Contamination of Phthalate Esters (PAEs) in Typical Wastewater-Irrigated Agricultural Soils in Hebei, North China

Yuan Zhang¹, Qiong Liang², Rutai Gao³, Haobo Hou¹, Wenbing Tan³, Xiaosong He³, Hui Zhang³, Minda Yu³, Lina Ma³, Beidou Xi³*, Xiaowei Wang³

¹ School of Resource and Environmental Science, Wuhan University, Wuhan 430079, China, ² Beijing Key Laboratory of New Technique in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China, ³ State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

* xibeidou@263.net

Abstract

The Wangyang River (WYR) basin is a typical wastewater irrigation area in Hebei Province, North China. This study investigated the concentration and distribution of six priority phthalate esters (PAEs) in the agricultural soils in this area. Thirty-nine soil samples (0–20 cm) were collected along the WYR to assess the PAE residues in soils. Results showed that PAEs are ubiquitous environmental contaminants in the topsoil obtained from the irrigation area. The concentrations of \( \Sigma_{6} \)PAEs range from 0.191 \( \mu \)g g\(^{-1}\) dw to 0.457 \( \mu \)g g\(^{-1}\) dw with an average value of 0.294 \( \mu \)g g\(^{-1}\) dw. Di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DnBP) are the dominant PAE species in the agricultural soils. Among the DEHP concentrations, the highest DEHP concentration was found at the sites close to the villages; this result suggested that dense anthropogenic activities and random garbage disposal in the rural area are possible sources of PAEs. The PAE concentrations were weakly and positively correlated with soil organic carbon and soil enzyme activities; thus, these factors can affect the distribution of PAEs. This study further showed that only dimethyl phthalate (DMP) concentrations exceeded the recommended allowable concentrations; no remediation measures are necessary to control the PAEs in the WYR area. However, the PAEs in the topsoil may pose a potential risk to the ecosystem and human health in this area. Therefore, the exacerbating PAE pollution should be addressed.

Introduction

Phthalate esters (PAEs) are widely used in different products, including industrial and commercial products, such as pharmaceutical pills, detergents, packaging, paints, and pesticides [1–2]. PAEs have caused considerable concern over their widespread distribution and potentially hazardous impact on the environment. The global production is more than 8.0 million tons of PAEs annually [3]. High PAE levels have been explained in terms of various...
environmental matrices [4–9]. The Environmental Protection Agency (EPA) of the USA and the Chinese State Environmental Protection Administration have also considered PAEs as environmental priority pollutants. Three phthalates (dimethyl phthalate (DMP), di-\textit{n}-butyl phthalate (DnBP), di-\textit{n}-octyl phthalate (DnOP)) are also in the black list of China’s water priority pollutants, as identified by the Chinese National Environmental Monitoring Center.

The Wangyang River (WYR) is situated in Shijiazhuang City, the capital of Hebei Province in North China. The 85 km long river is the tributary of the Ziya River, which is affiliated to the Haihe River Basin. Beijing and Tianjin also belong to the region of the Haihe River Basin. The industrial effluents from petrochemical, electronic, and pharmaceutical factories are discharged into the WYR after these effluents are subjected to secondary (biological) treatment. The river water has been used to irrigate farmland soils for more than 20 years [10]. The predominant cropping system is winter wheat (\textit{Triticum aestivum} L.)-summer maize (\textit{Zea mays} L.) rotation that covers 60% of the arable land in this area. However, the contribution of wastewater to the occurrence of PAEs in agricultural soils through irrigation and the biological effects of this contaminant have not yet been determined in the rivers receiving treated and untreated wastewater as the main water sources. Furthermore, the accumulation of PAEs in agricultural soils may contaminate vegetables and food chains; this phenomenon results in direct or indirect human exposure [8]. Moreover, the leaching, evaporation, and migration of PAEs are possible sources of atmospheric or groundwater contamination [3].

The environmental problem in this region has been extensively investigated by scientific and regulatory communities. In this study, the distribution and characterization of PAEs in WYR were determined; the potential PAE sources were also evaluated. Moreover, the effects of the PAE contamination on the soil microbial activities in the wastewater-irrigated fields were analyzed. This study provides the missing data on the distribution of PAEs in the Haihe River Basin and contributes to the assessment of the ecological risk of these pollutants.

### Materials and Methods

#### Sampling

No specific permissions were required for sampling locations/activities. The field studies did not involve endangered or protected species. The solid and liquid samples were collected in June 2013. The soil samples irrigated with river water were collected from thirteen sites along the WYR (Fig 1). The topsoil (0–20 cm) samples were collected in triplicate from each site with a 10 cm diameter soil core sampler. The field-moisture samples were passed through a 2 mm sieve to remove stone and plant residues and then divided into two parts. One part was frozen for biochemical analyses. The other part was air-dried at room temperature and ground for physical and chemical analyses. The river water and sediment samples were also collected from the thirteen sites in the WYR. Three water samples and four sediment samples were obtained at each site. The water samples were collected with a stainless steel bucket and stored in glass bottles at 4°C. Surface sediment samples (0–5 cm) were collected with a grab sampler. All of the samples were transported to our laboratory in an ice box and treated in 7 days.

#### Chemicals and materials

Reference PAEs including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-\textit{n}-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-\textit{n}-octyl phthalate (DnOP), di-(2-ethylhexyl) phthalate (DEHP) were purchased from AccuStandard Inc., USA. All solvents used for sample processing and analyses were chromatographic grade from Dikma Technologies Inc., USA.
Sample pretreatment

The water samples (1 L) were filtered at a flow rate of 5 mL min\(^{-1}\) by using solid-phase extraction (SPE) cartridges (HLB cartridges, 500 mg per 6 mL; Waters) pre-conditioned with 5 mL of dichloromethane, 5 mL of methanol, and 5 mL of ultra-pure water. The PAEs were eluted from...
the cartridge by using 10 mL of dichloromethane and collected in 10 mL glass tubes with stoppers. The eluate was evaporated under a stream of purified N₂ until the final volume of 1 mL was obtained for GC-MS analysis and quantified using the external standard method.

