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Title: Human migration activities drive the fluctuation of ARGs: case study of landfills in Nanjing, eastern China

Article Type: Research Paper

Keywords: Human migration; Waste dumping; Landfills; Antibiotic resistance genes; Mixed-compound bioaccessibility

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Abstract: Landfills are perfect sites to reflect the fluctuation of antibiotic resistance genes (ARGs) of one district as they are final destination for municipal waste. Large-scale human migration during holidays often is accompanied by changes in waste dumping, thereby impacting ARG abundance. Three landfills were selected to examine fifteen ARGs and Intl1 abundance fluctuation in Nanjing, eastern China for 14 months. Mass human migration, quantity of waste dumping and temperature exerted the most significant effects on bimonthly fluctuations of ARG levels in landfills. As a middle-sized cosmopolitan city in China, millions of college students and workers migrate during holidays, contributing to the dramatic increases in waste production and further ARG fluctuation. Mass migration was the main source of variation in waste dumping; then the waste production determined mixed-compound bioaccessibility; and compound bioaccessibility further positively impacted ARG level. The influence of various bioaccessible compounds on ARG level followed the order: antibiotics > nutrients > metals > organic pollutants. Concentrations of bioaccessible compounds were more highly correlated with ARG level than total concentrations, and could lead to the improvement of waste classification and management to control urban ARG dissemination by decreasing bioaccessible pollutants.

Dear Editor,

We would like to submit the original research article "**Human migration activities drive the fluctuation of ARGs: case study of landfills in Nanjing, eastern China**" for publication in *Journal of Hazardous Materials***.** No conflict of interest exits in the submission of this manuscript. All of the authors (*Mingming Sun*, Mao Ye, Arthur P. Schwab, Xu Li, Jinzhong Wan, Jun Wu*, Zhong Wei, Ville-Petri Friman, Kuan Liu, Da Tian, Manqiang Liu, Huixin Li, Feng Hu, and Xin Jiang*) mutually agree that the manuscript should be submitted to *Journal of Hazardous Materials*. The manuscript has not been previously submitted to *Journal of Hazardous Materials* or to any other international journal. There are 16 text pages, 4578 words (including text, figures, and table legends, but not references), 5 figures, 1 Table, 4 supplementary figures, 6 supplementary tables and 1 graphical abstract.

To our best knowledge, this is the first work to investigate the influence of human migration on the drive of the antibiotic resistance genes (ARGs) fluctuation in modern metropolises. As the final destination for municipal wastes, landfills are perfect sites to reflect the ARGs fluctuation of one district. Meanwhile, large-scale human migration during holidays often is accompanied by changes in waste dumping, thereby impacting ARG abundance. Therefore, three landfills in the present work were selected to examine fifteen ARGs and *IntI*1 abundance fluctuation in Nanjing, eastern China for 14 months. It was found that mass human migration and quantity of waste dumping exerted the most significant effects on bimonthly fluctuations of ARG levels in landfills. Mass migration was the main source of variation in waste dumping; then the waste production determined mixed-compound bioaccessibility; and compound bioaccessibility further positively impacted ARG level.

Additionally, the results of this macro-scale landfill investigation also contribute to a more thorough understanding of the fluctuations in ARG abundance in a modern metropolitan area and advance the development of best management practices to minimize ARG dissemination from urban landfills. This will be of great interest to the readers of *Journal of Hazardous Materials*.

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Responses to Editor Comments to Authors (in blue):

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Editor's comments:

Your submission entitled "Human Migration Activities Drive the Fluctuation of ARGs" has been received by Journal of Hazardous Materials. However, before we can proceed with your submission we ask you to address the following:

1. Cover letter should state the manuscript word count, which includes text, figure captions, and table legends, but not references. Also make sure manuscript should be no more than 5000 words including text, figures, and table legends, but not references.

Agree. Thanks for giving us this opportunity to revise the manuscript. There are 16 text pages, 4578 words (including text, figures, and table legends, but not references), 5 figures, 1 Table, 4 supplementary figures, 6 supplementary tables and 1 graphical abstract in this manuscript.

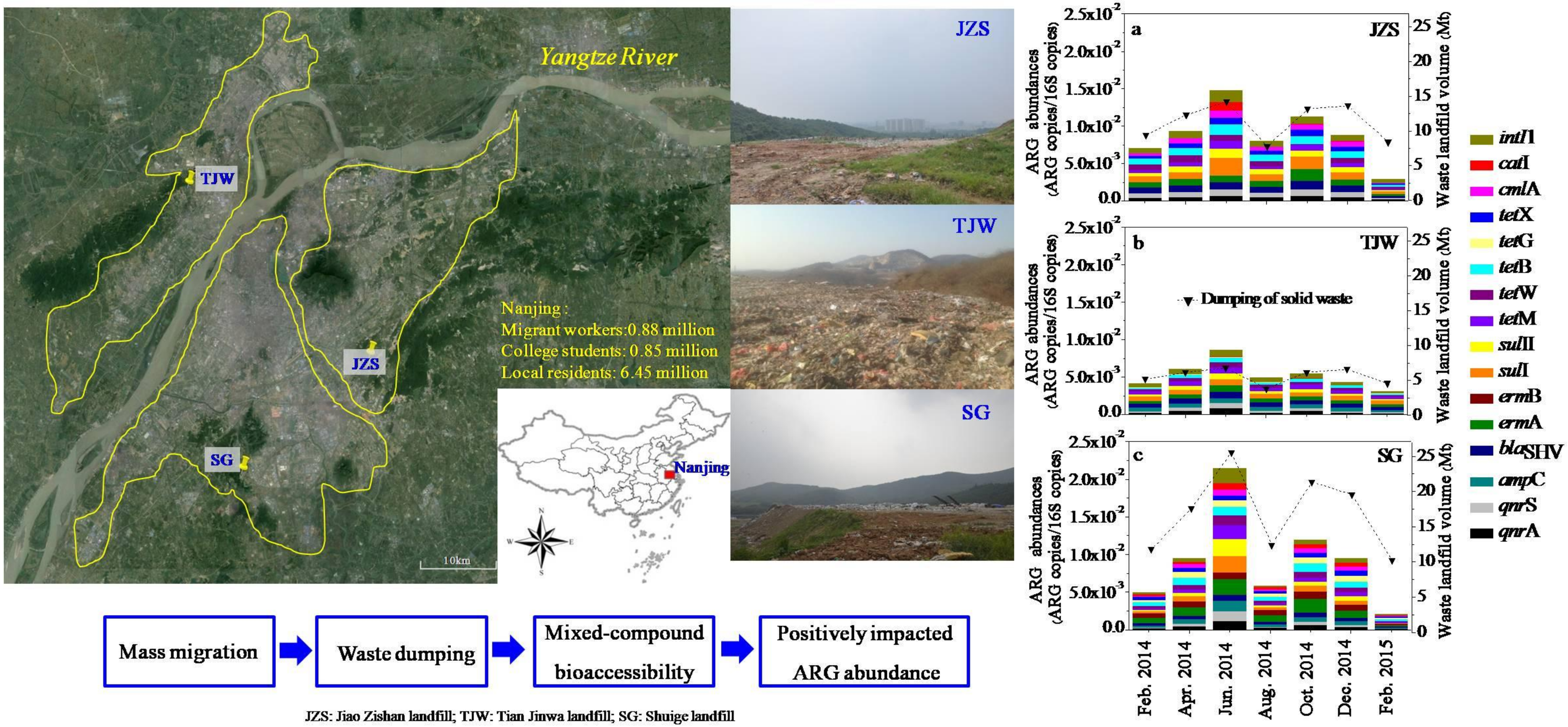
***Novelty Statement**

(1) This is the first comprehensive study to report that human mass migration acted as the major driving force to prompt the ARGs dissemination in landfills.

(2) Due to absence of waste classification and management, landfills in China was proved to be the hotspots of antibiotic resistance genes (ARGs) and mixed contaminants, including nutrient elements, heavy metals and organic pollutants, making which the perfect site to study ARG proliferation.

(3) Meanwhile, bioaccessible mixed pollutants exerted significant impact on the fluctuation of ARG level in landfills, following the orders of bioaccessible nutrients > bioaccessible heavy metals> bioaccessible organic pollutants.

Graphical Abstract



Highlights:

Mass migrations in a metro city greatly affect ARG dissemination.

Landfills are good sites to study the impact of mass migration on ARG proliferation.

Bioaccessible mixed contaminants clearly correlated with ARG levels in landfill.

Bioaccessible compounds effect on ARG was nutrient> heavy metal> organic pollutant.

Human migration activities drive the fluctuation of ARGs: case study of landfills in Nanjing, eastern China

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ABSTRACT

Landfills are perfect sites to reflect the fluctuation of antibiotic resistance genes (ARGs) of one district as they are final destination for municipal waste. Large-scale human migration during holidays often is accompanied by changes in waste dumping, thereby impacting ARG abundance. Three landfills were selected to examine fifteen ARGs and *IntI*1 abundance fluctuation in Nanjing, eastern China for 14 months. Mass human migration, quantity of waste dumping and temperature exerted the most significant effects on bimonthly fluctuations of ARG levels in landfills. As a middle-sized cosmopolitan city in China, millions of college students and workers migrate during holidays, contributing to the dramatic increases in waste production and further ARG fluctuation. Mass migration was the main source of variation in waste dumping; then the waste production determined mixed-compound bioaccessibility; and compound bioaccessibility further positively impacted ARG level. The influence of various bioaccessible compounds on ARG level followed the order: antibiotics > nutrients > metals > organic pollutants. Concentrations of bioaccessible compounds were more highly correlated with ARG level than total concentrations, and could lead to the improvement of waste classification and management to control urban ARG dissemination by decreasing bioaccessible pollutants.

Keywords: Human migration; Waste dumping; Landfills; Antibiotic resistance genes; Mixed-compound bioaccessibility

1. Introduction

Extensive use of antibiotics in household and animal husbandry has become a problem in China because it can lead to the over expression of antibiotic resistance genes (ARGs) in bacteria [1]. Meanwhile, ARGs have been found to be capable of spreading across bacteria through vertical and horizontal transfer with the assistance of integrons, transposons and plasmids etc [2]. As a consequence, the non-selective transfer of ARGs to both commensal and pathogenic bacteria makes ARGs an emerging environmental contaminant and a threat to human health and environmental security [3].

