Atrazine contamination in agricultural soils from the Yangtze River Delta of China and associated health risks

J. T. Sun[a,b] . L. L. Pan[a] . Yu Zhan[a] . Daniel C. W. Tsang[b] . L. Z. Zhu[a] . X. D. Li[b]

[a] Department of Environmental Science, Zhejiang University, Hangzhou 310058, Zhejiang, China

[b] Department of Civil and Environmental Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

ABSTRACT

Atrazine is one of the most widely applied and persistent herbicides in the world. In view of limited information on the regional contamination of atrazine in soils in China, this study investigated the spatial distribution and environmental impacts of atrazine in agricultural soils collected from the Yangtze River Delta (YRD) as an illustrative analysis of rapidly developing regions in the country. The results showed that the concentrations of atrazine in the YRD agricultural soils ranged from <1.0 to 113 ng/g dry weight, with a mean of 5.7 ng/g, and a detection rate of 57.7 % in soils. Pesticide factory might be a major source for the elevated levels of atrazine in Zhejiang Province. The contamination of atrazine was closely associated with land use types. The concentrations and detection rates of atrazine were higher in corn fields and mulberry fields than in rice paddy fields. There was no significant difference in compositions of soil microbial phospholipids fatty acids among the areas with different atrazine levels. Positive relationship ($R = 0.417$, $p < 0.05$, $n = 30$) was observed between atrazine and total microbial biomass. However, other factors, such as soil type and land management practice, might have stronger influences on soil microbial communities. Human health risks via exposure to atrazine in soils were estimated according to the methods recommended by the US EPA. Atrazine by itself in all the soil samples imposed very low carcinogenic risks (< 10-6) and minimal non- cancer risks (hazard index < 1) to adults and children.

INTRODUCTION

Synthetic chemicals used in modern agriculture have increased over the past 50 years. Excessive use of various pesticides has significantly impacted the environment quality. Atrazine (2-chloro-4-ethy- lamino-6-ethylamino-1,3,5-triazine) is one of the most widely applied, persistent and toxic

herbicides in the world to control pre- and post-emergent weeds in crops, such as maize, sorghum, sugarcane, and fruit crops (Satsuma 2009; Short and Colborn 1999). Because of its repeated applications, persistence and high water solubility (Moorman et al. 2001), atrazine can contaminate the environment for a long time (Schwab et al. 2006). Atrazine was commonly found as a water contaminant around the world, and residual concentrations was also found in soil after its application for agricultural purposes (Krutz et al. 2006; Vryzas et al. 2009).

Atrazine not only hinders the growth of sensitive succeeding crops, but also poses a potential threat to soil ecosystem and environmental health (Fang et al. 2015; Freeman et al. 2011). There is a growing concern about the risks of atrazine to human health including acute irritation of eyes and skin, and chronic effects on central nervous and immune systems (Hayes et al. 2002; Zaya et al. 2011). Atrazine can also act as an endocrine disrupting compound (EDC) affecting human endocrine system (Lasserre et al. 2009).

Many countries have classified atrazine in the list of controlled EDCs, and the European Union banned its use in 2004. In the United States (US), atrazine was classified as a restricted-use herbicide to minimize its potential impact to the environment. The US Environmental Protection Agency (US EPA) has initiated a comprehensive re-evaluation of atrazine to assess whether new restrictions are necessary to protect public health (US EPA 2013). However, atrazine is still widely used in some developing countries, such as China, without effectively regulatory control. Atrazine was introduced into China in the 1980s, and it has been extensively used in large area of farmland across the whole country (Deng et al. 2005). It was reported that the agricultural usage of atrazine in China in 2000 has reached 2800 tons, with an increase of 20 % each year (Li et al. 2007).

