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Analysis of Environmental Patterns and Leprosy in Minas Gerais, Brazil Using Spatial Statistics

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Georgia State University
2016

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

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By Shaiana Oliveira

Background: Brazil has the second highest number of new leprosy cases reported annually with the state of Minas Gerais (MG) having pockets of highly endemic leprosy. Transmission remains only partially understood, and in addition to a respiratory route, transmission may also be related to environmental conditions. Potentially viable *Mycobacterium leprae* has been found in water, soil, and armadillos.

Objective: To investigate the role of the environment on transmission of leprosy, specifically, (1) elevation, (2) normalized difference vegetation index (NDVI), (3) temperature, and (4) precipitation.

Methods: We conducted a cross-sectional study using Brazilian Notifiable Disease Surveillance System (SINAN) data in 853 municipalities in the state of Minas Gerais, Brazil from 2009 to 2013. Multivariable Poisson regression models were used to estimate the rate ratio (or incidence density ratio (IDR)) to compare incidence across municipalities. We then used spatial statistics (global autocorrelation, local indicator of spatial autocorrelation [LISA], Getis Ord Gi(d)* to analyze clustering of leprosy cases and incidence.

Results: Overall incidence decreased from 8.76 per 100,000 in 2009 to 5.04/100,000 in 2013 with the average municipality leprosy incidence at 7.11 per 100,000 annually. The local autocorrelation analysis identified 51 high-high clusters of leprosy incidence in the northeast and west of Minas Gerais. After controlling for clustering among all municipalities in Minas Gerais, temperature (IDR=1.76, 95% CI: 1.64, 1.89, $p < 0.0001$) and precipitation (IDR=1.06, 95% CI: 1.01, 1.12, $p = 0.0201$) were positively correlated with leprosy incidence, whereas elevation (IDR=0.53, 95% CI: 0.51, 0.55, $p < 0.0001$) was negatively correlated with leprosy incidence. NDVI (IDR= 0.98, 95% CI: 0.95, 1.00, $p = 0.18$) was negatively correlated with leprosy incidence, yet not significant.

Conclusions: The associations between leprosy and environmental predictors, especially higher temperatures, indicate that the role of the environment and geographical conditions need to be considered in the context of disease transmission and viability of *M. leprae* in the environment, especially in the era of global warming and climate change.

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A. Introduction

Background

Leprosy is an ancient disease that has been around for many centuries and has been referenced in ancient Egyptian, Roman, and Greek writings (O'Brien & Malik, 2017). Throughout history, leprosy was feared and misunderstood to the point that some believed it was a sinful and evil (Nations, Lira, & Catrib, 2009; O'Brien & Malik, 2017). Individuals who were diagnosed with the disease suffered from constant social stigma and were often excluded from society. Even though some of those outdated beliefs are no longer held today, patients with leprosy still suffer from social stigma.

A Norwegian scientist, Gerhard-Henrik Armauer Hansen, discovered that leprosy was caused by *Mycobacterium leprae* in 1873. This scientific discovery proved that the disease was not a sin or curse, but instead caused by bacteria. Today, leprosy is also known as Hansen’s disease (HD) to honor Hansen’s discovery, and to reduce social stigma (Gelber, 1993). The terms “leprosy” and “leper” had negative connotations and were associated with individuals that were excluded and shunned due to their physical disabilities caused by disease (Gelber, 1993; The Leprosy Mission, 2019). Lack of knowledge about disease treatment, diagnoses, and transmission perpetuated social stigma today.

Hansen’s disease is a chronic disease that affects the skin and peripheral nerves which can cause permanent disability, deformities and social stigma (Barbosa et al., 2018). Leprosy affect the nerves and mucous membrane leading to pale or red skin patches, dry skin, loss of sensation, numbness, muscle weakness, and facial lesions. If the disease is not treated immediately, the symptoms can escalate to debilitating symptoms, such as paralysis, deformed limbs and blindness (Centers for Disease Control and Prevention, 2017, January 6). These disabilities and deformities may decrease the individual's capacity to work, limit social life, and develop psychological problems (Ministério da Saúde do Brasil, 2016).