With a population of 1.4 billion, every national festival in China (i.e. the Spring Festival, the mid-Autumn Day, and the National Day, etc.) results in large-scale, world-wide migration. The combination of the huge population base and massive migration will result in great changes in the waste generation. Garbage classification and management systems have not been established in many Chinese cities; landfills are the common final destination of domestic waste, industrial waste, biohazard waste, and agricultural livestock waste [4]. The occurrence and fluctuation of ARGs in specific facilities, including landfills were detected, making which hotspots of ARG contamination [5-7]. Therefore, landfills are perfect sites to investigate the impact population migration and human activity on ARG abundance and fluctuations in a city and could serve as a bellwether of the proliferation of risk of ARGs across the region. However, detailed, systematic data on the fluctuation of ARG levels in landfill soils are limited.

Some studies detected the apparent correlation between antibiotics presence and their corresponding resistance genes in waste samples [8] or in the soils of facilities such as feedlots [9, 10], while others reported no significant correlation between ARG abundance and antibiotics/organic pollutants [7, 11]. Meanwhile, our previous study found apparent correlation can be detected between bioaccessible organic pollutants (OPs) and ARG levels in dairy farm soils [12]. Given that it is commonly the water-soluble fraction of chemicals exerts direct selective pressure on the bacterial gene expression [13]; therefore, the apparently contradictory results might be caused by inconsistent bioaccessibility of the pollutants among the different sites. The influence of the bioaccessibility of mixed pollutants on observed ARG levels in landfills has not been investigated.

The present work was performed to monitor the occurrence and diversity of ARGs, metals, OPs and nutrients in three landfills in Nanjing (population of 8.2 million) for 14 months. The objective was to: i) identify the factors impacting the abundance and fluctuation trend of ARGs in three landfills, such as population base and migration, waste dump quantity, antibiotic/OP/metal content and diversity, and nutrients contents; ii) explore the probable relationship between ARG abundance and bioaccessible fraction of detected pollutants. The results of this macro-scale landfill investigation will contribute to a more thorough understanding of the fluctuations in ARG abundance in a modern metropolitan area and advance the development of best management practices to minimize ARG dissemination from urban landfills.

2. Materials and methods

2.1. Site Description and Sample Collection

Nanjing, a middle-sized city in Southeast China, was selected for this study as representative of modern urban areas with large population base and drastic seasonal temperature variations. All three landfills in Nanjing were monitored for 14 consecutive months. As depicted in the Fig. 1, three landfill sites split the urban area of Nanjing into three districts, with one landfill in each district. Jiao Zishan landfill (JZS, built in 1992, 31°59'44'' N, 118°53'56'' E) accepts wastes produced from the eastern and northeastern Nanjing including Xianlin college town and Zhongshan Mountain. Tian Jinwa landfill (TJW, in operation between 1985 and 2015, 32°8'53'' N, 118°41'2'' E) corresponds to Pukou new downtown area in the north of Yangtze River. Shuige landfill (SG, built in 1993, 31°53'45'' N, 118°45'4'' E) covers the old downtown area and Jiangning new downtown in southern Nanjing. Populations in the three districts are significantly varied, in the order SG (4.33 million) > JZS (2.65 million) > TJW (1.23 million). Bi-monthly waste dumping quantity and temperature parameters in three landfill sites was provided by the City Council Department of Nanjing.

Soils were sampled bi-monthly from Feb. 2014 to Feb. 2015. Due to the long operating time, garbage mountains ranging in height from 50 to 100 meters had been formed at all three landfill sites. A soil layer covered the top refuse layer, and newly transported waste was dumped along the hillside. Therefore, check board pattern method was used to reflect the impact of newly transported waste on soil ARG fluctuation [14]. A square of 1600 m^2 (40 m×40 m) side length on hillside was set up, then the big square is divided into 16 small squares of 100 m^2 side length, in which we collect 0-15 cm surface soil sample (10 kg) at the right angle and mixed it thoroughly. Then twenty five soil samples were collected along the hillside per garbage dump each time. Soil samples were kept in polyethylene bags and put on dry ice during transport. After freeze drying, all soils samples were mixed thoroughly and sieved through a 2-mm mesh. The soil samples were stored at -20°C before DNA extraction and chemical analysis. Detailed information about the sampling sites and the physiochemical properties of soils are outlined in Fig. 1 and Supporting Information Table S1.

2.2. *DNA Extraction and ARG Determination*

Total DNA of soil samples were extracted using FastDNA kit for soil (MP Biomedicals, CA) according to the manufacturer's instruction. Extracted DNA samples were restored at -20 °C prior to subsequent quantitative PCR (qPCR) analysis. Sixteen target genes including two quinolone resistance genes (*qnr*A and *qnr*S), two β-lactams resistance genes (*amp*C and bla_{SHV}), two marcolides resistance genes (*erm*A and *erm*B), two *sul* genes (*sul*I and *sul*II), five *tet* genes (*tet*M, *tet*W, *tet*B, *tet*G, and *tet*X), two chlorampheicol resistance genes (*cml*A and *cat*I) and class 1 integron-integrase gene (*intl*1). The 16S rRNA gene abundance of each sample was also determined to normalize the target ARG abundance. Detailed primer information involved was listed as supplementary information (Table S2). The qPCR was performed to determine 16 target ARGs and 16S rRNA gene abundance in triplicate according to our previous study [15].

2.3. Chemical Analysis

Based on our preliminary investigation, six classes of antibiotics including oxytetracycline (OTC), chlortetracycline (CTC), sulfadiazine (SD), sulfamethoxazole (SMX), sulfadimidine (SM2), florfenicol (FF), chloramphenicol (CAP), norfloxacin (NFX), ofloxacin (OFX), roxithromycin (RTM), tylosin (TYL), amoxicillin (AMOX), and penicillin (PEN); eight OPs including phenanthrene (PHE), pyrene (PYR), hexachlorocyclohexane (HCH), pentachlorophenol (PCP), 2,4,4'-trichlorobiphenyl (PCB28), 2,2',5,5'-tetrachlorobiphenyl (PCB52), 2,4,4'-tribromodiphenyl ether (BDE28), 2,4,2',4'-tetrabromodiphenyl ether (BDE47) and six heavy metals, including Cd, Ni, Cr, Pb, Cu, and Zn. The analytical procedures for the total concentration of antibiotics, OPs and heavy metals in soil samples were analyzed using LC-MS-MS, GC-MS and Thermo Flame Atomic Absorption Spectrophotometer, respectively, following our previous methods [12, 16]. Environmental quality parameters including total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), hydrolysable nitrogen (N), total phosphorus (TP) and Olsen P were also analyzed according to the standard methods [17].

According to the theory of bioaccessibility of soil compounds, water-soluble antibiotics and, water-extracted heavy metals and DOC, hydrolysable N, and Olsen P were defined as bioaccessible fractions of their corresponding chemicals. Specifically, water-soluble antibiotics and heavy metals, and water-extractable OPs using Tenax TA extraction method were analyzed as described in our previous study [12].

2.4. Enumeration of total, fecal and antibiotic resistant coliforms

Total, fecal and antibiotic resistant coliform plate counts were performed on soil samples from three landfill sites according to the method described by Ahammad et al. [18]. Culturable bacteria were isolated from soil samples using a sterile buffer solution [0.2% (w/v) sodium pyrophosphate dissolved in ultrapure water]. Soil sample and buffer solution were mixed (1:1, v/v) in sterile 50-mL tube and set to shaker for 4 h at 120 rpm and 4 °C. Then the mixture was serially diluted with sterile phosphate buffer solution (PBS) and plated on Rapid Hi-chrome coliform agar (RHCA, Himedia, Shanghai) in triplicate at 37 °C for 24 h, and then at 44 °C for 48 h. The fecal and total coliform counts were calculated following manufacturer's instructions. Enumeration of antibiotic resistant coliforms was performed following the method described by Watkinson et al. [19].

2.5. Data Analysis

All data analyses were conducted using SPSS 13.0, Origin 8.0 and Canoco for Windows (Version 5.0). Pearson correlation and redundancy analysis (RDA) were carried out to determine the relationship between ARG levels and various environmental factors (population size, waste volume, antibiotics, heavy metals, organic pollutants, and nutrients, etc.).

3. Results and discussion

3.1. Seasonal fluctuation of ARGs in landfills

All six classes of ARGs were detected in the soil samples collected from three landfill sites, including two quinolone resistance genes (*qnr*A and *qnr*S), two β-lactams resistance genes (*amp*C and bla_{SHV}), two macrolides resistance genes (*erm*A and *erm*B), two *sul* genes (*sul*I and *sul*II), five *tet* genes (*tet*M、*tet*W、*tet*B、*tet*G, and *tet*X), and two chloramphenicol resistance genes (*cml*A and *cat*I) (Fig. 2). As depicted in the Fig. 1, three landfills cover three different districts of the city. Population in the three districts follows the orders of SG (4.33 million) > JZS (2.65 million) > TJW (1.23 million). As population base clearly determines the waste quantity produced in each district, monthly waste produced among three sites also correspondingly followed the same trends – SG > JZS > TJW. In addition, waste quantity was positively correlated with the amount of mixed pollutants transported to the site ($p < 0.05$). Meanwhile, the relative abundance of 15 detected ARGs varied significantly, with the highest values (2.2×10^{-2} ARG copies/16S copies) observed in

June, 2014 at SG, whereas the ARG accumulative abundance value in TJW was significantly lower over the same period (8.6×10^{-3} ARG copies/16S copies).

Thirteen classes of antibiotics, eight OPs, six metals and high contents of carbon/nitrogen/phosphorus (C/N/P) nutrients coexistence was also detected in three landfill samples. Similarly, mixed compounds' diversity and contents were the highest in the SG. However, only random fluctuation rather than apparent or consistent patterns in the diversity and contents of these mixed compounds was detected between seasonal collected samples (Fig. S1).

ARG levels in the three sites did share similar trend as summarized in Fig. 2. ARG levels increased from February, 2014 to June, 2014; decreased sharply in August; increased in the following two months, and declined to its lowest level in February, 2015. The average temperatures in February and August were the lowest and highest, respectively, in Nanjing (Fig. S2). Nanjing is the capital of the third-ranked gross domestic production province and has more than 30 colleges; thus, approximately 0.85 million college students and 0.88 million migrant workers will leave Nanjing for the Chinese Spring Festival around February (Fig. S3). Therefore, it seemed that significantly declined waste production (Fig. 2) due to large-scale human migration (Fig. S3) contributed to the lowest ARG level in the landfill sites. With the temperature increasing and million-population returning to Nanjing, waste dumping was enhanced significantly ($p < 0.05$), leading to the elevated ARG level correspondingly (Fig. 2). ARG level reached its highest in June, 2014. This was most likely caused by the most suitable temperature for bacterial propagation (average daily temperature at 26 °C in June) during this period (Fig. S2). Stable high population and ideal temperatures apparently acted as engines driving ARG increases. Waste dumping decreased sharply in August due at least in part to students leaving campus for summer and others leaving the city (Fig. 2, S2 and S3). Low disposal volumes accompanied by minimal soil moist contents in the landfill surface (caused by high temperature and reduced rainfall) coincided with declination of ARG abundance [17]. After two-month summer break, college students returned to campus, leading to a slight rebound of ARG level. Cold winter temperatures again were accompanied by reduced ARG level. The ARG level in February, 2015 was significantly lower than that in February, 2014 ($p < 0.05$).

