INVESTIGATION OF THE RELATIONSHIP BETWEEN WASTEWATER IRRIGATION AND TYPHOID INCIDENCE (2008-2019) IN MEKNES, MOROCCO: ENVIRONMENTAL AND HEALTH OUTCOMES

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Abstract. This study aims to explore the relationship between epidemiology and the spatial patterns of typhoid and wastewater irrigation in Meknes (Morocco). The methodological approach includes the analysis of health data of typhoid and the evaluation of the microbial quality of water and vegetables produced in smallholdings irrigated with wastewater selected from different sampling sites in the urban area and peri-urban area of Meknes. The results revealed that from 2008 to 2019, there were 879 confirmed typhoid cases with an average annual incidence of 11.07 and a peak in 2010, particularly affecting the age group 5 to 14 years (43.46%). The number of typhoid cases was higher in males compared to females, with no significant difference (p > 0.05). Bacteriological analysis of water samples indicates significant fecal contamination, not conforming to irrigation standards. The analysis of vegetables reveals a high level of fecal coliforms and the presence of Salmonella, without significant differences between the sampling sites (p > 0.05), representing a risk factor of infection for consumers. The spatial analysis highlights a correlation between typhoid incidence and the distribution of areas irrigated with wastewater. This further highlights the potential role of wastewater irrigation in typhoid transmission, pointing out the need to improve water management.

Keywords: wastewater, agriculture, waterborne diseases, microbial contamination, spatial analysis, geographic information system

Introduction

The degradation of environmental conditions can have a direct and negative impact on public health, leading to the development of severe pathologies (Warren et al., 2002). Water quality is a significant factor among environmental factors affecting public health (Tongesayi and Tongesayi, 2017; Lin et al., 2022; Ali et al., 2023). According to the World Health Organization, annually 12.6 million deaths are due to environmental factors, representing 23% of all deaths worldwide (Prüss-Üstün et al., 2016). A comprehension of the interaction between public health and the environment is essential for preventive measures to reduce health risks (CDC, 2018). Among the diseases linked to environmental factors, we are interested in water and food-borne diseases, which pose a significant public health challenge. These diseases are particularly pronounced in areas where urban wastewater reuse in irrigation is widespread (Cissé, 2019). Wastewater from

domestic, industrial, and stormwater runoff can contain a variety of pollutants, including pathogenic bacteria. Using wastewater in agriculture represents a potential risk of exposure to these pathogens. Such practices pose a risk to farmworkers and their families, handlers, consumers, and neighboring communities (WHO, 2006; Antwi-Agyei et al., 2016). Contamination occurs through contact with wastewater, improper post-harvest handling, and wastewater irrigated product consumption (Drechsel and Keraita, 2019).

Meknes, a city in Morocco with its patchwork of urban and peri-urban agriculture, has long been associated with wastewater irrigation practices. This area represents an important case study to explore the health impacts of wastewater irrigation. Prior studies have highlighted the health risks associated with wastewater irrigation in this area. A study revealed that smallholders in Meknes experienced a range of waterborne illnesses, such as skin infections, gastrointestinal issues, and diarrhea (El Addouli et al., 2012). Moreover, another study by Mouhaddach et al. (2015) identified a correlation between proximity to wastewater-irrigated areas and a high incidence of typhoid, especially in warmer months.

Our research builds upon these foundational studies to paint a more comprehensive picture of the health risks posed by wastewater irrigation in Meknes. Employing advanced Geographic Information Systems (GIS) for spatial analysis and microbiological evaluations, the study aims to map the spatial relationship between wastewater use in agriculture and typhoid. This infection is caused by *Salmonella enterica* serotype *Typhi* (Spanò, 2016). It is widely found where water supply and sanitation are substandard due to its transmission via the fecal-oral route. Characterized by prolonged fever, fatigue, headache, nausea, abdominal pain, and transit problems, typhoid can lead, in critical cases, to dangerous complications and even death. Young people are the most at risk (WHO, 2019).

The methodology consists of collecting and analyzing health data from 2008 to 2019 and environmental data from various water sources and agricultural sites. The study focuses on three main objectives:

- Analyses of the epidemiological situation of typhoid in Meknes
- Evaluation of microbial quality of irrigation water and irrigated crops
- Assessment of typhoid distribution in Meknes and their correlation with wastewater irrigated areas

Through this approach, this study intends to provide valuable insights for health professionals and researchers in decision-making to mitigate health risks associated with wastewater irrigation.