The environmental behavior of atrazine has become an interesting research topic. Most of the studies have focused on the sorption, biotransformation, toxicology, and remediation of atrazine

(Ji et al. 2015; Murphy et al. 2006; Vryzas et al. 2012; Wu et al. 2015). Valuable results were obtained for understanding atrazine's environmental fate, facilitating better management of the herbicide and avoiding its excessive application. Agricultural activities are a major source of atrazine contamination in the environment. The occurrence of atrazine in surface water and groundwater through water runoff and leaching has been reported (Byer et al. 2011; Panshin et al. 2000; Vonberg et al. 2014). Atrazine can enter water through a range of transport processes and compromise the water quality (Kurt-Karakus et al. 2010; Vonberg et al. 2014). However, our understanding of the contamination status of atrazine in soils is generally limited. Soil is an important reservoir for many organic pollutants (Zhong and Zhu 2013). In order to characterize the regional-scale spatial distribution of atrazine pollution in agricultural soils from rapidly developing regions, we conducted an extensive survey in the Yangtze River Delta (YRD) as an illustrative analysis of the relationship between land use and associated environmental impacts. A well-distributed sampling program composing of 241 sites was conducted to cover an area of nearly 45,800 km^2.

The aims of this work are (1) to reveal the contamination status and spatial distributions of atrazine in agricultural soils in the YRD region and (2) to assess the human health risk of atrazine residues in soils and to provide basic data for risk management and control of atrazine in China.

EXPERIMENTAL METHODS

Sampling

The YRD region consists of Shanghai, northern Zhejiang and southern Jiangsu, located in eastern China, which is one of the most populated and economically prosperous regions in China. The

87 loca- tions of the sampling sites for the study are shown in Fig. 1. A total of 241 topsoil samples
88 (0–15 cm depth) were obtained from various farmlands in the YRD region (approximately 45,800
89 km^2) during June 2014. Details of the collection procedures were described in the previous study
90 (Sun et al. 2016). The land-use type include, among others, rice paddy fields, corn fields, vegetable
91 fields, fruit gardens, mulberry fields, and uncultivated lands. The soil samples were collected using
92 a stainless steel scoop, packed in aluminum foil, sealed in Kraft bags, and freeze-dried at -50 °C
93 in the laboratory. They were then sieved to collect the <75 μm fraction and stored at -20 °C
94 before analysis.

95

96 **Analysis of atrazine**

97 The analysis of atrazine was adapted from a previous reported method (Cheng et al. 2015). An
98 aliquot of 10 g soil sample was spiked with surrogate standard di-2-ethylhexyl phthalate d_4 (20 ng
99 in dichloro-methane) and then ultrasonically extracted withdichloromethane/acetone (1:1; v/v) for
100 60 min. The extract was transferred to a flask, and the procedure was repeated two more times.
101 The extract was concentrated, the solvent exchanged with hexane and further reduced to 2.0 mL
102 with the use of a rotary evaporator (Heidolph 4000, Germany). Then, the extract was cleaned by
103 a multi-layered column (25 9 1.0 cm) consisting, from bottom to top with2 cm of Na_2SO_4, 6
104 cm of activated florisil, and 2 cm of Na_2SO_4. After prewashing with 30 mL of hexane, the extract
105 was loaded to the column. Hexane/acetone(9:1; v/v) (50 mL) was used to elute atrazine. Elution
106 was evaporated to nearly dryness and solvent exchanged with 0.5 mL of hexane prior to
107 instrumental analysis.

108 Atrazine was analyzed by a gas chromatographcoupled with mass spectrometer (GC–MS, 7890B/

5977A, Agilent Technologies, Santa Clara, CA, USA)equipped with a HP-5 capillary column (30 m, 0.25 mmi.d., 0.25 lm film thickness). Helium was used as carriergas at a constant flow of 1.0 mL/min. Injector and ionsource temperature were 250 and 230 °C, respectively.The oven program started at 80 °C for 1 min andincreased to 200 °C at a rate of 40 °C/min, then to 280 °C at 20 °C/min, kept for 3 min. The post-run wasset at 290 °C, held for 1 min. The instrument was operated using electron impact (EI) ionization and in the selected ion monitoring (SIM) mode. Quantification ionsfor atrazine were *m/z* 173, 200 and 215 respectively.