Armauer Hansen's scientific discovery was vital to understand the cause of the disease. Another fundamental development in leprosy control was the introduction of Multidrug Treatment (MDT) in 1980s, which rapidly became the standard form of treatment (The World Health Organization, 2019). Since the introduction of MDT, there have been significant improvements in health outcomes among leprosy patients and elimination of leprosy as a public health problem (< 1 / 10,000 population) was achieved in 2000 globally (The World Health Organization, 2016; White & Franco-Paredes, 2015). However, the use of MDT had a minimal impact on the effect of disease transmission, thus requiring further investigations into routes of transmission (Turankar et al., 2016). Efforts to eliminate leprosy are underway and the World Health Organization (WHO) developed a global strategy to reduce the burden of leprosy globally and locally. The 2016-2020 Global Leprosy Strategy aims at achieving the goal by strengthening government ownership, to stop leprosy and its problems, especially discrimination (The World Health Organization, 2016).

Even though prevalence of leprosy has decreased since early 2000, it still remains a public health issue in poor and endemic in countries in the world (World Health Organization, 2018). The World Health Organization (WHO) reported an estimate of 210,973 new cases of leprosy in countries around the world in 2017, where the majority of cases are concentrated in the Americas and Southeast Asia. Brazil, India and Indonesia are responsible for 81% of the new cases (Barbosa et al., 2018), with approximately a total of 168,949 new cases in 2017 (World Health Organization, 2018).

Brazil has the second largest number of new cases of leprosy in the world, approximately 26,875 new cases and detection rate of 12.84/100,000 in 2017 (World Health Organization, 2018). The number of new cases decreased between 2005 and 2010 from 38,410 new cases to 34,894 (World Health Organization, 2018). In response to the WHO's call for leprosy control strategies, the Brazilian Ministry of Health (MoH) established guidelines for surveillance, assistance and the elimination of leprosy as a public health problem in Brazil (Ministério da Saúde do Brasil, 2016). Increasing our understanding on pathogen viability, transmission and the interaction between agent, host and environment is important in order to develop feasible control strategies. In Minas Gerais, Brazil leprosy is still a problem due the continuous transmission and proximity to hyper-endemic states (Murto et al., 2013; Sampaio, Rossi, Cerutti Junior, & Zandonade, 2012).

Leprosy is a neglected tropical disease (NTD) commonly found in tropical and subtropical regions. The pathogen, *Mycobacterium leprae,* is a bacillus that cannot develop and multiply outside the animal or human host, but it can remain viable for up to 5 months in environment (Worobec, 2012). The bacteria is known for slow growth (about 12-13 days) and long incubation period, ranging from 2-12 years (Rodrigues & Lockwood, 2011). Due to its long incubation period, it is challenging to understand its transmission and source of exposure to pathogen.

Scientists suggests that the pathogen is transmitted by (1) discharge of bacilli by airborne droplets from the nasal and mouth of individual harboring the bacteria

(Turankar, Lavania, Singh, Siva Sai, & Jadhav, 2012); (2) skin-to-skin contact (Worobec, 2012); and (3) possible contact with infected soil, water sources, and vectors (Arraes et al., 2017; Turankar et al., 2016; Worobec, 2012). The rate of infection by *Mycobacterium leprae* is much higher than the rate of development of symptoms, which suggests that there are other sources of infection besides direct contact to people diseased patient (Tadesse Argaw et al., 2006). Additionally, there are many studies highlighting the relationship of low socioeconomic status (SES) and leprosy (Cabral-Miranda, Chiaravalloti Neto, & Barrozo, 2014; Rodrigues & Lockwood, 2011). However, the exact transmission route of *Mycobacterium leprae* remains largely unknown.

Environmental factors have been hypothesized to play a role in the spread of leprosy. Since the main mode of transmission is still uncertain, it is hard to fully comprehend how the disease is spread. However, live bacilli have been found in the water sources, soil, and animals (Arraes et al., 2017; Turankar et al., 2016). A study conducted in Ceará, Brazil found viable *M. leprae*, as measured by RNA, in several natural water sources (Arraes et al., 2017). Similarly, *M. leprae* DNA was found in water sources used for bathing and washing in Indonesia (Matsuoka, Izumi, Budiawan, Nakata, & Saeki, 1999).

Furthermore, other studies highlighted that *M. leprae* was found in soil samples around homes of leprosy patients. Turankar *et al.* in 2016 detected dead and live *M. leprae* DNA and mRNA (which suggest live bacteria) of leprosy patients around patient's home. There is possibility that the bacteria were found in soil near home of infected patients due to patient's bodily excretions (Turankar et al., 2016). Thus, this suggests that the environment may also be involved in the transmission of disease, possible due to aerosolization of the bacteria through soil of water after being contaminated by an infected individual.