Methods and materials

Study area

Meknes city is located in northwest Morocco, 140 km from the capital Rabat. It is part of the Fez-Meknes region (*Fig. 1*). Meknes is situated at an altitude of 516 m. The climate is semi-continental Mediterranean. Average temperatures range from 11° C to 24° C, with the warmest months being July and August. Annual precipitation averages around 576 mm. Winters are cool and rainy; the coldest month of the year is usually January (Haut Commissariat au Plan, 2017).



Figure 1. Geolocation of the study area

Urban and peri-urban agriculture in Meknes include, on one hand, the plain, where the predominance of large family-owned and commercial farms focus on specialization in commercial crops, where irrigation relies on groundwater accessed through wells and boreholes (Dugué et al., 2015b). On the other hand, the three valleys are distinguished by family-owned smallholdings where irrigation is provided locally through stream waters and wastewater.

Irrigation with wastewater extends mainly to three valleys around the three streams that cross the city (*Fig. 2*): Bouishak, Boufekrane, and Ouislane (RADEEM, 2003; Dugué et al., 2015a). They have always been the primary source of irrigation for smallholdings in urban and peri-urban agricultural areas. This water is highly contaminated by pollution resulting from domestic and industrial discharges without treatment. Additionally, the regular lack of yearly precipitation urges farmers to resort to untreated wastewater collected directly from urban sewers for irrigation to respond to the constant water demand (RADEEM, 2003; Dugué and Valette, 2015). Farmers take wastewater using motor pumps or through the destruction of pipe networks or the obstruction of wastewater pipes and spillways to divert water to stormwater pipelines that discharge mainly into the city's three streams.

Smallholdings in the city's urban area mainly produce market gardening crops, with some small plots of fruit farming. In the peri-urban areas, farms are larger in surface and combine market gardening, livestock, forage crops, and small plots of cereal farming (*Fig. 3*) (RADEEM, 2003; Dugué et al., 2016). Majority sold in the street local markets of neighborhoods bordering the irrigated fields (Mouhaddach et al., 2015). 39% of market gardening crops are supplied from these wastewater-irrigated farms and sold to local markets through the short food supply chain (Dugué et al., 2015b; Mouhaddach et al., 2015).

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Figure 2. Urban and peri-urban agricultural area of the city of Meknes



Figure 3. Wastewater irrigated crops in urban and peri-urban areas of Meknes

The reuse of wastewater in agriculture in the Meknes urban and peri-urban areas is vital for the socioeconomic dynamic. The livelihood of farmers is essentially based on agricultural production it associated activities. The agricultural income represents 85% of the total income of the smallholding (Dugué and Valette, 2015).

Data collection

Health data

Data were collected from the prefectural epidemiology cell of the prefecture of Meknes (Morocco) from 2008 to 2019. The data provided includes reports of confirmed cases of

typhoid, registered in 30 health centers of Meknes. The annual incidence for the period between 2008 and 2019 was calculated as follows:

Incidence =
$$\frac{\text{No. of new cases}}{\text{Size of population at risk}}$$
 (Eq.1)

Incidence measures the rate of new cases occurring over time, which can help pinpoint high-risk areas and provide insights into the trends and patterns of typhoid transmission (CDC, 2017).

Environmental data

Water and vegetable samples were collected using a composite sampling method to provide a single representative mean for microbial contamination at each site. Samples were collected three times over the course of one year, once during each of the following periods: July, October, and March.

For water samples, nine sites were selected. Water samples were taken from urban sewers (one site), ground water (one site) and surface water (three streams) (7 sites). At each site, three water samples were collected. The selection of sampling locations was based on their intended uses. Sites 1, 2, 3, 4, 6, and 8 were chosen due to their agricultural purposes. In contrast, sites 5 and 7 were chosen as comparison sites since they are not used for agricultural purposes, helping to distinguish the effects of agricultural practices. Site 9 was selected because it is commonly used for domestic water needs (*Fig. 2*).