Microbiological analysis

To reveal the impacts of atrazine on soil microbial communities, microbiological analysis was conducted in three selected groups of 10 soil samples after the chemical analysis. In group 1, all the ten soil samples were not contaminated by atrazine. In group 2, the concentrations of atrazine were all below or equal to 2.0 ng/g. In group 3, the concentrations of atrazine ranged from 4.0 to 85.2 ng/g. Group 1, 2 and 3 are representative soils of non-contaminated area, relatively low polluted area, and relatively high polluted area, respectively (Fig. 1). The microbial phospho-lipids fatty acid (PLFAs) were extracted from the soils with a single-phase mixture of chloroform–methanol– citrate buffer (He et al. 2013). Nonadecanoic acid methyl ester was used as the internal standard. PLFAs were analyzed by GC (Agilent 6890 N) fitted with MIDI Sherlock microbial identification system (Ver- sion 4.5, MIDI).

The total amount of PLFAs included all the detected 42 fatty acids, and it was used to indicate the total microbial biomass (Tunlid et al. 1989; Zogg et al. 1997). The fatty acids 15:0, 17:0, i15:0, i16:0, i17:0, a15:0, a17:0, 16:1w7, 18:1w7, cy17:0 and cy19:0w8c were chosen as an index of bacterial biomass. Gram-positive bacteria were indicated by the fatty acids i14:0, i15:0, i16:0,

i17:0, a15:0 and a17:0, whereas gram-negative bacteria were expressed by 16:1w5c, 16:1w7c, 16:1w9c, 17:1w8c, 18:1w5c, 18:1w7c, 19:1w11c, cy17:0 and cy19:0. The quantity of the PLFAs such as 18:2w6 and 18:1w9 was used as an indicator of fungal biomass. Actinomycete was identified by the fatty acids 10Me16:0, 10Me17:0, 10Me18:0 and 10Me19:0.

Quality assurance and quality control

A procedural blank, a spiked blank, and a sample duplicate were included in every batch of 15 samples to ensure the accurate quantification of atrazine. No atrazine was found in any of the blanks. The recovery rates of atrazine in the spiked samples ranged from 85.5 to 103.5 %. The variations in atrazine concentrations in duplicates were lower than 15 % ($n = 3$). Five-point standard calibration curves were employed for quantitative analysis. The recovery rates of surrogate standards di-2-ethylhexyl phthalate d_4 were 84.5 ± 12.2 %. The limit of detection (LOD) for atrazine, which was estimated based on a signal-to-noise ratio of 3 using the lowest concentration standard, was 1.0 ng/g dry weight in soils.

Statistical analysis

Statistical analyses were performed using SPSS 18.0, Origin 8.0 and R (R Development Core Team 2015). The concentrations of pollutants were log-transformed to approximately normal distributions prior to the statistical analyses, where half of the LOD was assumed as the concentrations below LOD. Statistical significance was considered as $p < 0.05$. Spatial distributions of atrazine were simulated using universal Kriging in ArcGIS 10.2 (ESRI, Redlands, CA, USA).

Risk assessment

A considerable number of soil samples in this study were collected from farmlands close to villages or even around farmers' houses, where people living in rural areas may have frequent contact with the soil. The non-cancer and carcinogenic risks of exposure to atrazine in the YRD farmland soils to human health were estimated using the methods recommended by the US EPA (1997). In general, soil ingestion, inhalation and dermal contact were the main exposure routes, as illustrated in recent studies (Alexakis 2016; Islam et al. 2015). As the reference dose of inhalation and unit risk of atrazine inhalation were not available in the recommended methods, only the risks via soil ingestion and dermal contact exposure routes were assessed. The average daily dose (ADD, mg/kg/day) were estimated as follows.

$$\mathrm{ADD}_{\mathrm{ingest}} = \frac{C_{\mathrm{soil}} \times IRS \times EF \times ED}{BW \times AT} \times CF \quad (1)$$

$$\mathrm{ADD}_{\mathrm{dermal}} = \frac{C_{\mathrm{soil}} \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times CF \quad (2)$$

where C_{soil} is the concentration of atrazine in the soil (mg/kg); IRS is the soil ingestion rate (mg/day); *EF* isthe exposure frequency (days/year); *ED* is the expo- sure duration (years); *BW* is the body weight (kg); *AT*is the average lifetime exposure (days); *SA* is the dermal surface area (cm^2/day); *AF* is the soil adher- ence factor (mg/cm^2); *ABS* is the fraction absorbed dermally from the soil (unitless); and *CF* is the conversion factor (kg/mg).