3.2. Mass human migration impacted ARG levels in three landfills

Previous studies have reported the seasonal variance of ARG levels caused by differentiating anthropogenic activity in surface waters camping areas or livestock waste lagoons [18, 20]. However, the relationship between mass human migration and ARG levels still remains unclear. Our previous results suggested that bioaccessibility of tetracycline and OPs in the dairy farm soils is the key factor impacting ARG fluctuation. However, compared with other reported ARG dissemination hotspots like livestock feedlots, and wastewater treatment plants, landfill sites absorb and restore more complex mixed compounds (i.e. antibiotics, OPs, heavy metals, and nutrient elements detected in this study) with diverse origins. Therefore, we assume that it is the variance of mixed compounds' bioaccessibility caused by mass migration that impact ARG levels in three landfills.

According to the definition in soil chemistry, the bioaccessible compound is the fraction that which is available to cross an organisms' membrane from the environment it inhabits [21]. Accordingly, water soluble antibiotics and metals, water extractable OPs, DOC, Olsen P, and hydrolysable N were determined as the bioaccessible fractions of their corresponding compounds in this work. As the world's largest waste generator, more than 90% of waste generated in China is still landfilled without any pretreatment [4]. Consequently, bioaccessibility of mixed substances in landfill soil is approximately 9.2-17.1% of the total (Fig. 3 and Fig. S1). Significant correlation was indeed observed between mass migration and the bioaccessible fractions of the above-detected compounds, with r^2 varying from 0.13 to 0.72 (Table S3). Generally, the larger the population, the higher the chemicals' bioaccessibility. In addition, fluctuation trends of bioaccessible mixed antibiotics, OPs, metals and nutrients (Fig. 3) resembled those of ARG abundance fluctuation (Fig. 2), with correlation coefficients (r^2) in the range of 0.12-0.78, 0.07-0.61, 0.08-0.27, and 0.16-0.82, respectively (Table 1 and Table S4-S6). In contrast, the total contents of various antibiotics, metals, OPs and nutrients being brought along with waste varied randomly among sampling time (r^2 at 0-0.38, Fig. S1, Table S4-S6), suggesting that it is the bioaccessbility of the mixed compounds that exerted apparent effect on ARG levels in three landfills (Table 1 and Table S4-S6).

To assess and classify the effect of various bioaccessible chemicals on ARG level, RDA analysis was carried out in this study. Seven environmental factors were analyzed, including population size, waste quantity, and average temperatures in Nanjing, and bioaccessible fractions of antibiotics, OPs, metals, and nutrients in three landfills. As depicted in Fig. 4, the lengths of the arrows stand for the strength of the correlation between the factor and ARG level, and the angles between arrows indicate the relationship between individual environmental variables. RDA results showed that: i) Three landfills in Nanjing exerted various effects on the potential transmission of ARG contamination in the city, with the orders of SG > JZS > TJW; and the ARG level fluctuation in SG site is impacted most significantly by the environmental variables ($p < 0.05$). ii) Although temperature exerted a strong effect, population size and waste quantity are the more important in accounting for the ARG level among seven analyzed environmental factors. In other words, instances greater population migration and the higher of waste production had the greatest influence on ARG fluctuation. This is especially true for the site of SG, while JZS and TJW sites still accountable just with lower strength. iii) Mixed OPs and nutrient elements in the landfills impacted the ARG level to different extents: antibiotics > nutrients > metals > OPs.

Antibiotics in soil are able to exert selective pressure on indigenous microbial consortia [22, 23]. Under such circumstances, antibiotic resistant bacteria (ARB) manage to sustain themselves by mutation or ARG overexpression [24, 25]. Moreover, the presence of some antibiotics not only results in their corresponding ARG over expression but can indirectly contribute to the acquisition of multi-resistance in ARB with the assistance of mobile genetic elements (such as plasmids, integrons, and transposons) [1, 26]. In the present work, high abundance of class 1 integron gene (*intl*I) was detected in all three landfills (Fig. 2). Pearson correlation analysis supports the theory that the presence of one class of antibiotic can be significantly correlated with more than one type of ARGs (Table 1). Moreover, in the present study, bioaccessible antibiotics exhibited stronger correlation with ARGs than total antibiotics. Similar correlation can be found between bioaccessible metals and various ARG levels (Table S4). Despite the fact that metals were often associated with ARG expression by acting as essential constitute of enzyme proteins involved [27], their total contents in soil were significantly correlated with ARG levels less than half as often as bioaccessible fractions of metals (Table S4).

For the detectable OPs in the three landfills (PAHs, OCPs, BDEs, and PCBs), total contents generally were not correlated with ARGs levels (Table S5). Interestingly, except for correlations between bioaccessible PHE, PHY, HCH, PCP and *tet* and *sul* genes ($p < 0.05$), all other bioaccessible OPs generally were not significantly correlated to any of the ARGs detected. Being hydrophobic, these compounds have low solubility in soil water (appx. 1.6 mg/L for PHE, appx. 0.4

mg/L for PCBs, appx. 0.1 mg/L for PYR and BDE 28, and <0.02 mg/L for HCH, PCP, and BDE 47). In addition, according to previous research [28], hydrophobic organic compounds commonly do not have a direct target to activate ARG expression in bacteria. As a consequence, non-polar organic compounds rarely induce ARG expression. But some of them can go through the cell membrane via diffusion, and activate or prompt efflux pumps in the cells [29-31]. Given that most efflux pumps carry genes that can efflux antibiotics and/or chemicals out the cell over the long evolution, some organic chemicals can induce indirectly ARG expression.

Nutrients also play a crucial role in ARG levels. As essential elements of microbial metabolisms, C, P, and N have previously been reported to impact ARG expression [1]. More importantly, the present study further demonstrated that more bioacessible forms of these elements (DOC, Olsen P, and hydrolysable N) were associated more significantly with ARG level than that of the total C, P, and N ($p < 0.05$, Table S6). Considering that it is the bioaccessible nutrient elements that have potential access to the organisms' cells, it is apparent that ARG abundance (especially the fraction expressed in the living bacterial cells) was more prone to be influenced by bioaccessible nutrients in the sites. Furthermore, among three investigated nutrient elements, hydrolysable N exerted the strongest impact on ARG abundance, followed by DOC and Olsen P. This can be caused by the much lower hydrolysable N than that of DOC, making it the comparatively rate-limiting factor among the three elements.

Apart from ARGs, the class 1 integron-integrase gene *intI*1 was also monitored in this work as a commonly accepted proxy for anthropogenic pollution [32]. Similar results were obtained. It was significantly correlated with mixed antibiotics, OPs, metals and nutrients to various extents, indicating its crucial role in manipulating ARG expression and dissemination in the landfills.

3.3. Antibiotic-resistant fecal coliforms as probable indicator of ARG fluctuation

Due to the poor implementation of garbage sorting in urban China, some unforeseen wastes other than municipal solid waste (i.e., human feces, manure, sludge from waste water treatment plants, and even some biohazard wastes) have been illegally dumped to landfill sites [4]. As an important endosymbiont of animals including human, coliform consortium exists in animal gut, and can be excreted out of the body and mainly ended up in landfills [33]. Therefore, changes in coliform enumeration in the landfills of one city can reflect the population fluctuation of this location. Considering the apparent correlation between population fluctuation and ARG level in this work, coliforms, including total, fecal, and antibiotic resistant coliforms were enumerated to investigate their potential as indicators of ARG fluctuation. As summarized in Fig. S4, despite the great variance of different groups of coliforms, two general trends can be observed in three landfills: i) their bimonthly counts following the orders of SG > JZS > TJW; ii) coliform isolates were the lowest during the periods of both December and February, and the elevated counts can be detected in April and June. Both phenomena correspond to fluctuations of ARG level (Fig. 1) and population in Nanjing (Fig. S3). Moreover, when the counts of three-group coliforms were fit into the correlation analysis with detected ARG abundance, they followed the orders of resistant coliforms > fecal coliforms > total coliforms (Fig. 4). Interestingly, despite a positive trend observed between total coliforms and ARG levels in three landfill sites, the correlation was not statistically significant ($p > 0.05$). However, both resistant and fecal coliforms significantly correlated with ARG levels ($p < 0.05$), with the higher r^2 value calculated for the resistant coliforms, suggesting that antibiotic resistant coliforms can act as an indicator of ARG fluctuation in landfills.

3.4. Environmental Implications

The results obtained in this work have two major implications. First, under-developed waste classification and management systems in landfills create incubators for ARG dissemination in the urban areas of China. Previous results tended to focus on facilities like feedlots, sewage management plants, which did not represent the whole urban district. However, more human migration events almost undoubtedly impact ARG level and proliferation at different levels- in the scenario-spot level, the middle-sized-city level as Nanjing described here, the larger-scale level like one country or even transnational district. More importantly, the larger the migration event, the greater influence it might exert on the ARG dissemination. Therefore, it is important for local government to facilitate waste classification and improve waste management facilities across the whole city. All the landfills eventually reach capacity, cease collections, undergo closure, and may be redeveloped as eco-park or similar facilities. For instance, TJW site was been closed in the middle of 2015. Considering its crucial role as ARG reservoir and incubator, the fluctuations of ARG level should be monitored over a long period even after its redevelopment. Meanwhile, a formalized risk assessment for antibiotic resistance genes should be established and carried out, and practical options should be put into effect.

Additionally, the present work also illustrated the need to employ bioaccessible fractions of pollutants (rather than total pollutant concentrations) to study the correlation between pollutants and ARG level. This is especially useful while carrying out relevant work on long-period contaminated site. As long as the precise content of bioaccessible fraction of pollutants can be determined, researchers can better determine the correlation between pollutant(s) and ARG levels. However, practices need to focus on reducing bioccessibile fractions of mixed pollutants and nutrients simultaneously to control the ARG fluctuation and further impede its dissemination.

