Without effective measures for disposal, aged plastics are more prone to adsorb estrogenic compounds, and accumulate in soil.

Studies have indicated that kaolin and montmorillonite have different adsorption capacities for E2 [63]. It has been reported that the wastewater discharged from mining areas contains nonylphenol, which is a chemical with estrogenic effects [64]. Mining activities can disrupt the original structure and distribution of ores, affecting the migration and transformation of estrogenic compounds in these areas. This may be a significant factor contributing to the substantial differences in estrogenic activity observed in the industrial village.

Freezing can provide a stable environment for soil and reduces the transportation of organic compounds, while creating a fluid environment in the thawed state and promoting the substances' transportation [65], resulting in a greater concentration of estrogen in soil in summer than in winter. Soil freezing can cause soil expansion and the formation of ice lenses, resulting in soil cracking and an increase in the soil infiltration coefficient [66]. As a result, the decrease of estrogenic active contaminants in winter soils may be due to low hydrophobicity.

Identification of estrogenic active compounds

The detection of two estrogenic compounds (TPhP and indole) in fraction G2, coupled with the ability of this fraction to induce estrogenic effects, suggests that these two compounds may be the primary substances responsible for the estrogenic activity in this fraction. Many bacteria and plants produce substantial amounts of indole, and higher concentrations of indole are found in the excrement of animals such as dogs, pigs, and cattle [67]. It has been reported that derivatives of indole may also contribute to various human diseases, including bacterial infections, gastrointestinal inflammation, neurological disorders, diabetes, and cancer [68]. TPhP, as a flame retardant widely used in various everyday chemical products, is frequently detected in the environment [69, 70]. TPhP accumulates in human and animal bodies, inducing endocrine disruption. It has been reported to induce toxicity to the reproductive systems of wild fish populations at environmental concentrations, pose ecological risk [71]. Moreover, studies have found a significant correlation between the lipid content in the human body and high levels of TPhP [72]. The estrogenic pollution induced by TPhP and indole deserves attention.

Although estrogenic compounds were detected in both G4 and G6, the absence of estrogenic effects in these two fractions may be attributed to their low concentrations, which may not be sufficient to induce estrogenic effects. Some natural estrogenic compounds may have been overlooked during the pretreatment process (Soxhlet extraction), and we will strive to consider these aspects in the future to detect a wider range of estrogen-active compounds. We will conduct further research on these natural estrogenic compounds in the future to explore their effects on the ecological environment and human health.

In the future, we will investigate other identified compounds to determine if they exhibit estrogenic effects and explore the mechanistic reactions they have in

comparison to the seven already established estrogen-active compounds.

Conclusions

In this study, the potential ecological risk in soil of the Northeast China was evaluated by cytotoxicity and estrogen effect, among different rural socioeconomic types and between summer and winter. The results indicated that the industrial and farming villages may be cytotoxic to H4IIE rat hepatoma cells, which the stronger cytotoxic effects were found in winter; whereas, the effects of estrogenic were found to be stronger in summer, with significantly difference observed from the farming village (0.1–11.3 EEQ μg/kg d.w.). The estrogenic active compounds were successfully identified by EDA, in which Indole and TPhP were identified from both raw sample and the fraction by NTS, with the explanation of estrogen activity accounting for 19.31% of the raw sample. Therefore, the current study is helpful for preparing measurements for estrogenic risk control.

Abbreviations

EDA	Effect-directed analysis
NTS	Non-targeted screening
TPhP	Triphenyl phosphate
E1	Estrone
E2	17β-Estradiol
E3	Estriol
17α	17α-Estradiol
DES	Ethinyl estradiol hexenestrol
β-HCH	β-Hexachlorocyclohexane
PCBs	Polychlorinated biphenyls
NP	4-Nonylphenol
LC–MS	Liquid chromatography and mass spectrometry
GC–MS	Gas chromatography and mass spectrometry
YES	Yeast estrogen screen assay
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
DMSO	Dimethyl sulfoxide
EEQ	Estrogenic equivalent
FY	FengYuan village
GM	Goumen village
DL	Daling village
DEHP	Bis (2-ethylhexyl) phthalate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-00885-x.

Additional file 1: Figure S1. Toxic effects of the soil extract on viability of H4IIE cells in the Daling village. **Figure S2.** Toxic effects of the soil extract on viability of H4IIE cells in the Fengyuan village. **Figure S3.** Toxic effects of the soil extract on viability of H4IIE cells in the Goumen village. **Figure S4.** Organic compounds detected in original sample and fractions. **Table S1.** Parameters set in the Compound Discoverer workflow for the non-target analysis. **Table S2.** Estrogen equivalents (EEQ) in the samples. **Table S3.** Non-target screening results of the selected sample DLS4.

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Author contributions
QF did the laboratory analyses, wrote—original draft, and prepared the figures. FF and JSG contributed to the conceptualization, funding acquisition and supervision. QF, LY, JC and FL processed the data. YS and ZLC edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agreed to publish the paper.

Competing interests
The authors declare that they have no competing interests.

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