Hazard quotient (*HQ*) was used to estimate the non-cancer risks of atrazine in agricultural soils via multiple routes, deriving from ADD and the specific reference dose (*RfD*) with the following equations (USEPA 1989).

$$HQ = \frac{\mathrm{ADD}}{RfD} \quad (3)$$

where *RfD* (mg/kg/day) is defined as the daily maximum permissible level of atrazine that will not pose non-cancer risks to residents during a lifetime, including the reference dose for ingestion of contam- inated food (RfD_o) and reference dose for dermal contact of contaminated food (RfD_{ABS}). RfD_{ABS} = - RfD_o 9 ABS_{GI}, where ABS_{GI} is the fraction absorbed in gastrointestinal tract in the critical toxicity study (US EPA 2015).

The non-cancer risks of atrazine via non-dietary and dietary pathways were presented as *HI*, which wascalculated as follows.

$$HI = \sum HQ_i \quad (4)$$

It is unlikely for the local residents to experience obvious adverse effect if HI < 1. The cancer risk assessment of each pollutant was calculated as follows.

$$\mathrm{RISK} = \mathrm{ADD} \times SF \quad (5)$$

where *SF* (mg/kg/day) consists of oral slope factors *SFO* for ingestion and *SFO* 9 ABS_{GI} for dermal contact.

It is considered that the carcinogenic risks of the pollutant is very low, while the risk value is less than 10^{-6}, low in the range of 10^{-6} and 10^{-4}, moderate from 10^{-4} to 10^{-3}, high from 10^{-3} to 10^{-1}, and very high if it is over 10^{-1} (Niu et al. 2013). All parameters used in the human exposure risk assess- ment are listed in Supporting Information (Table SI- 1), which are widely adopted in the literature (Niuet al. 2013; Rovira et al. 2015; Wang et al. 2015). This is a standard approach as a part of tiered risk assessment, which serves in this study to identifycontaminant hotspots and provide a screening refer- ence for site-specific detailed assessment if needed inthe future.

RESULTS AND DISCUSSION

Contamination status

All concentrations reported are on a dry weight (dw) basis. Atrazine was detected in 139 soil samples, i.e., a detection rate of 57.7 % of 241 sites. The concentrations of atrazine in all soil samples ranged from < 1.0 to 113 ng/g dry weight, with a mean of 5.7 ng/g and a median of 2.0 ng/g. Relatively high concentrations of atrazine were found in Changxing County of Zhejiang Province. Wang and Zhao (2000) monitored the residue of atrazine in agricultural soils from four villages of Liaoning Province, Northeast China, where an atrazine contamination accident happened. The level of atrazine still reached 362.1 ng/g one year after the pollution source had been eliminated. Atrazine residues were also investigated in crop– soil–groundwater system in Baiyangdian Lake area in North China (Ye et al. 2001). The concentrations of atrazine in soils from 18 sampling sites ranged from 10.2 to 86.9 ng/g in 9 months. Overall, the concentrations of atrazine in the YRD region were lower than those in North and Northeast China regions, suggesting the generally lower potential risk on human health in the YRD region. As atrazine was mostly used in cornfields, which was mainly based in Northeast China, the highest amount of atrazine usage in these regions may correlate to the higher concentrations of atrazine than other regions in China.

Soil physicochemical properties may affect the occurrence and behavior of atrazine. In this study, weak relationships were observed between the pairs of atrazine and total organic carbon (TOC) ($R = 0.166$, $p < 0.01$, $n = 241$; R correlation coefficient, p significance level, n sample size), as well as atrazine and pH ($R = -0.316$, $p < 0.01$, $n = 241$). It inferred that other factors such as pollution source and farmland practice might have greater effects on the spatial distributions of atrazine than soil pH and organic matter content did.