4. Conclusions

Antibiotic resistance genes have caused great threat against human health considering its dissemination among different environments. The present work demonstrated that landfills in major cities are hotspots of ARGs and mixed contaminants. Among different factors investigated in this work the mass human migration was the most significant one impacting the fluctuation of ARG levels in landfill soils, following by the factors of nutrient elements, heavy metals, and organic pollutants. Moreover, greater significant correlation between bioaccessible fractions nutrients and mixed pollutants and ARG abundances in landfills could be detected than that of total fractions. As a consequence, researchers need to focus on reducing the bioaccessibility of mixed pollutants in landfills to decrease the proliferation of ARGs from indigenous bacteria to human pathogens in metro cities, therein declining the ever-increasing threats to human health.

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Table

Table 1 Correlation Analysis between Total/Bioaccessibile Concentrations of Antibiotics and Target ARGs [a]

	∑ OTC+CTC		∑ SD+SMX+SM2		∑ FF+CAP		∑ NFX+OFX		∑ RTM+TYL		∑ AMOX+PEN	
	T [b]	B [c]	T	B	T	B	T	B	T	B	T	B
*qnr*A	-	-	-	-	-	-	-	**0.42**	-	-	-	-
*qnr*S	-	0.12	-	-	-	-	0.14	**0.51**	-	-	-	-
*amp*C	-	-	-	0.05	-	-	-	-	-	-	0.16	**0.62**
bla_{SHV}	-	-	-	-	-	-	-	-	-	-	-	**0.52**
*erm*A	0.12	0.21	0.06	0.14	-	0.16	-	-	0.33	**0.68**	-	-
*erm*B	0.08	0.13	0.11	0.18	-	0.21	-	-	-	**0.58**	-	-
*sul*I	0.15	0.22	**0.34**	**0.78**	-	0.17	-	0.05	-	0.19	-	0.03
*sul*II	0.09	0.18	-	**0.67**	-	0.13	-	-	-	-	-	-
*tet*M	**0.12**	**0.56**	0.03	0.11	-	0.19	-	0.17	-	0.23	-	-
*tet*W	**0.24**	**0.68**	-	-	-	-	-	0.12	-	-	-	0.18
*tet*B	nd	**0.57**	0.08	0.16	-	-	-	-	-	-	-	-
*tet*G	0.19	**0.47**	-	0.08	-	-	-	-	-	-	-	-
*tet*X	-	**0.62**	-	-	-	-	-	-	-	-	-	-
*cml*A	-	-	-	-	0.18	**0.46**	-	-	-	-	-	-
*cat*I	-	0.08	-	-	-	**0.36**	-	-	-	-	-	-
∑ Total ARGs	-	0.12	-	0.17	-	-	-	-	-	-	-	-
*intI*1	0.24	**0.62**	0.35	**0.68**	0.14	**0.57**	0.18	**0.51**	0.12	**0.64**	0.08	**0.68**

[a] Values of the Pearson correlation coefficient (r^2) were indicated respectively (values in **bold** $p < 0.01$, values $p < 0.05$, values $p > 0.05$ were not shown). [b] T means total concentration. [c] B means bioaccessible concentration.

Figure Captions

Fig. 1. Samping location of the three landfills in Nanjing city,easten china. Jiao Zishan (JZS) landfill absorbs wastes produced from the eastern and northeastern Nanjing including Xianlin college town and Zhongshan Mountain scenic spot; Tian Jinwa (TJW) landfill corresponds to Pukou new downtown area in the north of Yangtze River; and Shuige (SG) landfill covers the old downtown area and Jiangning new downtown in southern Nanjing. The population of JZS, TJW and SG is 2.65, 1.20 and 4.33 million, respectively. JZS: 0.20 million migrant workers, 0.45 million college students, 2.00 million local residents; TJW: 0.08 million migrant workers, 0.12 million college students, 1.00 million local residents; SG: 0.60 million migrant workers, 0.25 million college students, 3.48 million local residents.

Fig. 2. Soil ARG relative abundance (ARG copies/16S copies) and waste landfill volume (Mt) in different sampling months from the three landfills. JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

Fig. 3. Bioaccessibile concentration (mg/kg) of antibiotics, organic pollutants, metals and nutrients in different sampling months from the three landfills. JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

Fig. 4. Redundancy analysis (RDA) of ARGs and the environmental variable factors. Circle, rectangle and diamond symbols represent the ARG relative abundance in different sampling months from JZS, TJW and SG landfills, respectively. The size of the sample symbols corresponding to the ARGs richness (number of ARGs in the soil samples). Environmental variables in three purple arrows represent population, waste dumpling and temperature factors, respectively. Environmental variables in four orange arrows represent bioaccessibile antibiotics, bioaccessibile POPs, bioaccessibile metals and bioaccessibile nutrients, respectively. The lengths of the arrows reveal the strength of the relationship and the angles between arrows indicate the correlation between individual environmental variables. The percentage of variation explained by each axis is shown, and the relationship is significant (p <0.01). RDA was performed with Canoco for Windows (Version 5.0).

Fig. 5. Relationships between ARG relative abundance (ARG copies/16S copies) and total, fecal and antibiotic resistant coliforms (CFU/mL). JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

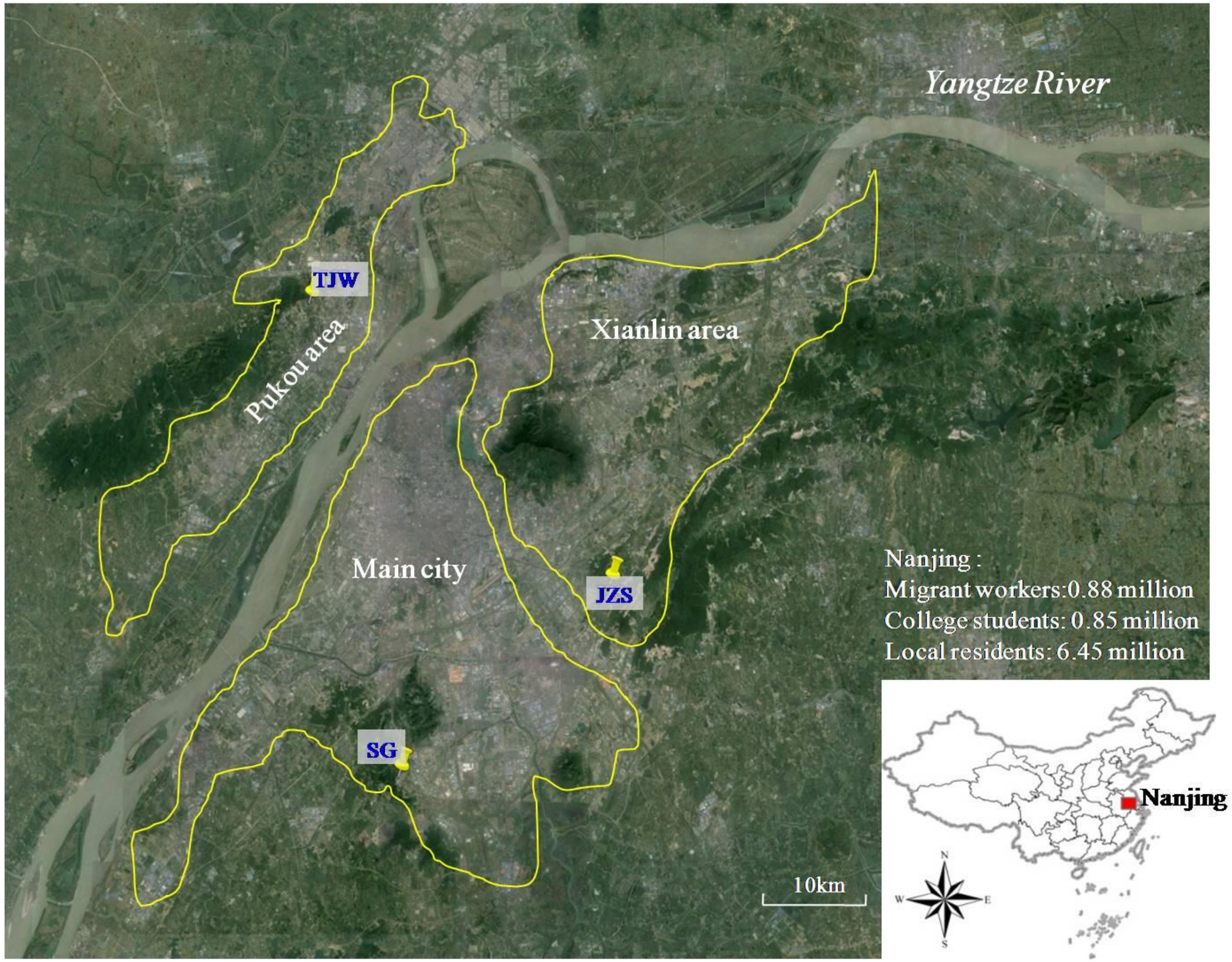

Yangtze River
TJW
Pukou area
Xianlin area
Main city
JZS
SG
Nanjing :
Migrant workers:0.88 million
College students: 0.85 million
Local residents: 6.45 million
10km
Nanjing
N
S
W
E

Fig. 1.