The sediment and soil samples were dried using a freeze-drying apparatus and ground with a 100-mesh sieve. In brief, 5 g of the dry sample was combined in a 250 mL conical flask with an acetone-dichloromethane mixture (1/2, v/v) as the extraction solvent for 20 min of ultrasonic extraction. After the extracts were allowed to stand for several minutes, the supernatants were transferred to 50 mL centrifuge tubes. The final extraction volume was approximately 20 mL in three extraction cycles. The extracts were completely condensed to approximately 10 mL and then subjected to SPE for cleanup and concentrated to 1 mL for further analysis.

**Instrumental analysis**

The concentrations of PAEs in water, sediment, and soil samples were analyzed with an Agilent 6890N gas chromatograph with a 5973C mass selective detector (GC/MSD), equipped with an Agilent 7683B automatic liquid sampler and a DB-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas with a column flow rate of 1.2 mL min⁻¹ in a constant-flow mode. The injector, ion source, and transfer line temperatures were set at 280, 290, and 260°C, respectively. The GC oven temperature was programmed from 70°C (2 min) to 130°C (1 min) at 20°C min⁻¹, increased to 240°C at 5°C min⁻¹, and held constant for 1 min; the temperature was further increased to 260°C at 5°C min⁻¹ and held constant for 1 min; the temperature was subsequently increased to 270°C at 5°C min⁻¹ and held constant for 1 min; afterward, the temperature was increased to 280°C at 10°C min⁻¹ and held constant for 7 min. The electron impact energy was set at 70 eV, and 2 μL of each sample was injected in the splitless mode. The PAEs were analyzed in the selected ion monitoring mode.

**Quality control and assurance**

The blanks covering the entire analytical procedure, including extraction and GC analysis, were analyzed. The PAE levels in water, sediment, and soil blanks were not detected or were much lower than the detection limits of the respective method; in particular, the PAE levels in water, sediment, and soil were 0.08–1.1 ng L⁻¹, 0.12–1.9 ng g⁻¹, and 0.13–2.1 ng g⁻¹, respectively. The recovery rates of DMP, DEP, D₈BP, BBP, D₉OP, and DEHP were 103.47%, 91.93%, 109.58%, 98.65%, 84.81%, and 85.34%, respectively.

**Soil physico-chemical analysis**

Soil pH was determined using a PHS-3C pH meter (REX Instrument Factory, Shanghai, China) in a 1:2.5 suspension in a 0.01 M CaCl₂ solution. Soil electrical conductivity (EC) was measured using a DDS-307 conductivity meter (REX Instrument Factory, Shanghai, China) by mixing 10 g of soil with 50 mL of distilled water. Soil texture was evaluated via the pipette method [11]. The soil samples for soil organic C (SOC) measurements were pretreated with 0.5 M HCl to remove the carbonates and ball-milled [12]. The SOC concentration was determined through dry combustion (Analytik Jen, Germany).

Soil microbial biomass C (MBC) was determined via the chloroform fumigation–extraction method on fresh soil samples [13]. The fumigated and unfumigated soils were shaken for 30 min with 0.5 M K₂SO₄ and analyzed for C by using a Multi 3100N/C TOC analyzer (Analytik Jen, Germany). To obtain the soil MBC, we divided the difference of the extractable C between fumigated and unfumigated soils by a conversion factor of 0.45 [14]. The unfumigated samples were used to estimate the background dissolved organic C (DOC) values [15]. Permanganate oxidizable C (KMnO₄-C) was estimated in accordance with a previously described method [16].
Catalase activity was determined via a titration method [17]; the activities of dehydrogenase, alkaline phosphatase, β-glucosidase, arylsulphatase, and urease were determined in accordance with previously described methods [18]. The dehydrogenase activity was evaluated with triphenyltetrazolium chloride as a substrate; the samples were incubated for 24 h at 37°C. The activities of β-glucosidase, alkaline phosphatase, and arylsulphatase were determined with p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl phosphate, and p-nitrophenyl sulfate as substrates, respectively; the samples were incubated for 1 h at 37°C for the analyses. The urease activity was evaluated with urea as a substrate; the samples were incubated for 2 h at 37°C and examined through steam distillation. All of the measured parameters were calculated the basis of dry matter.

Statistical analysis

Analytical results were calculated on the basis of the oven-dried (105°C) weight of soil. Data were analyzed using SPSS 19.0 for Windows. The PAE concentrations among the sampling sites were analyzed through one-way ANOVA to evaluate the effects of wastewater irrigation. Differences were evaluated and considered significant at \( p < 0.05 \).

Results and Discussion

Levels of PAEs in water, sediments, and agricultural soils of the WYR

The individual concentrations of the six priority PAEs in water, sediments, and agricultural soils of the WYR are shown in Table 1. PAEs were detected in all of the river water samples, thereby indicating that PAEs are ubiquitous environmental contaminants in the WYR. The total concentrations of the six PAEs in the river water ranged from 2.42 \( \mu \text{g L}^{-1} \) to 6.67 \( \mu \text{g L}^{-1} \) with an average value of 4.01 \( \mu \text{g L}^{-1} \). These results were comparable to those of several previous studies; the levels of the compounds are often of the same order of magnitude as those found in other countries (Table 2) [4,19–22]. Nevertheless, a comparison with internal studies (Table 2) [23–25] indicated that these levels were below the average levels. This result may be related to the different sources of the river. Moreover, PAE contamination is currently low in irrigation water, but further studies should be conducted to trace the flow of PAEs in the WYR.