220 **Spatial distributions and sources estimation**

221 The concentrations of atrazine were higher in Zhejiang Province than those in Jiangsu Province
222 and Shanghai Municipality (Fig. 1). In large areas between the Yangtze River and the Taihu
223 Lake, no atrazine was found in soils and these areas were not polluted by atrazine, possibly
224 reflecting little usage of this herbicide because of the land use and cropping need. Nevertheless,
225 in the area southwest of the Taihu Lake, the contamination of atrazine was the highest among the
226 studied YRD sites, and thus the potential source of atrazine was further investigated. These
227 sampling sites were located close to a chemical plant in Changxing County, Zhejiang Province,
228 where atrazine was one of the major products, and it was one of the largest atrazine-producing
229 factories in China. The latest study in Southwest China (Tang et al. 2016) has reported that the
230 concentrations of pesticides were extraordinarily high in the topsoil from pesticide-producing
231 factories, and the pesticide concentrations in soils surrounding the pesticide factories were 1–2
232 orders of magnitude higher than those in the agricultural areas away from pesticide factories.
233 Possible release of atrazine products to the surrounding environment may occur via pathways
234 such as sewage discharge, waste disposal, atmospheric transmission and deposition, which may
235 have affected a large area of agricultural soils in northwest Zhejiang Province, as shown in Fig.
236 1. Although widespread presence of atrazine was detected in most soils from Zhejiang Province,
237 atrazine residues in areas other than Changxing County were relatively low, which may not pose
238 serious threat to the environment and people.

239 The spatial distribution of atrazine was also highly associated with the land-use types. In rice
240 paddy fields, atrazine was generally non-detectable or at low concentrations (mean of 1.9 ng/g,
241 detection rate = 45 %, n = 44). In view of pesticide application and agricultural practice, atrazine
242 was advised not to be used for weed control in rice paddy fields, due to its toxic effect on rice

243 seedlings (Zhang et al. 2014). This could explain why atrazine was rarely detected in rice paddy
244 fields. The higher concentrations of atrazine were found in corn fields (mean of 7.7 ng/g,
245 detection rate = 93 %, n = 29) and mulberry fields (mean of 15.1 ng/g, detection rate = 100 %, n
246 = 9). Since atrazine has been recommended suitable for weeding without hazard to corn plants
247 (Vonberg et al. 2014), this study revealed that its application for weed control could be important
248 sources of atrazine in the YRD region. On the other hand, it is worth noting that the highest
249 concentration of atrazine was found in a land planted with both rice and vegetables. It was
250 unlikely that farmers applied atrazine in this site on purpose for weed control. However, rice
251 cultivation needs abundant water, which is often taken from nearby river or reservoir. Surface
252 water was frequently recognized as secondary emission source of various pollutants, including
253 atrazine (Vonberg et al. 2014). In the vicinity of the above-mentioned pesticide production
254 factory, the contamination of atrazine may occur regardless of cropland uses, while the farmers
255 may be unaware of such pollution in water and soil.
256
257 **Impacts of atrazine on microbial communities**
258 Atrazine could undergo interactions with microbes in agricultural soil, thus inducing changes in
259 the composition and diversity of soil microbial communities (Fang et al. 2015; Godoi et al. 2014).
260 On the one hand, atrazine might be toxic to soil microbes and affect the microbial communities
261 (Freeman et al. 2011); on the other hand, some kinds of microbes can degrade atrazine and reduce
262 its concentration, while the soil microbial quantity increased (Fang et al. 2015). Comparison of
263 microbial PLFAs and classes associated with contamination are presented in Fig. 2. The bacterial
264 PLFAs of group 1, 2 and 3 were 9.5 ± 5.0, 5.8 ± 5.7, and 9.3 ± 5.4 nmol/g, respectively.
265 Nevertheless, there was no significant difference in PLFA compositions in soils among the areas