Vegetables were collected from the farms in the same agricultural area of Meknes (Site 1, 2, 3, 4). Vegetable samples chosen were irrigated with untreated wastewater and mixed wastewater. 48-h interval between irrigation and vegetable sampling, in order to capture a more accurate representation of microbial contamination levels. Vegetable samples were collected exclusively from wastewater-irrigated smallholdings, representing the predominant irrigation practice in Meknes area (over 95%), to directly assess the environmental health risks that are most relevant to the local population (RADEEM, 2003). Vegetables from non-wastewater irrigated sites were not included as they do not significantly contribute to the agricultural practices under investigation in relation to typhoid incidence.

Environmental analyses

Water and vegetable samples were collected aseptically and transported to the laboratory for bacteriological analysis within 24 h. Water analyses were carried out based on standard methods described in Rodier (2009) using the membrane filtration method (cellulosic membrane 0.45 µm in diameter) using selective media. All water samples had undergone serial dilutions prior to filtration. Fecal Coliforms were determined using Lactose TTC Agar with Tergitol-7 media. Enterococci were determined using Slanetz and Bartley media. Vegetable samples were put in Buffered Peptone Water for pre-enrichment. Three decimal dilutions homogenate for each sample were prepared. Fecal coliforms were counted using Red Bile Lactose Agar Violet and then incubated respectively at 44°C for 24h. Vegetables analyses was based on the standards methods described in Da Silva et al. (2018).

For the search for salmonella, the pre-enrichment was done in buffered peptone water. Pre-enrichment was done in Rappaport Vassiliadis soya broth and into Muller-Kauffmann tetrathionate novobiocin broth. Selective differential plating was done with xylose lysine desoxycholate (XLD) agar. The purification was done in Nutrient Agar. The identification was made using biochemical tests. The confirmation was done using molecular method.

Water microbiological parameters were compared with World Health Organization (WHO) guidelines for wastewater use in agriculture, United States Environmental Protection Agency (USEPA) for water reuse and the Moroccan Water Quality Standard for Irrigation. The Guidelines for interpretations of water quality for irrigation were summarized in *Table 1*.

	Limits recommended (CFU/100 ml)		
	WHO guidelines (WHO, 2006)	USEPA standards (USEPA, 1992)	Moroccan standards (S.E.E.E, 2007)
Fecal Coliform	 10³ Unrestricted irrigation of crops to be eaten raw 10⁵ Restricted irrigation of cereal crops 	≤200 food crops commercially processed 0 food crops not commercially processed	10 ³
Salmonella	-	_	Absence in 5 L

 Table 1. Guidelines of water quality for irrigation

Spatial analysis

Quantum GIS (Qgis) software was used to create the maps and analyze the distribution of wastewater irrigated areas and typhoid incidence in Meknes. We overlayed wastewater irrigated areas map with dot distribution maps of disease incidence to assess the potential spatial correlations, with the integration of surface water data. To calculate the distances between health centers and sampling sites, we utilized the QGIS distance matrix tool, enabling the quantification of the spatial proximity of health centers to wastewater irrigation fields.

Statistical analysis

The Kruskal-Wallis test was used to compare and discern significant differences in incidence rates across various health centers and microbial quality across different sampling sites. Spearman rank correlation analysis was employed to explore the relationship between water quality and typhoid incidence, as well as to examine the dynamics between typhoid incidence and the geographic proximity to wastewater irrigation areas. The significance level was 95% ($\alpha = 0.05$), and p-values below 0.05 generated are considered statistically significant. The data processing and analysis were done using Microsoft Office Excell 2019 and Stata software Stata/IC 14.2.

Ethics statement

All health data collection was carried out with the permission of the Director General of Health, Delegation of Fes Meknes region granted on January 2021.

Results

Epidemiological profile of typhoid (2008-2019)

During the period from 2008 to 2019, there were 879 confirmed cases of typhoid.

Demographic distribution of typhoid cases

For typhoid, 432 cases were female (49.15%), and 447 were male (50.85%). The most cases of typhoid were recorded among the 5-14-year age group, with 382 cases (43.46%). The 15-29-year age group recorded the second-highest number of typhoid cases, 244 (27.76%). The youngest age groups, 0-4 years, and the oldest, +60 years, recorded the lowest typhoid rate, with 29 cases (3.30%) and 32 cases (3.64%) respectively (*Fig. 4*).