In the sediments, the total content of the six PAEs varied considerably, ranging from 0.367 \( \mu \text{g g}^{-1} \) to 0.729 \( \mu \text{g g}^{-1} \) based on dry weight (dw), with an average value of 0.536 \( \mu \text{g g}^{-1} \) dw (Table 1). Among the individual PAEs, DnBP, DEHP, and DnOP with the respective mean concentrations of 0.084, 0.307, and 0.077 \( \mu \text{g g}^{-1} \) dw were present in all of samples; by contrast,

| PAEs | River watera (\( \mu \text{g L}^{-1} \)) | Sedimentsb (\( \mu \text{g g}^{-1} \) dw) | Soilsb (\( \mu \text{g g}^{-1} \) dw) |
|------|---------------------------------|---------------------------------|---------------------------------|
| DMP  | Range 0.31–1.87 Mean 0.64 ± 0.45 Median 0.43 | Range ND–0.067 Mean 0.020 ± 0.021 Median 0.026 | Range ND–0.034 Mean 0.024 ± 0.008 Median 0.024 |
| DEP  | Range 0.23–1.00 Mean 0.34 ± 0.15 Median 0.28 | Range ND–0.069 Mean 0.022 ± 0.021 Median 0.032 | Range 0.023–0.034 Mean 0.026 ± 0.003 Median 0.026 |
| DnBP | Range 0.32–3.65 Mean 0.95 ± 0.69 Median 0.81 | Range 0.042–0.159 Mean 0.084 ± 0.033 Median 0.072 | Range 0.035–0.054 Mean 0.045 ± 0.005 Median 0.045 |
| BBP  | Range 0.39–3.36 Mean 1.18 ± 0.68 Median 1.06 | Range ND–0.111 Mean 0.028 ± 0.029 Median 0.034 | Range ND–0.116 Mean 0.022 ± 0.025 Median 0.029 |
| DEHP | Range 0.26–0.94 Mean 0.48 ± 0.15 Median 0.43 | Range 0.161–0.465 Mean 0.307 ± 0.078 Median 0.304 | Range 0.066–0.263 Mean 0.143 ± 0.052 Median 0.132 |
| DnOP | Range 0.31–0.65 Mean 0.40 ± 0.07 Median 0.39 | Range 0.043–0.142 Mean 0.077 ± 0.021 Median 0.077 | Range ND–0.069 Mean 0.036 ± 0.019 Median 0.041 |
| \( \Sigma \text{PAEs} \) | Range 2.42–6.67 Mean 4.01 ± 1.16 Median 3.74 | Range 0.367–0.729 Mean 0.536 ± 0.096 Median 0.529 | Range 0.191–0.457 Mean 0.294 ± 0.060 Median 0.287 |

DF, detectable frequency (%). ND, concentration was lower than the MDL. dw, dry weight.
a reported concentrations were corrected by subtracting the mean blank values.

Table 1. Summary of the individual concentrations of PAEs in the WYR water, sediments, and agricultural soils (n = 39).

doi:10.1371/journal.pone.0137998.t001
the other PAEs exhibited relatively low concentrations and detectable frequencies. The concentrations of the six PAEs in sediments samples from WYR were lower than those from the Songhua River in China ($\Sigma_6$PAEs ranged from 0.381 μg g$^{-1}$ to 2.044 μg g$^{-1}$) [2] and the suburban southern Jiangsu River ($\Sigma_6$PAEs ranged from 2.3 μg g$^{-1}$ to 80.1 μg g$^{-1}$) [26]. The differences in the PAE profiles of sediments from different regions may reflect the predominantly utilized PAE formulation. Therefore, the sources and modes of transfer to the river require investigation in future studies [26].

The sum of the concentrations of the six PAEs in the agricultural soils along the WYR considerably varied, ranging from 0.191 μg g$^{-1}$ dw to 0.457 μg g$^{-1}$ dw, which are within the less stringent grade II limits of PAEs (10 μg g$^{-1}$ dw) in arable soils recommended by the Environmental Quality Standard for soil in China (GB-15618-2008) [27]. Compared with the PAE concentrations in the rural soils of the Yellow River Delta (Σ$_6$PAEs ranged from 0.716 μg g$^{-1}$ dw to 3.25 μg g$^{-1}$ dw) [7] and those in black soils of northeast China (Σ$_{16}$PAEs ranged from 1.37 dw to 4.90 dw) [9], the results of the present study were relatively low. This difference may be attributed to the different cropping systems and field management practices. The predominant cropping system in the WYR area is winter wheat-summer maize rotation, with a large vegetable or orchard land area in the Yellow River Delta and black soil in northeastern China; these conditions may have introduced more PAE contaminants to the topsoil by intensive management practices, such as greenhouses for vegetables, plastic mulching for cotton, apple bagging, and pesticide application [7]. In the present studied area, the wastewater discharged into the river mainly originated from the effluent of wastewater treatment plants (WWPT) in Shijiazhuang, which is located in the upstream region of the WYR; the said wastewater was used for irrigation of this agricultural field [28]. Irrigation with wastewater from domestic and industrial sources has been known to contribute significantly to the amount of toxic contaminants, such as heavy metals, PAEs, and polychlorinated biphenyls, in the soils [29–30], even after the samples were treated by the WWPT. Thus, the wastewater irrigation might be an important source of PAE content in agricultural soils. This observation is supported by the study of Niu et al. [31], who found that application of plastic films, wastewater irrigation, and fertilizers were the predominant sources of PAEs in arable soils across China.