266 with different pollution levels (i.e., not contaminated, below 2.0 ng/ g, and from 4.0 to 85.2 ng/g),
267 although large differences in total microbial PLFAs were found in this study. There was no
268 significant difference in soil properties, such as TOC and pH in soils among the three areas.
269 A matrix of correlation coefficients between atrazine residues, microbial community
270 characteristic and soil property is shown in Table 1. Positive relationships were observed between
271 atrazine and total microbial biomass ($R = 0.417$, $p < 0.05$, $n = 30$), which suggested that the
272 current atrazine pollution level may not significantly affect soil microbial communities, while it
273 may stimulate the reproduction of some atrazine- degrading microbes. Microorganisms had the
274 ability to adapt and eventually utilize the present atrazine after long-term contamination (Sutton
275 et al. 2013). Nevertheless, the correlation coefficient (R) between atrazine residues and microbial
276 biomass was only 0.417, possibly because other factors such as soil types and land management
277 practices could impose greater effects on the structure of microbial communities in soils (Bossio
278 et al. 1998).
279 As shown in Table 1 and Fig. 2, no clear correla- tion was found between atrazine concentration
280 and the ratio of fungi to bacteria. Similarly, no clear correla- tion was found between atrazine
281 concentration and the ratio of gram-negative to gram-positive bacteria. These results indicated
282 that atrazine in the YRD soils showed no apparent effect on the composition of microbial
283 communities. Thus, the compositions of PLFAs in soils that had been exposed to atrazine were
284 not very sensitive indicators, although microbial communities might be influenced by the
285 introduction of atrazine to agricultural soil.
286
287 **Health risk assessments**
288 The health risks of atrazine to residents via multiple pathways were assessed based on the

289 concentrations of atrazine in the YRD farmland soils. Figure 3 illus- trates that soil ingestion was
290 the primary pathway of atrazine exposure, which contributed to 70–80 % of the total risk.
291 Overall, the non-cancer and carcinogenic risks to children were higher than those to adults.
292 Children are more susceptible to given dose of toxin and likely to inadvertently ingest significant
293 amount of soil by virtue of pica behavior and hand or finger sucking (Rasmussen et al. 2001).
294 Regarding the non-cancer risks via different path- ways, the estimated intake doses of atrazine
295 in all the samples were within acceptable levels (HI < 1). The average HI of atrazine for
296 children and adults were 1.46×10^{-6} and 2.79×10^{-7}, respectively, indicating minimal non-
297 cancer risks of atrazine in the YRD agricultural soils. Likewise, the estimated mean
298 carcinogenic risks posed by atrazine in soils via soil ingestion and dermal contact exposure
299 routes for children and adults were 1.03×10^{-9} and 4.81×10^{-10}, respectively. The risks
300 in all the samples were less than $<10^{-6}$, implying very low cancer risks of atrazine to both
301 children and adults.

302 However, it should be noted that the risks via dietary pathway had not been considered as the
303 data regarding intake of agricultural products harvested from the soils were not available. The
304 risks of chronic exposure to a mixture of atrazine and other pollutants were also not included in
305 this assessment. The actual contents of atrazine in various crops from the farm- lands and
306 metabolism of atrazine in human bodies would require additional assessment in the future.

307

308 **CONCLUSION**

309 A total of 241 topsoil samples collected from farm- lands in the YRD region were analyzed to
310 investigate the spatial distribution, possible sources, impacts on microbial communities, and
311 human health risks of herbicide atrazine. The concentrations of atrazine ranged from <1.0 to 113
312 ng/g dry weight (with a detection rate of 57.7 %). Relatively high concentration of atrazine was
313 found in Changxing County of Zhejiang Province where an herbicide factory was located. The

contamination of atrazine was closely related with land-use types. Atrazine was detectable at low concentrations in less than half of the rice paddy fields, but it was widely found at higher levels in corn fields and mulberry fields. There were no differences in PLFA composition and diversity in soils among the studied areas with different atrazine levels, although positive relationship was observed between atrazine and the total microbial biomass. The risk assessment of atrazine by itself to farmland residents via ingestion and dermal contact pathways showed minimal non-cancer risks and very low carcinogenic risks to adults and children in all the agricultural soil samples.