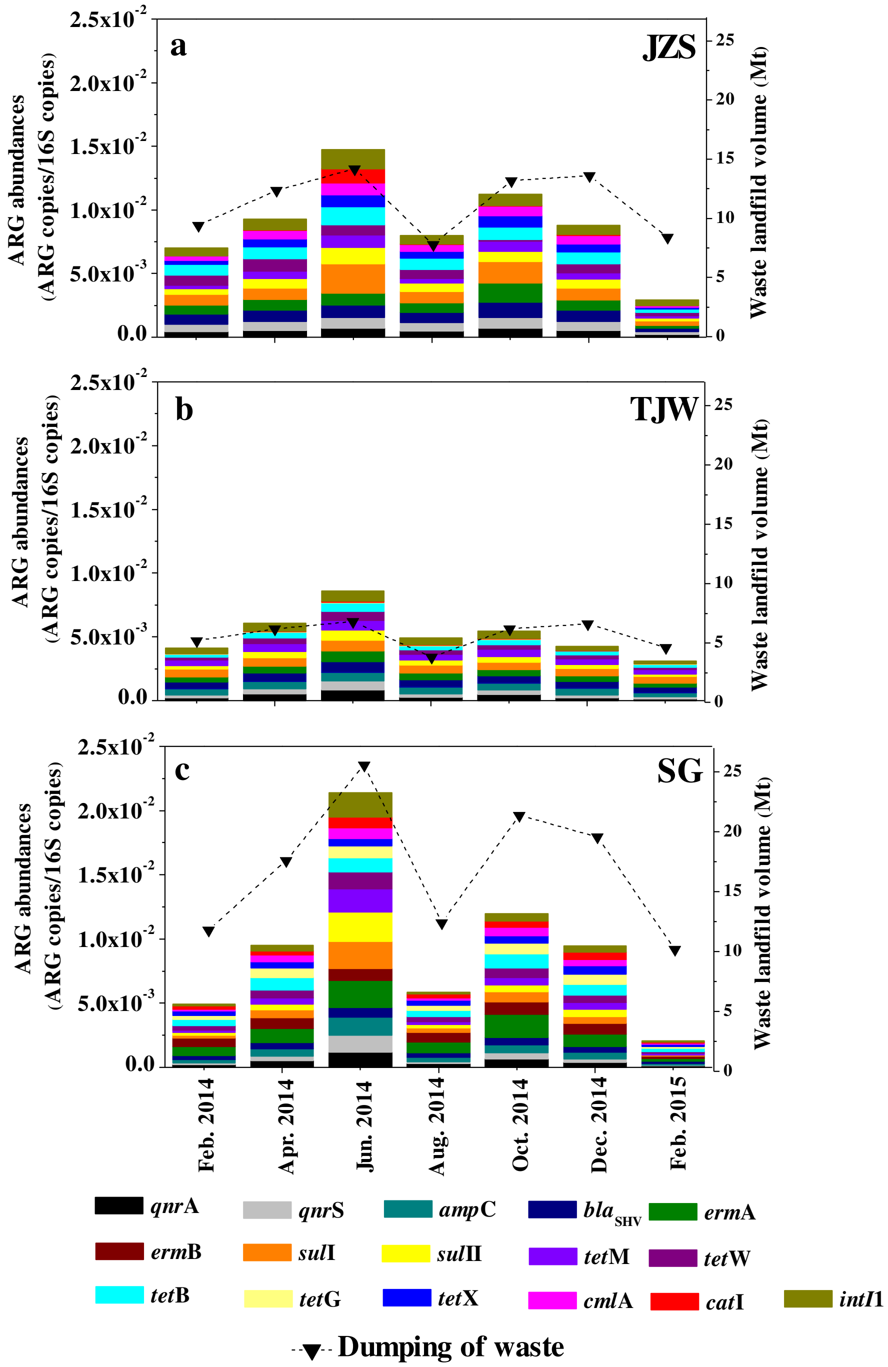

a
JZS
b
TJW
c
SG
ARG abundances
(ARG copies/16S copies)
Waste landfild volume (Mt)
0.0
5.0x10^-3
1.0x10^-2
1.5x10^-2
2.0x10^-2
2.5x10^-2
0
5
10
15
20
25
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015
qnrA
qnrS
ampC
bla SHV
ermA
ermB
sulI
sulII
tetM
tetW
tetB
tetG
tetX
cmlA
catI
intI1
Dumping of waste

Fig. 2.

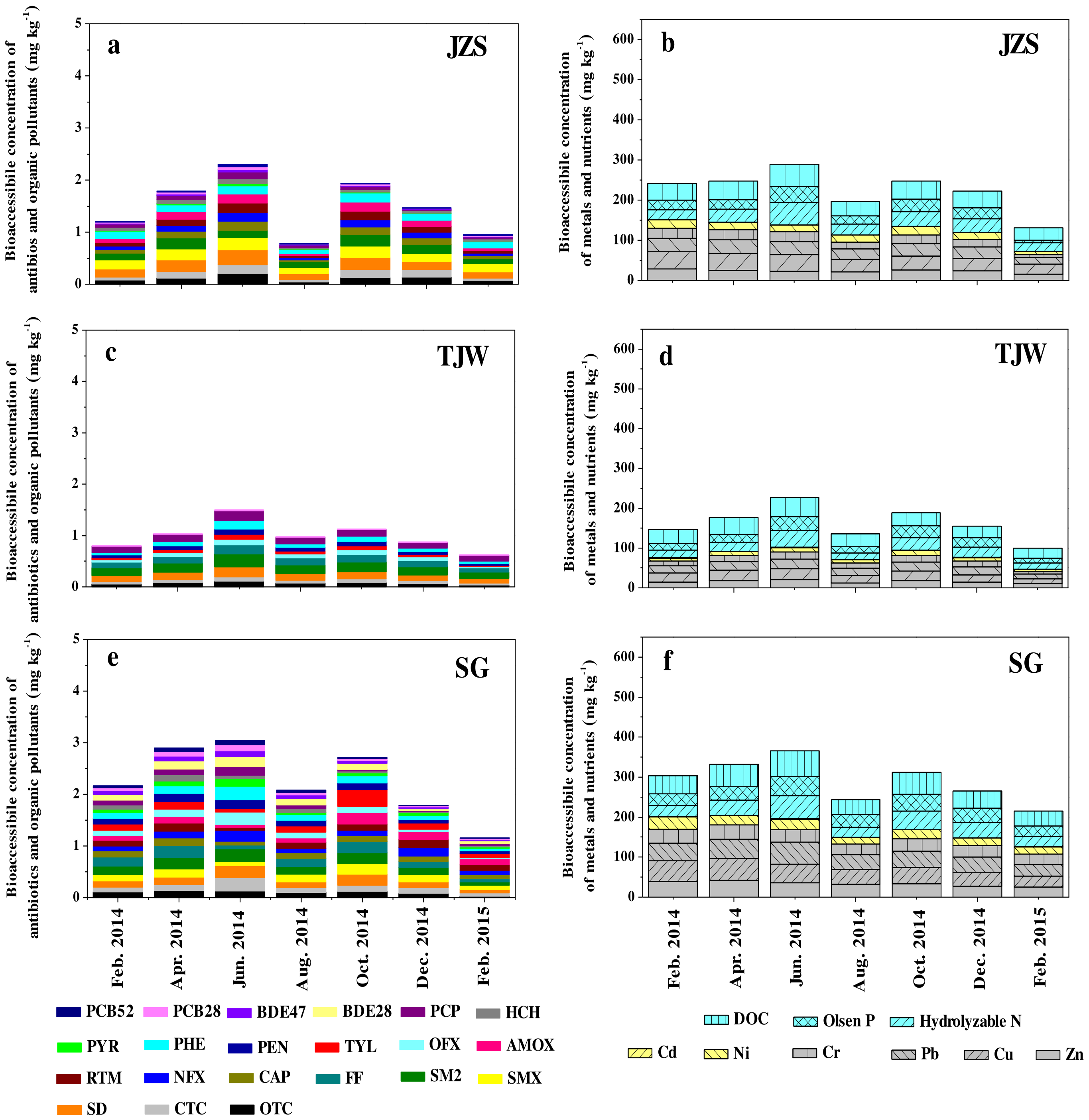
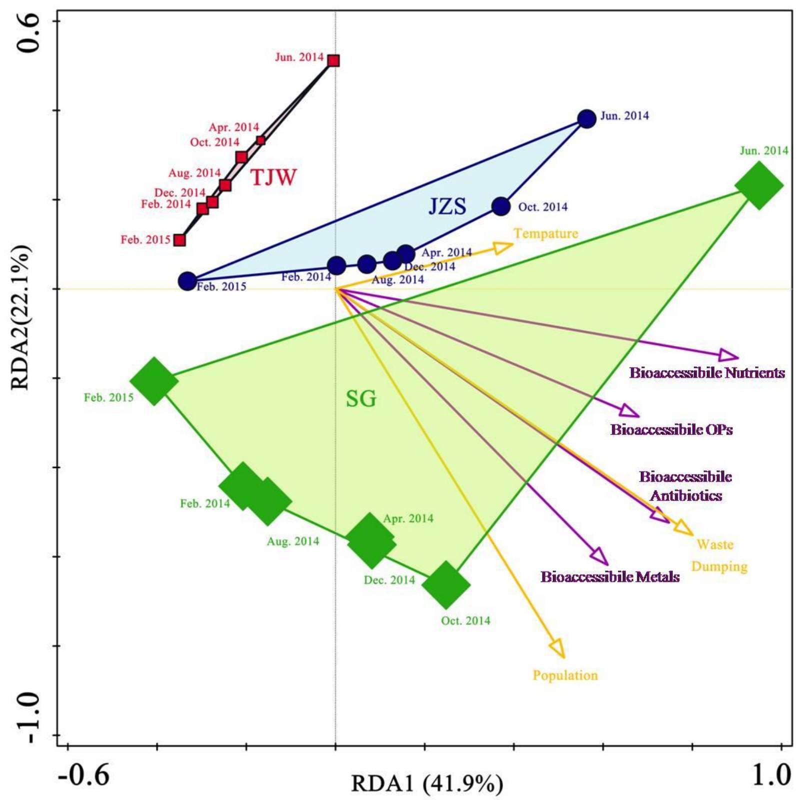
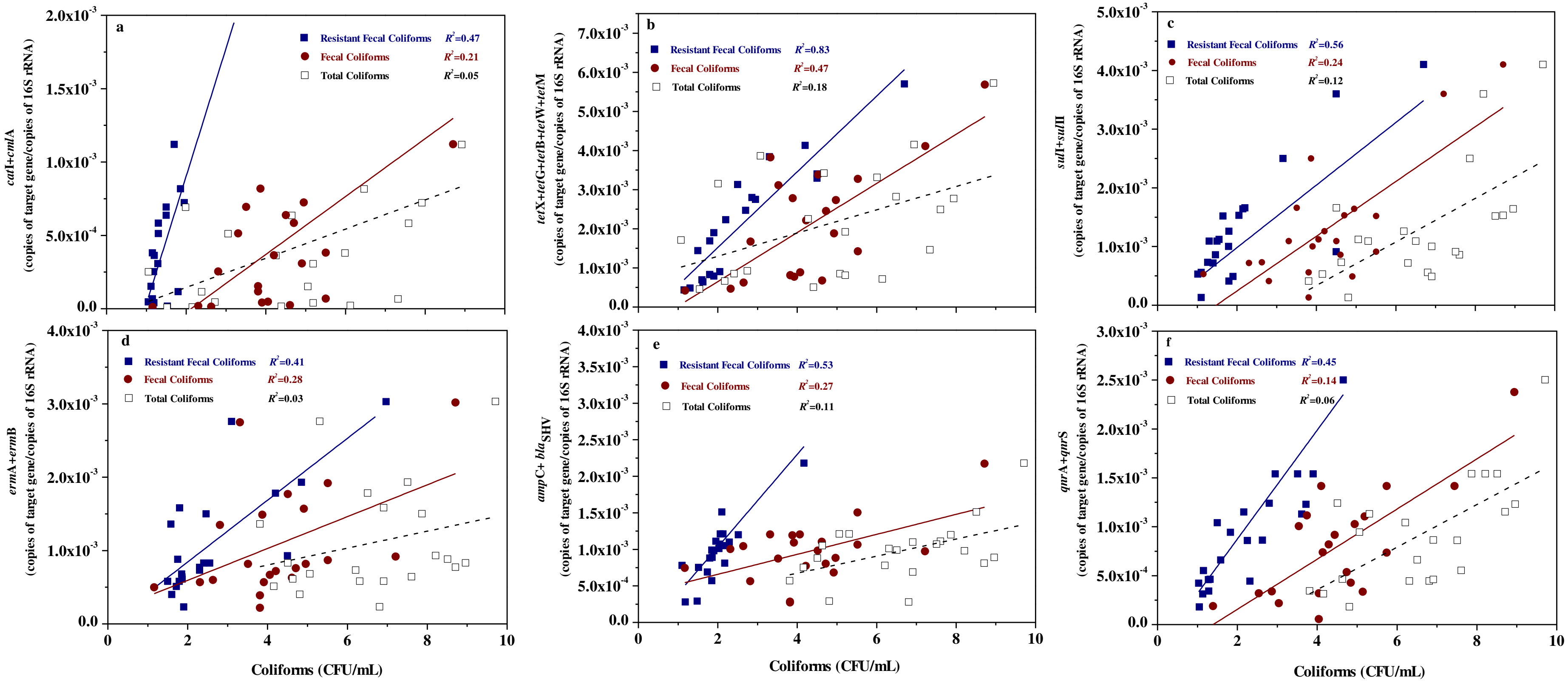