### PAE congener profiles and distribution in agricultural soils

The PAE congener profiles and distribution in agricultural soils from different areas along the WYR are shown in Table 1 and Fig 2. DEP, D$n$BP, and DEHP were present in all of the

| Location                        | DMP  | DEP  | DBP  | DEHP | DNOP | Reference, Year |
|--------------------------------|------|------|------|------|------|-----------------|
| Klang River Basin, Malaysia     | ND–0.1 | ND–0.2 | 0.8–4.8 | 3.1–64.3 | ND–1.5 | Tan [19], 1995  |
| Velino River, Italy             | —    | ND–3.2 | ND–44.3 | ND–31.2 | ND–11.3 | Vitelli et al. [4], 1997 |
| Ebro River, Spain               | ND   | 0.26  | —    | 0.7   | —    | Penalver et al. [20], 2000 |
| East London Port, South Africa  | 0.03–31.7 | 0.03–33.1 | 2.8–12.19 | 0.06–19.74 | —    | Fatok and Noma [21], 2002 |
| Dutch aquatic, Netherlands      | 0.05–0.19 | 0.07–0.23 | 0.07–3.1 | 0.9–5  | < 0.01–0.08 | Vethaak et al. [22], 2005 |
| Guiyang, China                  | ND   | ND–4.5 | 3.1–13.9 | 14.8–235 | —    | Seth et al. [23], 1999 |
| Yellow River, China             | ND–0.58 | 0.012–1.093 | ND–26 | 0.35–24 | ND–7.1 | Sha et al. [24], 2007 |
| Haihe River, China              | —    | —    | 0.35–40.68 | 3.54–101.1 | —    | Chi [25], 2009  |
| This article                    | 0.31–1.87 | 0.25–1.00 | 0.32–3.65 | —    | 0.26–0.84 |

ND, concentration was lower than the MDL.

| Location          | DMP  | DEP  | DBP  | DEHP | DNOP | Reference, Year |
|-------------------|------|------|------|------|------|-----------------|
| Klang River Basin | ND–0.1 | ND–0.2 | 0.8–4.8 | 3.1–64.3 | ND–1.5 | Tan [19], 1995  |
| Velino River      | —    | ND–3.2 | ND–44.3 | ND–31.2 | ND–11.3 | Vitelli et al. [4], 1997 |
| Ebro River        | ND   | 0.26  | —    | 0.7   | —    | Penalver et al. [20], 2000 |
| East London Port  | 0.03–31.7 | 0.03–33.1 | 2.8–12.19 | 0.06–19.74 | —    | Fatok and Noma [21], 2002 |
| Dutch aquatic     | 0.05–0.19 | 0.07–0.23 | 0.07–3.1 | 0.9–5  | < 0.01–0.08 | Vethaak et al. [22], 2005 |
| Guiyang, China    | ND   | ND–4.5 | 3.1–13.9 | 14.8–235 | —    | Seth et al. [23], 1999 |
| Yellow River      | ND–0.58 | 0.012–1.093 | ND–26 | 0.35–24 | ND–7.1 | Sha et al. [24], 2007 |
| Haihe River       | —    | —    | 0.35–40.68 | 3.54–101.1 | —    | Chi [25], 2009  |
| This article      | 0.31–1.87 | 0.25–1.00 | 0.32–3.65 | —    | 0.26–0.84 |

ND, concentration was lower than the MDL.
agricultural soil samples along the WYR; the average concentrations of the three substances were 0.0260, 0.0450, and 0.143 μg g⁻¹ dw, respectively (Table 1); conversely, the detection frequencies of other PAEs showed a decreasing pattern: DMP (89.7%), DnOP (82.1%), and BBP (51.3%). The dominant PAE species in the agricultural soils was DEHP that accounted for 48.6% of the total PAEs, followed by DnBP, which accounted for 15.3%. This trend is inconsistent with that described in previous reports, which indicated that DEHP and DnBP are the dominant components of the PAE distribution pattern in the environment [22,32–34]. In addition, DEHP and DnBP are among the most commonly produced PAEs used as plastic additives [4]. Furthermore, the dominant PAE species in the river water was BBP (Table 1), which was inconsistent with those in sediment and agricultural soils. This discrepancy may be possible because the species and concentrations of the PAEs detected in the river water were more dependent on the instantaneous concentrations in the wastewater than on other parameters; by contrast, the concentrations of the PAEs in the sediment and agricultural soils mainly resulted from long-term accumulation.

The high proportion of DEHP and DnBP in the soils was similar to the results detected in the sediment samples (Table 1), thereby indicating the same accumulation characteristics of the PAEs in the two environmental matrices. This result suggested that the sediments may be a potential pollution source of PAEs in the agricultural soils. A part of the sediments may be introduced using pumps during irrigation and accumulate in the topsoil of farmlands because of the shallow depth of the river. This mechanism of irrigation is similar to that in sediment-laden water, which may introduce organic matter and contaminants to soils [35]. The increased DEHP and DnBP levels in agricultural soils derived from wastewater irrigation in China have been discussed. Zeng et al. [6] detected the PAE levels in the agricultural soils from the peri-urban areas of Guangzhou City; these soils are heavily polluted by long-term industrial wastewater irrigation and sludge application; DEHP and DnBP concentrations ranged from 0.107 μg g⁻¹ dw to 29.4 μg g⁻¹ dw and from 0.009 μg g⁻¹ dw to 2.74 μg g⁻¹ dw, respectively. Kong et al. [36] studied the diversity of PAEs in suburban agricultural soils under wastewater irrigation for more than 40 years in Tianjin; their group showed DEHP and DnBP concentrations of 0.026–4.17 and 0.007–0.285 μg g⁻¹ dw, respectively. Wang et al. [37] studied the accumulation and distribution of phthalate ester residues in soils from the Sanjiang Plain (3.1–110.7 ng g⁻¹ dw for DEHP, 1.5–133.6 ng g⁻¹ dw for DnBP); the residual concentrations of PAEs in the paddy field were possibly caused by the surface coverage of irrigation water. Compared with
the concentrations in the other areas, the concentrations of DEHP and DnBP in the agricultural soils along the WYR (0.066–0.263 μg g⁻¹ dw for DEHP, 0.035–0.054 μg g⁻¹ dw for DnBP) were relatively low. These trends may be attributed to the dilution effect of the PAEs in the river water, and this effect could decrease the PAE concentrations in the irrigation water and reduce the accumulation rate in soils.