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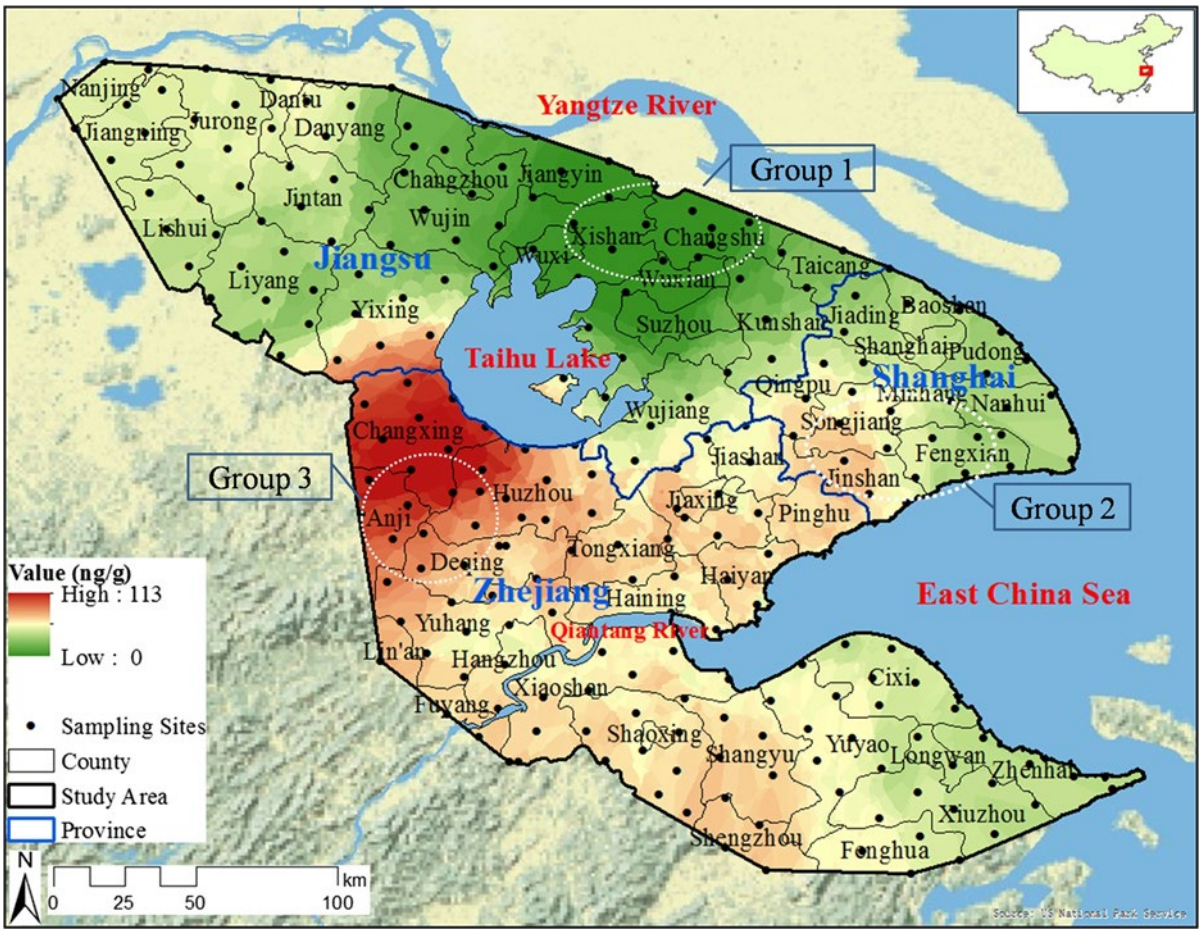

Fig. 1 Spatial distributions of atrazine in agricultural soils of the YRD region. Group 1 (non-contaminated area), group 2 (low polluted area) and group 3 (high polluted area) were selected for microbiological analysis