Zoonotic transmission of *M.leprae* has also been described. In 2011, Truman *et al.* detected that armadillos and many leprosy patients in the gulf states of the United States share the same strain of *M. leprae*, leading to the discovery that armadillos are an important non-human reservoir for *M. leprae* (Truman et al., 2011). Furthermore, Domozych *et al.* (2016) continued to investigate the link between nine-banded armadillos and leprosy in Central Florida. Likewise, a study in Espírito Santo, Brazil confirmed the association of direct contact with armadillos and the incidence of leprosy, leading to the conclusion that direct contact with armadillos is a potential risk factor for contracting leprosy (Deps et al., 2008). In 2008, Lahiri et al. supported the hypothesis that free-living amoebae might facilitate the survival of the *M. leprae* in the environment (Lahiri & Krahenbuhl, 2008; Wheat et al., 2014). These findings support that there are environmental reservoirs of *M. leprae*.

Spatial analysis, conducted through the use of geospatial information systems (GIS), have been previously used to assess of risk factors for leprosy worldwide (Bakker, Scheelbeek, & Van Beers, 2009; Fischer, Pahan, Chowdhury, & Richardus, 2008; Queiroz et al., 2010). In the 2016-2020 Global Leprosy Strategy, the WHO recommends using GIS to strengthen surveillance and health information systems to assess secular time-trend and spatial analysis (The World Health Organization, 2016). Spatial analyses allow researchers to visualize patterns and trends within a certain geographic location to infer associations between disease and potential environmental predictors. In 2006, Tadesse *et al.* assessed the influence of environmental factors, such as NDVI, maximum temperature, and climate, on prevalence of leprosy in Ethiopia (Tadesse Argaw et al., 2006). The findings suggest that thermal-hydrologic regime of the environment is a vital factor in the transmission of leprosy due to the conditions that facilitates the viability of leprosy bacillus in the environment. However, there is a lack of literature aimed at understanding the role of geographical conditions that may facilitate or hinder the transmission of leprosy. Thus, it is important to analyze the temporal associations between weather patterns over time and disease incidence to investigate the effects of climate on these infections and inform how we understand the transmission of these infections.

Purpose of Study

The purpose of this study was to conduct spatial analysis to examine the associations between environmental factors and incidence of leprosy in cases/100,000 person-years. Specifically, we examined the role of elevation, vegetation, temperature, and precipitation on leprosy. The main aims of the study were to: 1) examine the clustering of total leprosy incidence in Minas Gerais, Brazil on a spatial scale to identify clusters with high incidence and hot-spots and 2) to identify environmental factors of leprosy incidence associated with increased incidence of leprosy.

B. Methods

Study Area, Population, and Design

The State of Minas Gerais is located in the southeastern part of the country, with a territorial extent of 586,520,732 km², and with a demographic density of 33,41 habitants/km² (Instituto Brasileiro de Geografia e Estatística, 2018). Minas Gerais has 853 municipalities with a state population of approximately 21 million (Instituto

Brasileiro de Geografia e Estatística, 2018). New cases of leprosy are recorded by the Brazilian Notifiable Disease Surveillance System (*Sistema de Informação de Agravos de Notificação* – SINAN). The study population consisted of new incident cases of leprosy reported from 2009 to 2013 in inhabitants in Minas Gerais. We performed a cross-sectional study using SINAN data, analyzing the association of new leprosy cases and 4 environmental factors. To calculate municipality incidence, population data was obtained from the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística* – IBGE) for 2009 - 2013.

Descriptive Analysis

We performed descriptive analyses on leprosy count and incidence case data annually. We calculated HD incidence rates using estimated municipality population from 2009 through 2013. Population data from 2011 was not available for analysis. We averaged the estimated population from 2010 and 2012. We assumed municipality population size for 2011 was close to previous year population. Incidence was reported as a number of cases per 100,000 person-years. Maps representing incidence and number of cases over the years were created to visualize areas with high incidence and number of cases. Maps were created using SIRGAS 2000 UTM Zone 24S projection in ArcGIS 10.4 (ESRI, 2011).