The spatial distribution of PAEs showed that the DEHP concentration fluctuated in the agricultural soils along the WYR, whereas other PAEs showed no significant differences among the different sites (Fig 2). The highest DEHP concentration was found at site 7 (0.225 μg g⁻¹ dw), followed by the DEHP concentration at site 10 (0.199 μg g⁻¹ dw); both sites were immediately close to villages, where a significant amount of agricultural waste and garbage (including plastic films, wrapping materials, pesticide bottles, and construction waste) were discarded at the bank of the river. Garbage and its leachate have been reported as important sources of PAEs in the surface water and soils [38]. Consequently, the high concentrations of PAEs in the topsoil probably resulted from the dense anthropogenic activity and random dumping of garbage, especially in the rural areas. This result is consistent with that of Kong et al. [36], who found that the leachate from stacked wastes, including building materials and domestic garbage, may contribute a considerable amount of PAEs to the soil environment. Their group pointed out that disordered piling of waste on suburban soil should be cleaned and prohibited in the future. Thus, the concentration and contribution of PAEs to the agricultural soils along the WYR were attributed to the application of wastewater, sewage sludge, plastic films, pesticides, and agricultural management practices; the concentration and contribution of PAEs were also caused by human activities and garbage pollution in rural areas. The PAEs in agricultural soils may remain on the soil surface or may be transferred to deeper soil layers; both conditions can cause long-term harmful effects on ecosystems and human health.

Characteristics of agricultural soils

The distribution of PAEs is closely related to the nature of the soil; thus, we analyzed the soil components. The properties of the agricultural soils along the WYR, including the pH, EC, moisture content, clay content, soil organic carbon fractions, and enzyme activity are presented in Table 3. The soils along the WYR are weakly basic, with pH (CaCl₂) values ranging from 7.57 to 7.90. The soil EC varied between 93 and 430, thereby reflecting the slightly salinity of the soils. The texture of the agricultural soils was silt loam or silt clay loam, and the clay content ranged from 14.3% to 39.8%. The average SOC content was 13.75 g kg⁻¹, whereas the mean content of labile organic C in terms of DOC, MBC, and KMnO₄-C was 0.120, 0.320, and 3.07 g kg⁻¹, which accounted for 0.87%, 2.33%, and 22.33% of the SOC, respectively. The activity of alkaline phosphatase, arylsulphatase, β-glucosidase, urease, dehydrogenase, and catalase varied and depended on the different kinds of enzymes. The spatial distribution was similar to the gradient of labile organic C fractions, which were higher in the downstream soils than in the upstream soils (data not shown). Therefore, the availability of labile C is an important determinant of the enzyme activities in soils.

Correlations of PAE concentrations with SOC fractions and soil enzyme activities

The concentrations and distribution of PAEs in the soil could be influenced by various factors, such as the pH, clay content, organic matter, humic substances, and the microbial activity. PAEs also have significant effects on soil biological and microbiological properties because of their toxicity. The importance of soil organic matter has been shown in the mobility of
hydrophobic organic contaminants in soils, such that PAEs are likely adsorbed by soil organic matter [7, 35]. Therefore, the SOC content could affect the PAE concentrations in the soil. The relationship was analyzed between the concentrations of the S6PAEs and the soil organic carbon fractions in the studied soil samples. Fig 3 shows that the correlation coefficient ($R^2$) was 0.559 (n = 39), thereby indicating a positive correlation between S6PAEs and SOC in the investigated soils. Similar results were reported by Zeng et al. [6] and Yang et al. [7]. The present results also show that the concentrations of S6PAEs were weakly and positively correlated with the MBC and KMnO4-C contents, with $R^2$ of 0.454 and 0.26, respectively. These trends suggested that labile SOC fractions may affect the distribution of PAEs in the studied area. However, the concentrations of S6PAEs were poorly correlated with DOC, with an $R^2$ of only 0.008 (n = 39; Fig 3). The low correlation may be attributed to the small size, highly labile nature, and mobility of DOC. However, other researchers previously showed that DOC may play an important role in the coexistence of PAEs and heavy metals in the sediments and enhance the PAE sorption to sediments [39].

Soil enzyme activities can respond to changes more quickly in soil management, fertilization, and environmental pollution than other soil properties [40]. Thus, the soil enzyme activities can be used as soil quality and contamination indicators. The relationship between $Σ_6$PAE concentrations and soil enzyme activities is shown in Fig 4. The weakly positive correlations of the $Σ_6$PAEs and soil enzyme activities ($R^2$ ranged from 0.164 for arylsulphatase to 0.391 for dehydrogenase, n = 39) indicated that the existence of the PAEs in the soils did not decrease microbial activities in the studied area. Although PAEs are considered toxic to microorganisms [1, 41], their inhibitory effects on microbial activities were weaker than the promoting effects of soil organic matter in our study.