a
JZS
b
JZS
c
TJW
d
TJW
e
SG
f
SG
Bioaccessibile concentration of antibios and organic pollutants (mg kg-1)
Bioaccessibile concentration of antibiotics and organic pollutants (mg kg-1)
Bioaccessibile concentration of metals and nutrients (mg kg-1)
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015
PCB52
PCB28
BDE47
BDE28
PCP
HCH
PYR
PHE
PEN
TYL
OFX
AMOX
RTM
NFX
CAP
FF
SM2
SMX
SD
CTC
OTC
DOC
Olsen P
Hydrolyzable N
Cd
Ni
Cr
Pb
Cu
Zn

Fig. 3.



0.6
-1.0
-0.6
1.0
RDA1 (41.9%)
RDA2(22.1%)
TJW
JZS
SG
Jun. 2014
Apr. 2014
Oct. 2014
Aug. 2014
Dec. 2014
Feb. 2014
Feb. 2015
Tempature
Bioaccessibile Nutrients
Bioaccessibile OPs
Bioaccessibile Antibiotics
Waste Dumping
Bioaccessibile Metals
Population

Fig. 4.



a
Resistant Fecal Coliforms R^2=0.47
Fecal Coliforms R^2=0.21
Total Coliforms R^2=0.05
catI+cmlA
(copies of target gene/copies of 16S rRNA)
b
Resistant Fecal Coliforms R^2=0.83
Fecal Coliforms R^2=0.47
Total Coliforms R^2=0.18
tetX+tetG+tetB+tetW+tetM
(copies of target gene/copies of 16S rRNA)
c
Resistant Fecal Coliforms R^2=0.56
Fecal Coliforms R^2=0.24
Total Coliforms R^2=0.12
sulI+sulII
(copies of target gene/copies of 16S rRNA)
d
Resistant Fecal Coliforms R^2=0.41
Fecal Coliforms R^2=0.28
Total Coliforms R^2=0.03
ermA+ermB
(copies of target gene/copies of 16S rRNA)
e
Resistant Fecal Coliforms R^2=0.53
Fecal Coliforms R^2=0.27
Total Coliforms R^2=0.11
ampC+ bla_{SHV}
(copies of target gene/copies of 16S rRNA)
f
Resistant Fecal Coliforms R^2=0.45
Fecal Coliforms R^2=0.14
Total Coliforms R^2=0.06
qnrA+qnrS
(copies of target gene/copies of 16S rRNA)
Coliforms (CFU/mL)

Fig. 5.

Supporting Information (Tables)

Table S1 Physiochemical Properties of Soils from three sampling sites

Site		Feb. 2014	Apri. 2014	Jun. 2014	Aug. 2014	Oct. 2014	Dec. 2014	Feb. 2015
JZS [a]	Sand (%)	25.6	22.4	24.7	26.3	28.3	21.1	25.3
	Silt (%)	63.7	65.3	61.2	64.2	61.1	61.8	62.4
	Clay (%)	10.7	12.3	14.1	9.5	10.6	17.1	12.3
	pH	5.9	6.2	6.1	6.9	6.7	6.3	6.1
	Organic matter (%)	2.6	2.8	3.1	2.7	2.5	2.4	2.5
	Soil moisture content (%)	15.9	19.2	19.7	5.6	17.6	16.6	14.8
TJW [b]	Sand (%)	27.8	26.5	28.3	23.9	24.7	22.4	27.4
	Silt (%)	57.9	59.6	61.2	62.2	65.3	64.4	58.5
	Clay (%)	14.3	13.9	10.5	13.9	10	13.2	14.1
	pH	6.9	6.5	6.3	6.4	6.5	6.2	6.7
	Organic matter (%)	1.6	1.4	2.2	1.6	1.8	1.9	1.4
	Soil moisture content (%)	15.3	18.1	19.1	6.3	17.4	16.1	15.5
SG [c]	Sand (%)	22.1	26.7	28.4	28.7	21.6	24.3	22.2
	Silt (%)	66.4	64.4	69.2	65.5	60.2	59.6	64.5
	Clay (%)	11.5	8.9	2.4	5.8	18.2	16.1	13.3
	pH	6.3	6.5	6.7	6.6	6.3	6.7	6.5
	Organic matter (%)	2.7	2.5	3.5	2.2	3.1	2.8	2.6
	Soil moisture content (%)	15.4	18.7	19.3	4.7	16.6	17.2	15.1

[a] JZS: Jiao Zishan landfill, [b] TJW: Tian Jinwa landfill, [c] SG: Shuige landfill.

Table S2 Target Genes, primers sequence and amplicon size for ARGs and *intI*1 amplification.

Target genes	Primer sequence (5'-3')	Amplicon size (bp)	Annealing temperature (°C)	Amplification program
*tet*W	R- GGG CGT ATC CAC AAT GTT AAC F- GAG AGC CTG CTA TAT GCC AGC	168	60	Q-PCRwas performed using a Bio-Rad IQ5 instrument (Bio-Rad Company, U.S.) and a 25-μL reaction mixture (0.2 μM of each primer, and 1 μL of template) with a temperature program of 15 min at 95°C (initial denaturing and Hot Start Taq activation), followed by 50 cycles of the following: 15 s at 95°C; 30 s at the annealing temperature; and 30 s at 72°C (optical window on) followed by a final dissociation stage.
*tet*X	R- TTC TTA CCT TGG ACA TCC CG F- AGC CTT ACC AAT GGG TGT AAA	278	60	
*tet*M	R- TGG CGT GTC TAT GAT GTT CAC F- ACA GAA AGC TTA TTA TAT AAC	171	55	
*tet*B	R- ACT GCC GTT TTT TCG CC F- CCT TAT CAT GCC AGT CTT GC	391	55	
*tet*G	R- CGATTACAGCTGTCAGGTGGG F- CAGCTTTCGGATTCTTACGG	844	60	
*sul*I	R-CTG AAC GAT ATC CAA GGA TTY CC F-AAA AAT CCC ACG GRT C	239	50	
*sul*II	R-GCG TTT GAT ACC GGC ACC CG F- GCG CTC AAG GCA GAT GGC AT	293	60	
*erm*A	R-TTC GCA AAT CCC TTC TCA AC F-AAG CGG TAA ACC CCT CTG A	190	60	
*erm*B	R- GGA ACA TCT GTG GTA TGG CG F-CAT TTA ACG ACG AAA CTG GC	450	60	
*cat*I	R- CCG CCC TGC CAC TCA TC F- GGC ATT TCA GTC AGT TG	585	55	
*cml*A	R- GGCCACCTCCCAGTAGAA F- GCCAGCAGTGCCGTTTAT	158	55	
*amp*C	R- CCY TTT TAT GTA CCC AYG A F-TTC TAT CAA MAC TGG CAR CC	550	60	
bla_{SHV}	R- CGC AGA TAA ATC ACC ACA ATG F- TCG CCT GTG TAT TAT CTC CC	304	60	
*qnr*A	R- CCA TCC AGA TCG GCA AA F- TTC TCA CGC CAG GAT TTG	521	55	
*qnr*S	R- TTY GCB GYY CGC CAG TCG F- GCA AGT TCA TTG AAC AGG GT	428	55	
*intI*1	R-GAG GAT GCG AAC CAC TTC CAT F-ACC AAC CGA ACA GGC TTA TG	286	60	
16S rRNA	R-ATT ACC GCG GCT GCT GG F-ACT CCT ACG GGA GGC AGC AG	200	60	

Table S3 Correlation Analysis between Human Migration and Bioaccessibile Concentrations of Compounds [a]

Bioaccessibile compounds	Human migration								
	JZS [b]			TJW [c]			SG [d]		
	Migrant workers	College students	Local residents	Migrant workers	College students	Local residents	Migrant workers	College students	Local residents
∑ Bioaccessibile antibiotics	**0.48**	**0.58**	-	**0.38**	**0.45**	-	**0.61**	**0.72**	-
∑ Bioaccessibile metals	**0.41**	**0.56**	-	0.35	0.42	-	**0.52**	**0.64**	-
∑ Bioaccessibile organic pollutants	0.35	0.44	-	-	0.13	-	0.45	0.45	-
DOC	**0.46**	**0.52**	-	**0.16**	**0.32**	-	**0.51**	**0.66**	-
Olsen P	**0.37**	**0.49**	-	**0.22**	**0.31**	-	**0.47**	**0.62**	-
Hydrolyzable N	**0.40**	**0.55**	-	**0.23**	**0.39**	-	**0.56**	**0.69**	-

[a] Values of the Pearson correlation coefficient (r^2) were indicated respectively (values in **bold** p < 0.01, values p < 0.05, values p > 0.05 were not shown). [b] JZS: Jiao Zishan landfill, [c] TJW: Tian Jinwa landfill, [d] SG: Shuige landfill.