Figure 4. Distribution of typhoid in different population age groups

Temporal variation of typhoid

Between 2008 and 2019, there were 879 confirmed cases of typhoid, with an average incidence of 11.71 per 100,000 inhabitants (*Fig. 5*). Notably, the highest incidence of typhoid, 23.38 cases per 100,000 inhabitants, was recorded in 2010, whereas the lowest incidence of 1.51 cases per 100,000 inhabitants occurred in 2019.



Figure 5. Evolution of the incidence (1/100.000) of typhoid and hepatitis A in Meknes over the period (2008-2019)

Geospatial distribution of Typhoid

Over the 12 years, the health center with the highest mean typhoid incidence was Bab Belkari, reporting 185.86 cases per 100,000 inhabitants. Ras Aghil followed with an incidence rate of 53.15, while Bab Rha reported the third-highest mean incidence rate at 40.21 cases per 100,000 population. Conversely, Saada, Sidi Bouzekri, and Zahoua had the lowest rates at 0.58, 1.7 and 1.709 per 100,000, respectively (*Fig. 6*). The Kruskal-Wallis test confirmed that the variation in annual typhoid incidence across the 30 health centers was statistically significant (p-value = 0.0001).



Figure 6. Geographical distribution of the typhoid incidence (2008-2019) for each health center in the city of Meknes

Relationship between typhoid wastewater irrigation

Typhoid and water quality

The microbial examination of water samples revealed a significant contamination, exceeding the established guidelines for irrigation. Sites 1 and 2 were the most contaminated, with no statistically significant difference (p > 0.05). The mean concentrations of fecal coliforms were 4.7.10⁶ CFU/100 ml and 3.42.10⁶ CFU/100 ml, respectively. Similarly, the average concentrations of the enterococci were 2.08.10⁵ CFU/100 ml for site 1 and 1.7.10⁵ CFU/100 ml, for site 2. Site 5 exhibited the lowest mean bacterial load, with 8.13.10³ CFU/100 ml for fecal coliforms and 5.10² CFU/100 ml for enterococci. Salmonella was detected on sites 1, 2 and 7 (*Fig. 7*).

The analysis of Spearman rank correlation between microbial water quality parameters and typhoid incidence rates revealed a moderate positive correlation for fecal coliforms ($\rho = 0.42$, p < 0.05) observed with the incidence of typhoid. Similarly, a positive correlation was noted between mean enterococcus levels and typhoid incidence ($\rho = 0.6$, p < 0.05).

The spatial analysis revealed an association between high incidences of typhoid in the health centers of Ras Aghil, Sidi Baba, and Um Rabie and the proximity to high levels of bacterial contamination, mainly at agricultural sites 1, 2, 3, and 8 (*Fig. 7*). The presence of Salmonella for site 1 and 2 shows that these areas may pose a high risk of typhoid infection.



Figure 7. Relationship between the average typhoid incidence (2008-2019), wastewater irrigated farms and water microbial quality

Typhoid and vegetables quality

The evaluation of fecal contamination in wastewater-irrigated farms showed that the highest fecal coliform load was recorded in site 1, particularly in lettuce, with a fecal coliform count of $1.08.10^4$ CFU/g (*Fig. 8*). The application of the Kruskal-Wallis test across the data set revealed no statistically significant differences in mean fecal coliform counts between sampling sites for any of the vegetable types examined (p > 0.05). This could be explained by untreated wastewater directly drawn from urban sewers. Site 4 had the lowest coliform load. Salmonella contamination in vegetables was found only in sites 1 and 2 (*Fig. 9*). The consumption of these vegetables could lead to a risk of disease transmission.

Typhoid incidence and proximity to wastewater-irrigated fields

Assessing the link between typhoid incidence and proximity to wastewater-irrigated fields is vital for understanding how land use influences disease patterns (*Fig. 10*). The Spearman rank correlation analysis revealed a significant strong negative correlation between the proximity to wastewater irrigated sites and typhoid incidence rates ($\rho = -0.76$, p < 0.05), suggesting that health centers located closer to these sites had

higher typhoid incidence. Conversely, a moderate positive correlation was observed between the proximity to non-agricultural sites and typhoid incidence ($\rho = 0.62$, p < 0.05), implying that health centers closer to non-agricultural sites tend to report higher rates of typhoid.