### Potential risk assessment of the agricultural soils

The six PAEs in the present study have attracted much attention worldwide and are listed as priority pollutants and endocrine-disrupting compounds by the US EPA, Chinese National Environmental Monitoring Center, and other regulatory bodies. The concentrations of PAEs

---

**Table 3. Major characteristics of agricultural soils along the WYR (n = 39).**

|                     | Range          | Mean ± SD       | Median |
|---------------------|----------------|-----------------|--------|
| pH$_{CaCl_2}$       | 7.57–7.90      | 7.71 ± 0.08     | 7.69   |
| EC (μS cm$^{-1}$)   | 93–430         | 201 ± 91        | 169    |
| θ$_m$ (g g$^{-1}$) | 0.14–0.28      | 0.21 ± 0.04     | 0.21   |
| Clay (%)            | 14.3–39.8      | 23.6 ± 6.6      | 22.4   |
| SOC (g kg$^{-1}$ dw)| 6.07–33.11     | 13.75 ± 8.14    | 10.12  |
| DOC (g kg$^{-1}$ dw)| 0.07–0.17      | 0.12 ± 0.03     | 0.11   |
| MBC (g kg$^{-1}$ dw)| 0.09–0.71      | 0.32 ± 0.16     | 0.27   |
| KMnO4-C (g kg$^{-1}$ dw)| 1.13–5.25   | 3.07 ± 0.92     | 3.18   |
| Alkaline phosphatase (μg PNP g$^{-1}$ dw h$^{-1}$) | 234.8–723.2 | 521.4 ± 102.0 | 513.1  |
| Arylsulphatase (μg PNP g$^{-1}$ dw h$^{-1}$) | 13.3–132.1 | 64.8 ± 27.0 | 61.6   |
| β-Glucosidase (μg PNP g$^{-1}$ dw h$^{-1}$) | 20.9–234.5 | 127.5 ± 53.5 | 120.7  |
| Urease (μg NH$_4^+$-N g$^{-1}$ dw 2 h$^{-1}$) | 69.1–486.7 | 238.3 ± 105.0 | 215.7  |
| Dehydrogenase (μg TPF g$^{-1}$ dw 24 h$^{-1}$) | 96.8–317.4 | 213.0 ± 48.1 | 213.0  |
| Catalase (mL (20 mM KMnO4) g$^{-1}$ dw h$^{-1}$) | 0.42–1.29 | 0.72 ± 0.22 | 0.67   |

EC, electrical conductivity; θ$_m$, soil water content; SOC, soil organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon; KMnO4-C, permanganate oxidizable carbon; dw, dry weight.

doi:10.1371/journal.pone.0137998.t003
Fig 3. Correlations of PAEs concentrations with the soil organic carbon fractions in the agricultural soils along the WYR. SOC, soil organic carbon; KMnO₄-C, permanganate oxidizable carbon; MBC, microbial biomass carbon; DOC, dissolved organic carbon.

doi:10.1371/journal.pone.0137998.g003

Fig 4. Correlations of PAEs concentrations with the soil enzyme activities in the agricultural soils along the WYR.

doi:10.1371/journal.pone.0137998.g004
recorded in the present study were used to assess the potential risk from the agricultural soils along the WYR. The PAE concentrations were compared with the soil cleanup guidelines used in New York, USA because of the shortage of local standards for soil pollution identification [42].

In the agricultural soil samples from the WYR area, only the DMP levels exceeded the recommended allowable concentrations (0.02 μg g⁻¹ dw), which is 75% more than the standard value. However, the DMP concentrations in the present study were far below the recommended soil cleanup levels set by the USA standards (2.0 μg g⁻¹ dw). The concentrations of the other PAEs in the soil samples were below the recommended allowable concentrations (0.071 μg g⁻¹ dw for DEP, 0.081 μg g⁻¹ dw for DnBP, 1.215 μg g⁻¹ dw for BBP, 4.35 μg g⁻¹ dw for DEHP, and 1.20 μg g⁻¹ dw for DnOP). Therefore, no remediation measures are needed in terms of the PAEs in the WYR area. However, the relatively lower concentrations of PAEs may pose a potential long-term risk to the ecosystem and human health through the food chain [35]. Therefore, further studies should be conducted on the basis of the possible biological magnifications of PAEs.

**Conclusion**

This study reports the concentrations and distribution of PAEs in the agricultural soils from the WYR basin. The six priority PAEs were ubiquitous in the topsoil of the wastewater irrigation areas; the total concentrations range from 0.191 μg g⁻¹ dw to 0.457 μg g⁻¹ dw. As the dominant PAEs in agricultural soils, DEHP and DnBP accounted for 63.9% of the total PAE concentration. Positive correlations were observed between the PAE concentrations and the soil organic carbon fractions and soil enzyme activities; therefore, these factors can affect the distribution of PAEs. The predominant cropping system of the study area was wheat-maize rotation; plastic films are applied to a low extent during agricultural management. The wastewater irrigation and stacked domestic garbage were considered as the major sources of PAE contaminants in this area. Compared with the results of other studies, our findings show that the agricultural soils in the WYR wastewater irrigation area were weakly polluted by PAEs; despite this finding, no remediation measures were required. However, the PAE pollution in this area should be addressed because this phenomenon may pose a potential long-term risk to the ecosystem and human health.

**Acknowledgments**

The authors would like to thank the academic editor and the anonymous reviewers for their helpful comments and suggestions.

**Author Contributions**

Conceived and designed the experiments: YZ BDX HBH. Performed the experiments: YZ QL MDY LNM. Analyzed the data: YZ RTG HZ. Contributed reagents/materials/analysis tools: YZ WBT XSH. Wrote the paper: YZ QL XWW.