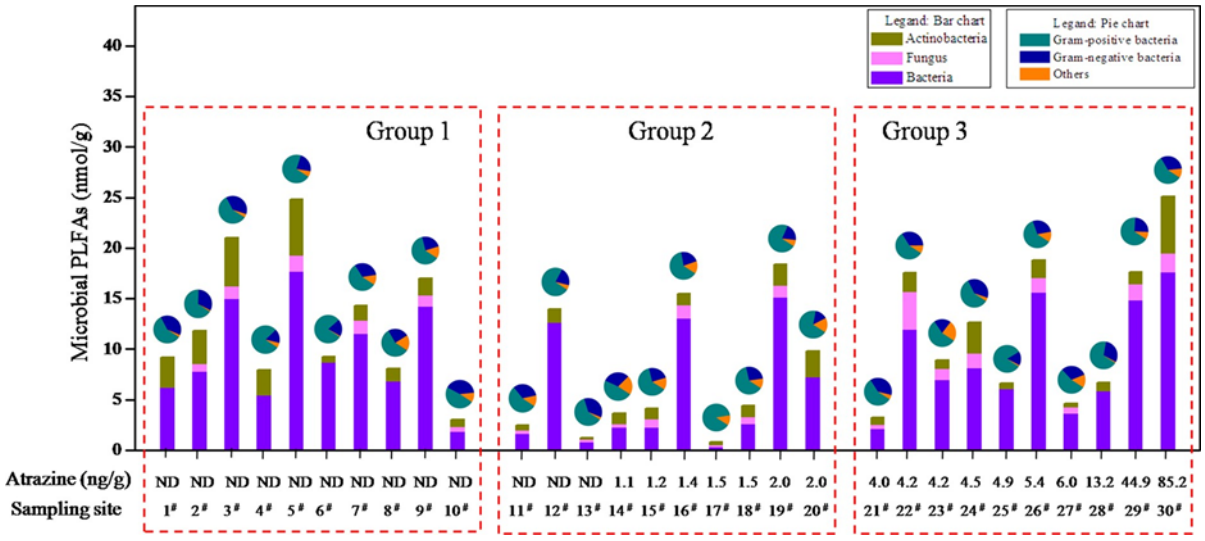

Fig. 2 Comparison of microbial PLFAs and classes associated with contamination. PLFAs of bacteria, fungi and actinomycete are given in the bar graph using y axis. Pie charts are included for gram-positive bacteria and gram-negative bacteria distribution

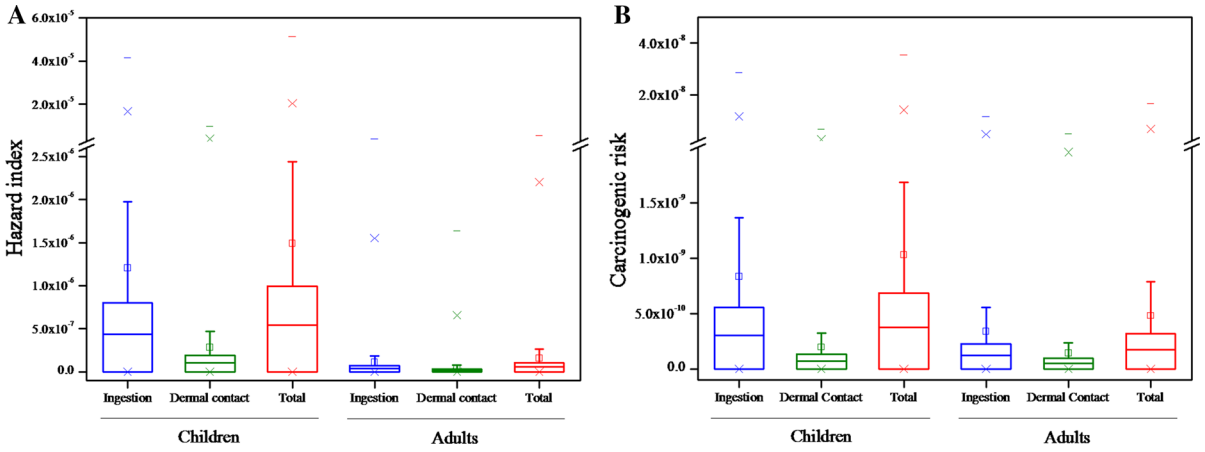

Fig. 3 Non-cancer risks (a) and carcinogenic risks (b) of atrazine to children and adults via dermal contact and ingestion exposure pathways

Table 1 Correlation coefficients (R, $n = 30$) between atrazine residues and microbial communities

	Atrazine	Biomass[a]	F/B[b]	GN/GP[c]
Atrazine	1			
Biomass	0.417*	1		
F/B	−0.068	−0.387*	1	
GN/GP	0.058	−0.110	0.067	1

[a] Biomass, the total biomass of bacteria, fungi and actinomycete

[b] F/B, PLFAs ratio of fungi to bacteria

[c] GN/GP, PLFAs ratio of gram-negative bacteria to gram-positive bacteria

* $p < 0.05$

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