Figure 8. Mean fecal count in produced vegetables in wastewater irrigated farms (error bars represent standard deviation) (p > 0.05)



Figure 9. Relationship between the typhoid cumulative incidence (2008-2019), wastewater irrigated farms and vegetable microbial quality

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Figure 10. Spearman rank correlation scatter plot of typhoid incidence with wastewater irrigated agricultural and non-agricultural sites (ρ : Spearman rank correlation coefficients)

Discussion

The analysis of typhoid cases from 2008 to 2019 revealed distinct patterns in age distribution. The most affected age group by both diseases is 5-14. Children are more likely to be affected by this infection, as they are less immune to the infectious agent and lack practice in terms of hygiene measures compared to older people. The lowest number of cases was among + 60 years. This is due to the fact that typhoid shows less severity with age after childhood. Typhoid incidence did not show any differences between both genders. The gender distribution, being almost equal, suggests that both are exposed to environmental risk factors, likely related to water and food sources.

The typhoid distribution across the 30 health centers of Meknes showed that the highest typhoid incidences were in Bab Belkari, Ras Aghil, Bab Rha, Um Rabie, Sidi Baba, and Toulal, respectively.

The analysis of the spatial distribution of typhoid showed high incidence rates in areas near wastewater irrigation areas (Ras Aghil, Um Rabie, Sidi Baba, and Toulal). A clear correlation was observed between the proximity to wastewater irrigation areas and the increase in typhoid incidence. These results are consistent with the findings of Mouhaddach et al. (2015), which revealed the presence of a spatial gradient in the distribution of typhoid in relation to wastewater-irrigated areas, with clusters at high risk of infection mainly in the northwest of the city near wastewater-irrigated areas and clusters at low risk in the southeast.

Wastewater irrigation fields can be the biggest risk factor for waterborne disease transmission, as several studies in Morocco have shown these areas present a high risk of infection (Melloul and Hassani, 1999; Melloul et al., 2001; Amahmid and Bouhoum, 2005). The spatial and microbial analysis from different sites shows that wastewater irrigation is associated with significant levels of contamination in both water sources and vegetable crops. The irrigation water quality assessment showed a high fecal contamination that did not conform to the limits set for irrigation by the WHO and Moroccan standards. It conforms with previous studies conducted on the quality of surface water in the city of Meknes, as they reported high bacteriological contamination of fecal origin of the three watersheds (Aboulkacem et al., 2007; El Addouli et al., 2009;

Abrid et al., 2011; Larif et al., 2013; Ouarrak et al., 2019). The presence of Salmonella and fecal coliforms in high concentrations suggests that untreated wastewater used in agriculture can be a vector for waterborne infections.

The quality of irrigation water and the type of irrigation system affect the microbial quality of produced vegetables (Brackett, 1999). Several studies have shown that irrigation water can be a source of microbial contamination of vegetables (Khalid et al., 2018; Ali et al., 2023). Vegetables produced in wastewater-irrigated farms had high fecal coliform loads, especially lettuce. The presence of Salmonella in vegetables at Sites 1 and 2 raises concerns, as these products are often consumed raw. This contamination can pose an infection risk to consumers as most of the vegetables produced in these farms are sold in local markets nearby. These results are consistent with the one found in Morocco, which reported that wastewater irrigation has contributed to vegetable contamination (Ibenyassine et al., 2007; Talouizte et al., 2008; Hajjami et al., 2012; El Hassani et al., 2023). Contaminated water and street-vended vegetables have been identified as vehicles of typhoid and paratyphoid outbreaks (Kabwama et al., 2017; Giri et al., 2021; Zhang et al., 2023). A study in the Hongta district in China, observed a decrease in typhoid cases by distancing from markets that sold wastewater-irrigated produce and contaminated watercourses (Cheng et al., 2013). Moreover, asymptomatic infections can serve as a reservoir for typhoid transmission (Senthilkumar et al., 2014). Chronic exposure to low doses of pathogens from wastewater irrigation can lead to long-term health problems and the development of antibiotic-resistant strains (Arsène et al., 2022). It can lead to lowlevel persistent inflammation and other subtle health changes that can have cumulative effects over time, which can lead to chronic gastrointestinal problems and other health problems (Suzuki et al., 2020).