**References**

1. Staples CA, Peterson DR, Parkerton TF, Adams WJ (1997) The environmental fate of phthalate esters: a literature review. Chemosphere 35:667–749.

2. Gao DW, Li Z, Wen ZD, Ren NQ (2014) Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China. Chemosphere 95:24–32. doi: 10.1016/j.chemosphere.2013.08.009 PMID: 24001662
3. Wang M, Yang XD, Bi WT (2015) Application of magnetic graphitic carbon nitride nanocomposites for the solid-phase extraction of phthalate esters in water samples. Journal of Separation Science 38: 445–452. doi: 10.1002/jssc.201400991 PMID: 25421601

4. Vitali M, Guidotti M, Macilenti G, Creminini C (1997) Phthalate esters in freshwater as markers of contamination sources in a site study in Italy. Environment International 23: 337–347.

5. Tier M, Blanchard M, Chevreul M (2006) Atmospheric fate of phthalate esters in an urban area (Paris-France). Science of the Total Environment 354: 212–223. PMID: 16398997

6. Zeng F, Cui KY, Xie ZY, Wu LN, Liu M, Sun GQ, et al. (2008) Phthalate esters (PAEs): Emerging organic contaminants in agricultural soils in peri-urban areas around Guangzhou, China. Environmental Pollution 156: 425–434. doi: 10.1016/j.envpol.2008.01.045 PMID: 18343547

7. Yang HJ, Xie WJ, Liu Q, Liu JT, Yu HW, Lu ZH (2013) Distribution of phthalate esters in topsoil: a case study in the Yellow River Delta, China. Environmental Monitoring and Assessment 185: 8489–8500. doi: 10.1007/s10661-013-3190-7 PMID: 23609921

8. Chai C, Cheng H, Ge W, Ma D, Shi Y (2014) Phthalic Acid Esters in Soils from Vegetable Greenhouses in Shandong Peninsula, East China. PLoS ONE 9(4): e95701. doi: 10.1371/journal.pone.0095701 PMID: 24747982

9. Zhang Y, Wang PJ, Wang L, Sun GQ, Zhao JY, Zhang H, et al. (2015) The influence of facility agriculture production on phthalate esters distribution in black soils of northeast China. Science of the Total Environment 506–507: 118–125. doi: 10.1016/j.scitotenv.2014.10.075 PMID: 25460946

10. Jiang YH, Li M.X, Guo CS, An D, Xu J, Zhang Y, et al. (2008) Phthalate esters (PAEs): Emerging organic contaminants in agricultural soils in peri-urban areas around Guangzhou, China. Environmental Pollution 156: 425–434. doi: 10.1016/j.envpol.2008.01.045 PMID: 18343547

11. Jiang YH, Xie WJ, Liu Q, Liu JT, Yu HW, Lu ZH (2013) Distribution of phthalate esters in topsoil: a case study in the Yellow River Delta, China. Environmental Monitoring and Assessment 185: 8489–8500. doi: 10.1007/s10661-013-3190-7 PMID: 23609921

12. Chai C, Cheng H, Ge W, Ma D, Shi Y (2014) Phthalic Acid Esters in Soils from Vegetable Greenhouses in Shandong Peninsula, East China. PLoS ONE 9(4): e95701. doi: 10.1371/journal.pone.0095701 PMID: 24747982

13. Zhang Y, Wang PJ, Wang L, Sun GQ, Zhao JY, Zhang H, et al. (2015) The influence of facility agriculture production on phthalate esters distribution in black soils of northeast China. Science of the Total Environment 506–507: 118–125. doi: 10.1016/j.scitotenv.2014.10.075 PMID: 25460946

14. Jiang YH, Li M.X, Guo CS, An D, Xu J, Zhang Y, et al. (2008) Phthalate esters (PAEs): Emerging organic contaminants in agricultural soils in peri-urban areas around Guangzhou, China. Environmental Pollution 156: 425–434. doi: 10.1016/j.envpol.2008.01.045 PMID: 18343547

15. Jiang YH, Xie WJ, Liu Q, Liu JT, Yu HW, Lu ZH (2013) Distribution of phthalate esters in topsoil: a case study in the Yellow River Delta, China. Environmental Monitoring and Assessment 185: 8489–8500. doi: 10.1007/s10661-013-3190-7 PMID: 23609921

16. Schinner F, Öhlinger R, Kandeler E, Margesin R (1996) Methods in Soil Biology. Springer, Berlin.

17. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass: calibration of the kEC value. Soil Biology & Biochemistry 28: 25–31.

18. Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kögel-Knabner I, et al. (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology & Biochemistry 41: 1622–1631.

19. Vieira FBC, Bayer C, Zanatta JA, Dieckow J, Mielniczuk J, He ZL (2007) Carbon management index based on physical fractionation of soil organic matter in an Acrisol under long-term no-till cropping systems. Soil and Tillage Research 96: 195–204.

20. Zhou QH, Wu ZB, Cheng SP, Li HW, Fan MS, Kuzyakov Y (2009) Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. Soil and Tillage Research 108: 85–94.

21. Schinner F, Öhlinger R, Kandeler E, Margesin R (1996) Methods in Soil Biology. Springer, Berlin.

22. Chen HQ, Hou RX, Gong YS, Li HW, Fan MS, Kuzyakov Y (2009) Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. Soil and Tillage Research 106: 85–94.

23. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass: calibration of the kEC value. Soil Biology & Biochemistry 28: 25–31.

24. Dannenmann M, Simon J, Gasche R, Holst J, Naumann PS, Kögel-Knabner I, et al. (2009) Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology & Biochemistry 41: 1622–1631.