Table S4 Correlation Analysis between Total/Bioaccessibile Concentrations of Metals and Target ARGs*[a]

	Cd		Ni		Cr		Pb		Cu		Zn	
	T [b]	B [c]	T	B	T	B	T	B	T	B	T	B
*qnr*A	-	-	-	-	-	-	-	0.15	-	0.12	-	0.15
*qnr*S	-	-	-	-	-	-	-	0.17	-	0.13	-	0.17
*amp*C	-	-	-	-	-	-	-	-	-	-	-	-
bla_{SHV}	-	-	-	-	-	-	-	0.19	-	0.12	-	0.22
*erm*A	0.08	0.36	-	0.19	-	0.08	-	0.16	-	0.19	-	0.27
*erm*B	-	0.07	-	-	-	-	-	-	-	-	-	-
*sul*I	0.17	**0.44**	0.14	0.24	-	0.21	**0.17**	**0.42**	0.27	**0.58**	0.22	**0.38**
*sul*II	-	0.05	-	-	-	-	0.19	**0.27**	0.14	0.27	0.12	0.23
*tet*M	0.15	**0.36**	0.12	0.23	-	0.17	0.21	**0.51**	0.38	**0.61**	0.24	**0.52**
*tet*W	0.06	0.14	-	0.22	-	0.16	0.26	**0.47**	0.22	**0.47**	**0.14**	**0.47**
*tet*B	-	-	-	-	-	-	-	0.15	-	0.26	-	0.25
*tet*G	0.01	0.09	-	0.26	-	-	-	0.18	-	0.16	-	0.12
*tet*X	-	-	-	-	-	-	-	0.13	-	0.21	-	0.16
*cml*A	-	0.21	-	-	-	-	-	0.07	-	0.14	-	-
*cat*I	-	0.16	-	-	-	-	-	-	-	-	-	-
∑ Total ARGs	0.12	**0.26**	0.14	0.23	-	0.18	0.25	**0.49**	0.32	**0.59**	0.28	**0.53**
*intI*1	0.18	**0.39**	-	-	-	-	0.29	**0.58**	0.38	**0.51**	0.32	**0.62**

[a] Values of the Pearson correlation coefficient (r^2) were indicated respectively (values in **bold** p < 0.01, values p < 0.05, values p > 0.05 were not shown). [b] T means total concentration. [c] B means bioaccessible concentration.

Table S5 Correlation Analysis between Total/Bioaccessibile Concentrations of Organic Pollutants and Target ARGs [a]

	∑ PHE+PYR		∑ HCH+PCP		∑ BDE28+BDE47		∑ PCB28+PCB52	
	T [b]	B [c]	T	B	T	B	T	B
*qnr*A	-	-	-	-	-	-	-	-
*qnr*S	-	-	-	-	-	-	-	-
*amp*C	-	-	-	-	-	-	-	-
bla_{SHV}	-	-	-	-	-	-	-	-
*erm*A	-	0.13	-	-	-	-	-	-
*erm*B	-	-	-	-	-	-	-	-
*sul*I	0.11	**0.24**	0.15	0.27	-	-	-	0.05
*sul*II	-	-	-	-	-	-	-	-
*tet*M	0.12	**0.27**	0.11	**0.25**	-	-	-	-
*tet*W	-	0.08	-	**0.16**	-	-	-	-
*tet*B	-	-	-	-	-	-	-	-
*tet*G		0.09	-	-	-	-	-	-
*tet*X	-	-	-	-	-	-	-	-
*cml*A	-	0.21	-	-	-	-	-	-
*cat*I	-	0.16	-	-	-	-	-	-
∑ Total ARGs	-	**0.20**	0.04	**0.19**	-	-	-	-
*intI*1	-	**0.31**	-	**0.29**	-	-	-	-

[a] Values of the Pearson correlation coefficient (r^2) were indicated respectively (values in **bold** p < 0.01, values p < 0.05, values p > 0.05 were not shown). [b] T means total concentration. [c] B means bioaccessible concentration.

Table S6 Correlation Analysis between Total/Bioaccessibile Concentrations of Nutrient Elements and Target ARGs [a]

	TOC [b]	DOC [c]	Total P	Olsen P	Total N	Hydrolyzable N
*qnr*A	0.12	**0.46**	-	**0.37**	-	**0.52**
*qnr*S	-	0.42	-	**0.29**	-	**0.35**
*amp*C	-	0.34	-	**0.22**	-	0.43
bla_{SHV}	-	0.24	-	0.35	-	0.34
*erm*A	0.18	**0.61**	0.16	**0.31**	0.11	**0.67**
*erm*B	0.15	0.54	0.04	0.38	-	**0.59**
*sul*I	0.21	**0.67**	0.08	**0.45**	0.15	**0.82**
*sul*II	0.15	**0.63**	-	0.32	0.06	**0.74**
*tet*M	0.22	**0.62**	0.13	**0.27**	0.18	**0.68**
*tet*W	0.18	**0.56**	0.04	0.32	-	**0.62**
*tet*B	0.08	0.48	-	0.40	0.08	**0.53**
*tet*G	0.06	0.42	-	**0.36**	-	**0.47**
*tet*X	-	0.34	-	0.39	-	**0.34**
*cml*A	-	0.42	-	0.44	-	0.36
*cat*I	-	0.16	-	0.32	-	0.26
∑ Total ARGs	0.18	**0.56**	0.08	**0.42**	0.12	**0.58**
*intI*1	-	**0.64**	-	**0.53**	-	**0.68**

[a] Values of the Pearson correlation coefficient (r^2) were indicated respectively (values in **bold** $p < 0.01$, values $p < 0.05$, values $p > 0.05$ were not shown). [b] TOC means total organic carbon. [c] DOC means dissolved organic carbon.

Supporting Information (Figures)

Figure Captions

Fig. S1. Total concentration (mg/kg) of antibiotics, organic pollutants, metals and nutrients in different sampling months from the three landfills. JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

Fig. S2. Temperature (°C) changes JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

Fig. S3. Population migration in different sampling months from Xianlin area, Pukou area and Main city.

Fig. S4. Total, fecal and antibiotics resistant coliforms levels (CFU/mL) in different sampling months from the three landfills. JZS: Jiao Zishan landfill, TJW: Tian Jinwa landfill, SG: Shuige landfill.

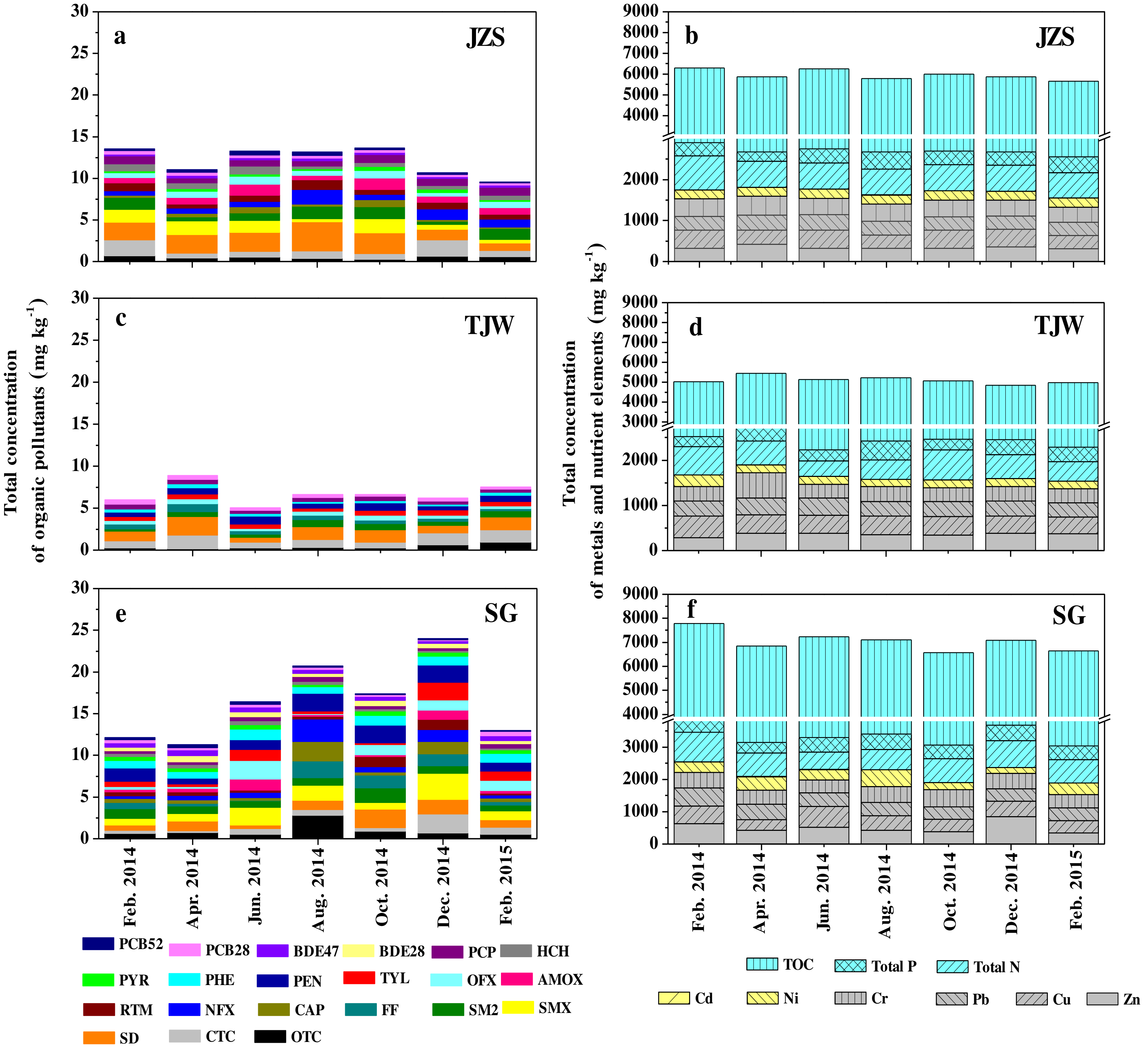

a
JZS
b
JZS
c
TJW
d
TJW
e
SG
f
SG
Total concentration of organic pollutants (mg kg-1)
Total concentration of metals and nutrient elements (mg kg-1)
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015
PCB52
PCB28
BDE47
BDE28
PCP
HCH
PYR
PHE
PEN
TYL
OFX
AMOX
RTM
NFX
CAP
FF
SM2
SMX
SD
CTC
OTC
TOC
Total P
Total N
Cd
Ni
Cr
Pb
Cu
Zn

Fig. S1.