There is a high rate of typhoid incidence in Bab Belkari and Bab Rha, which are located in the ancient medina and not particularly close to wastewater irrigation farms. It could be due to underlying local risk factors, such as the existence of individual non-controlled urban wells. The analysis of water samples from these wells (Site 9) revealed that they do not conform to the recommended standards for drinking and irrigation use (*Fig.* 7). This contamination could be due to leaks in the sewerage network, contamination by runoff of untreated wastewater used in urban agriculture, punctual sources of fecal contamination such as septic tanks and landfill as it was reported by Benhida et al. (2012). During periods of reduced water supply in Meknes, households in these areas may have resorted to using these wells for domestic purposes. Using water from an unimproved water source, such as unprotected dug wells or springs, for drinking or domestic purposes have been identified as a risk factor for typhoid (Mogasale et al., 2018; Kim et al., 2023).

Socioeconomic conditions are another critical risk factor in typhoid transmission in Meknes. As shown in a study conducted among smallholders in Meknes, these areas are characterized by the predominant use of conventional production methods and inadequate infrastructure, as well as a lack of training and poor work hygiene (El Ghazi et al., 2021). Furthermore, poor housing conditions and overcrowding can aggravate the risk due to neglected hygiene practices and increased person-to-person contact, enabling the spread of infectious diseases (Siddiqui et al., 2008; Mogasale et al., 2014; Hechaichi et al., 2023; Ren et al., 2023). Moreover, limited access to healthcare and a lack of disease prevention awareness in poor communities can lead to a higher incidence of typhoid (Kaljee et al., 2018).

During the period 2008 to 2019, the average yearly incidence of typhoid was estimated at 11.71 per 100,000 population. The examination of the annual evolution of typhoid

incidence showed a peak incidence of typhoid in 2010, followed by a decrease by 2019. It could reflect changes in public health policies, with the ongoing efforts, on one hand, of the National Office of Electricity and Drinking Water (ONEE) to increase the rate of drinking water distribution supply (Sadeq et al., 2020). On the other hand, the health delegation and the Municipal Office of Hygiene (BMH) of the city of Meknes with the consistent public well chlorination in urban areas, especially in the ancient medina (Mouhaddach et al., 2015). In addition, there is an increase in public health consciousness due to awareness campaigns, which have had a substantial impact on the behavior of the population by adopting precarious measures such as hand and fresh produce washing as well as safe cooking methods...

The limitations of the study are mainly related to the underestimation of the actual number of cases. Due to the presence of asymptomatic carriers, people who do not seek health centers when they are sick, and the fact that private medical facilities do not report cases of typhoid, a mandatory reporting disease, all contribute to under-reporting. The impact and spread of typhoid could be much more extensive. Monitoring typhoid incidence patterns and taking effective measures for prevention and control are necessary to maintain the downward trend.

Conclusion

The objective of this study was to assess the relationship between typhoid incidence and urban and peri-urban agricultural areas irrigated by wastewater by exploring environmental factors contributing to typhoid transmission. The epidemiology and spatial trends of typhoid infection in the Meknes region of Morocco in a 12-year period have shown that the disease affects younger age groups the most. The typhoid temporal distribution showed a decrease over time, underlining the importance of prevention efforts. Spatial analysis revealed a significant correlation between the highest incidence rates of typhoid and the distribution of wastewater-irrigated areas. This analysis provided information on potential hot spots and their association with typhoid incidence. Environmental analyses in the context of agricultural settings have shown high fecal pollution in irrigation water and vegetables. Salmonella was also detected in irrigated water and the produced vegetables, which could be a risk factor for the transmission of typhoid disease. The superposition of the different layers of information allowed us to obtain new maps that collect information on urban and peri-urban agriculture irrigated with wastewater, the geographical distribution of typhoid cases, and the bacteriological quality of water resources and vegetables irrigated with wastewater. The maps could help local public health authorities assess the spatial risk for typhoid incidences in Meknes and prepare for targeted interventions. This approach can have broader implications for similar urban and peri-urban environments where wastewater reuse is practiced. Overall, the study highlights the importance of considering spatial dimensions in public health analysis, particularly in areas where environmental factors such as wastewater irrigation are prevalent.

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