25. Tabatabai MA (1982) Soil enzymes. In: Page A.L., Miller R.H., Keeney D.R. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Soil Science Society of America, Madison, 903–947.

26. Tan GH (1995) Residue levels of phthalate esters in water and sediment samples from the Klang river basin [J]. Bulletin of Environmental Contamination and Toxicology 54(2): 171–176. PMID: 7742623

27. Penalver A, Pocurull E, Borrell F, Marce RM. (2000) Determination of phthalate esters in water samples by solid-phase microextraction and gas chromatography with mass spectrometric detection [J]. Journal of Chromatography A 872(1): 191–201.

28. Fatoki O, Noma A (2002) Solid phase extraction method for selective determination of phthalate esters in the aquatic environment [J]. Water, Air, & Soil Pollution 140(1): 85–98.

29. Vethaak AD, Lahr J, Schrap SM, Belfroid AC, Rijs GBJ, Gerritsen A, et al. (2005) An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of the Netherlands. Chemosphere 59: 511–524. PMID: 15788174

30. Seth R, Mackay D, Muncie J (1999) Estimating the organic carbon partition coefficient and its variability for hydrophobic chemicals [J]. Environmental Science & Technology 33(14): 2390–2394.

31. Sha Y, Xia X, Yang Z, et al. (2007) Distribution of PAEs in the middle and lower reaches of the Yellow River, China [J]. Environmental Monitoring and Assessment 124(1): 277–287.

32. Chi J (2009) Phthalate acid esters in Potamogeton crispus L. from Haihe River, China [J]. Chemosphere 77(1): 48–52. doi: 10.1016/j.chemosphere.2009.05.043 PMID: 19545887
26. Wang J, Bo LJ, Li LN, Wang DJ, Chen GC, Christie P, et al. (2014) Occurrence of phthalate esters in river sediments in areas with different land use patterns. Science of the Total Environment 500–501: 113–119. doi: 10.1016/j.scitotenv.2014.08.092 PMID: 25217750
27. China National Environmental Protection Agency, 2008. Environmental Quality Standard for Soils. China National Environmental Protection Agency, China.
28. Liang Q, Gao RT, Xi BD. Zhang Y, Zhang H (2014) Long-term effects of irrigation using water from the river receiving treated industrial wastewater on soil organic carbon fractions and enzyme activities. Agricultural Water Management 135:100–108.
29. Chen Y, Wang CX, Wang ZJ (2005) Residues and source identification of persistent organic pollutants in farmland soils irrigated by effluents from biological treatment plants. Environment International 31:778–783. PMID: 16005065
30. Mapanda F, Mangwayana EN, Nyamangara J, Giller KE (2005) The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agriculture, Ecosystem and Environment 107: 151–165.
31. Niu LL, Xu Y, Xu C, Yun LX, Liu WP (2014) Status of phthalate esters contamination in agricultural soils across China and associated health risks. Environmental Pollution 195: 16–23. doi: 10.1016/j.envpol.2014.08.014 PMID: 25194267
32. Mackintosh CE, Maldonado JA, Ikonomou MG, Gobas FAPC (2006) Sorption of phthalate esters and PCBs in a marine ecosystem. Environmental Science and Technology 40: 3481–3488. PMID: 16786683
33. Cai QY, Mo CH, Wu QT, Katsoyiannis A, Zeng QY (2008) The status of soil contamination by semivolatile organic chemicals (SVOCs) in China: a review. Science of the Total Environment 389: 209–224. PMID: 17936334
34. Meng ZM, Wang Y, Xiang N, Chen L, Liu ZG, Wu B, et al. (2014) Flow of sewage sludge-borne phthalate esters (PAEs) from human release to human intake: Implication for risk assessment of sludge applied to soil. Science of the Total Environment 476–477: 242–249. doi: 10.1016/j.scitotenv.2014.01.007 PMID: 24468498
35. Wang XJ, Piao XY, Chen J, Hu JD, Xu FL, Tao S (2006) Organochlorine pesticides in soil profiles from Tianjin, China. Chemosphere 64: 1514–1520. PMID: 16466771
36. Kong SF, Ji YQ, Liu LL, Chen L, Zhao XY, Wang JJ, et al. (2012) Diversities of phthalate esters in sub-urban agricultural soils and wasteland soil appeared with urbanization in China. Environmental Pollution 170: 161–168. doi: 10.1016/j.envpol.2012.06.017 PMID: 22813629
37. Wang XL, Lin GX, Wang J, Lu XG, Wang GP (2013) Effect of wetland reclamation and tillage conversion on accumulation and distribution of phthalate esters residues in soils. Ecological Engineering 51: 10–15.
38. Hu XX, Han ZH, Liu BY, Zhang FB, Li F, Wang WH (2007) Distribution of phthalic acid esters in environment and its toxicity. Environmental Science and Management 32: 37–40. (in Chinese)
39. Xu N, Ni JR, Sun WL, Borthwick AGL (2007) Role of dissolved organic carbon in the cosorption of copper and phthalate esters onto Yellow River sediments. Chemosphere 69: 1419–1427. PMID: 17560627
40. Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31: 1471–1479.
41. He W, Qin N, Kong XZ, Liu WX, He QS, Ouyang HL, et al. (2013) Spatio-temporal distributions and the ecological and health risks of phthalate esters (PAEs) in the surface water of a large, shallow Chinese lake. Science of the Total Environment 461–462: 672–680. doi: 10.1016/j.scitotenv.2013.05.049 PMID: 23770548
42. Department of Environmental Conservation, New York, USA. (1994) Determination of soil cleanup objectives and cleanup levels (TAGM 4046). http://www.dec.ny.gov/regulations/2612.html.