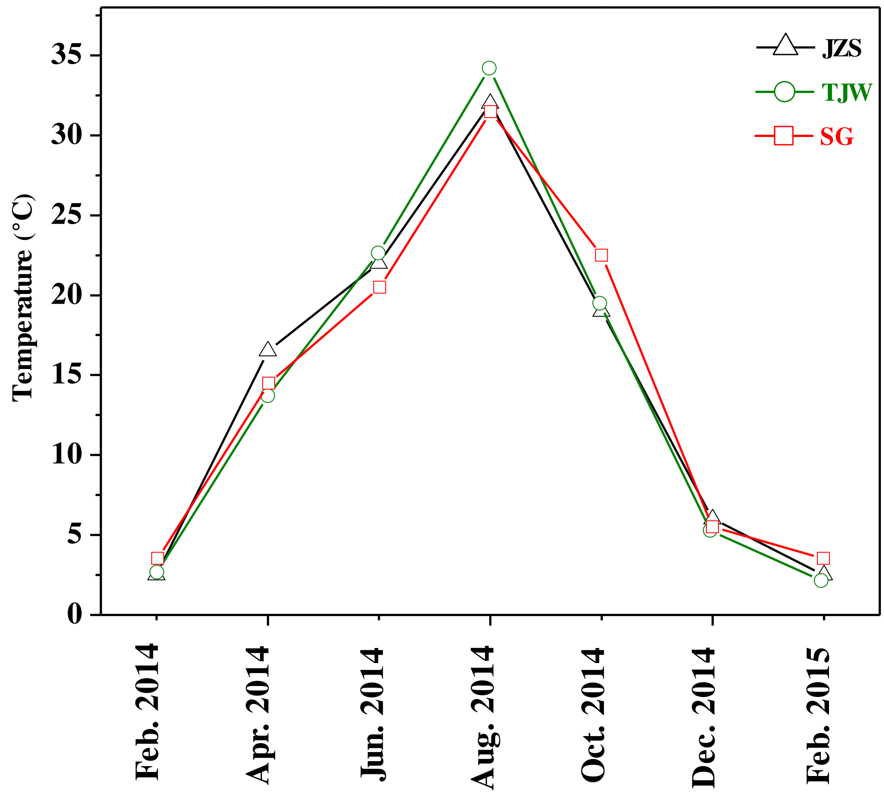
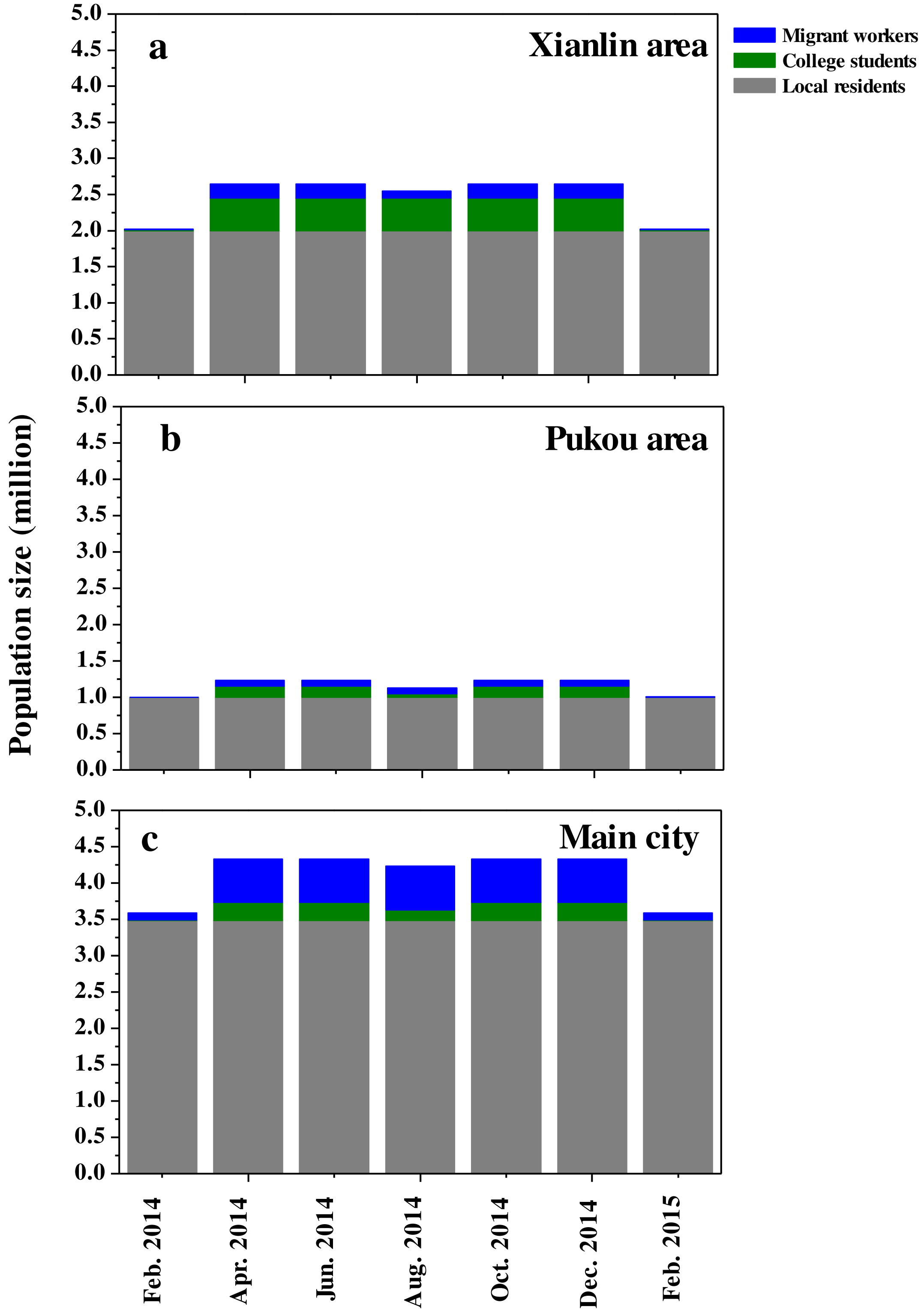
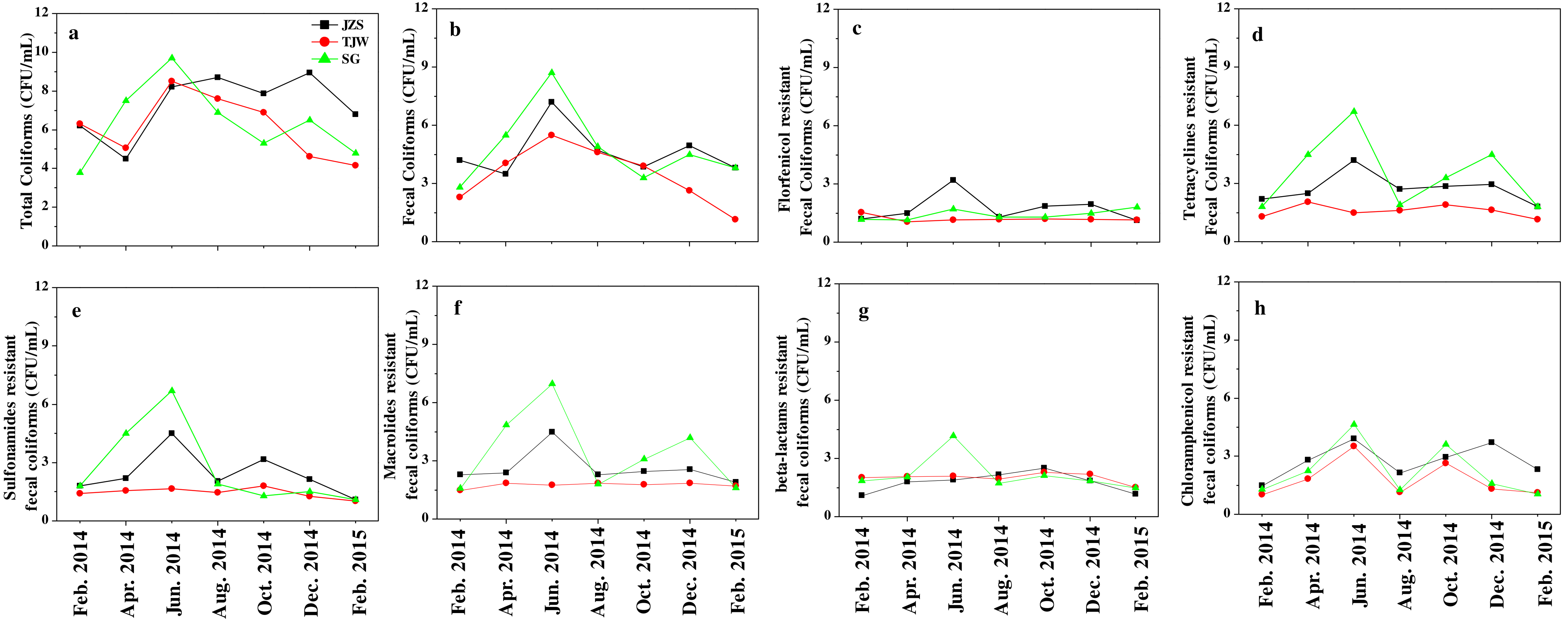

Temperature (°C)
0
5
10
15
20
25
30
35
JZS
TJW
SG
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015

Fig. S2.



a
Xianlin area
b
Pukou area
c
Main city
Population size (million)
Migrant workers
College students
Local residents
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015

Fig. S3.



a
b
c
d
e
f
g
h
JZS
TJW
SG
Total Coliforms (CFU/mL)
Fecal Coliforms (CFU/mL)
Florfenicol resistant Fecal Coliforms (CFU/mL)
Tetracyclines resistant Fecal Coliforms (CFU/mL)
Sulfonamides resistant fecal coliforms (CFU/mL)
Macrolides resistant fecal coliforms (CFU/mL)
beta-lactams resistant fecal coliforms (CFU/mL)
Chloramphenicol resistant fecal coliforms (CFU/mL)
Feb. 2014
Apr. 2014
Jun. 2014
Aug. 2014
Oct. 2014
Dec. 2014
Feb. 2015

Fig. S4.