Spatial Data Analysis

Spatial autocorrelation measures the effect of distance on the distribution of leprosy cases. According to Tobler's first law of Geography, "everything is related to everything else, but near things are more related than distant things" (Tobler, 1970). We used spatial autocorrelation analysis to investigate geographic patterns of leprosy distribution in the state of Minas Gerais. The distribution of leprosy is described as either dispersed, clustered, or random pattern within a specific area or space. Leprosy incidence rate and number of cases over 5 years were taken as the attribute value. In order to minimize the problem of small numbers that leads to statistical instability, we also computed a spatially empirical Bayes (SEB) incidence rate to smooth the differences between neighboring areas, thereby increasing the stability of the data (Waller & Gotway, 2004). We conducted local and global spatial autocorrelations and Getis Ord G* (L. Anselin, 1995; D. A. Griffith, 2009; Getis & Ord, 1992). All spatial statistical analyses were performed using ArcGIS 10.1 by ESRI (ESRI, 2011), ClusterSeer 2.0 (BioMedware, 2003), and GeoDa 1.12.1.161 (L. Anselin, Ibnu Syabri and Youngihn Kho 2006).

Global Autocorrelation

Spatial autocorrelation analysis measures the degree to which a location's incidence is related to nearby locations. The null hypothesis states that there is complete randomness, therefore the leprosy rates are independent and randomly distributed among the municipalities in Minas Gerais. Spatial autocorrelation analysis was applied to analyze the patterns of leprosy distribution in all municipalities in Minas Gerais by calculating Moran's I global indices using ClusterSeer 2.0 (BioMedware, 2003; D. A. Griffith, 2009). Global Moran's Index assessed the spatial autocorrelation of municipalities locations and leprosy incidence rate over a 5-year period. Global Moran's I ranges from -1 to +1, which $I > 0$ indicates a clustered pattern, $I = 0$ indicates a random pattern, and $I < 0$ indicates a dispersed pattern. The distribution pattern is determined by Z-value and the level of significance (p-value). Queen contiguity was used to build the spatial weights, and significance was determined using at a 95% significance cutoff ($p<0.05$) with 999 repeated Monte Carlo (MC) simulations. In order to avoid the issue of "neighbor less areas", we used a precision threshold of 0.0005.

Local Spatial Autocorrelation

Local indicators of spatial autocorrelation (LISA) detects local spatial autocorrelation in group-level data (L. Anselin, 1995). Similar to the Global Moran test, the Local Moran's Index is calculated for every municipality. The Local Moran's I identify local clusters and local spatial outliers. Local Moran's I was conducted to test for local autocorrelation of incidence rates of leprosy using a queen contiguity to create the spatial weights in GeoDa 1.12.1.161 (L. Anselin, 1995; L. Anselin, Ibnu Syabri and Youngihn Kho 2006). Moran's I values close to +1 indicate that a municipality and its neighbors have similar high or low incidence rate of leprosy, which is designated as a cluster. Moran's values close to -1 indicate that municipality has neighbors with dissimilar incidence rates, which is considered an outlier. Significance was assessed with 999 permutations and a p-value of 0.05 to identify clusters or outliers.

Hot-Spot Analysis

Getis-Ord Gi* statistic identifies statistically significant hot-spots and cold-spots for each municipality (Getis & Ord, 1992). This function considers each municipality within the context of neighbors. Getis-Ord Gi* was performed with a queen contiguity weighting (and a precision threshold of 0.0005) to assess the study area for hot- and cold-spots of leprosy incidence using GeoDa 1.12.1.161 (L. Anselin, Ibnu Syabri and Youngihn Kho 2006; Getis & Ord, 1992). The Getis-Ord Gi* statistic significantly separates hot-spots clusters from cold-spots clusters. To be a statistically significant hot-spot, a municipality will have a high value and be surrounded by other municipalities with high values. Statistical significance was based on 999 permutations Monte Carlo randomizations to identify hot-spots and cold-spots.

Environmental Predictors

Environmental predictors were identified from a review of relevant literature and were acquired from different sources (Table 1). The environmental factors selected for analysis were (1) elevation, (2) total precipitation, (3) maximum temperature, and (4) normalized difference vegetation index (NDVI) of state of Minas Gerais. These factors were analyzed for the association of new cases of leprosy in Minas Gerais, Brazil. We used zonal statistics to calculate the mean value by municipality for each predictor variable. All analyses were performed using the statistical software SAS 9.4 software.

Elevation

The ASTER Global Digital Elevation Model (GDEM) was obtained from the online EarthData, courtesy of the NASA. ASTER GDEM is a product of METI and NASA. Ninety individual granules with 30-meter postings and 1 x 1 degree from the ASTER product were obtained. The digital elevation bands for each tile were extracted, mosaiced, and clipped to the extent of the state of Minas Gerais. The merged dem files were transformed into raster a file. The mean elevation for each municipality was extracted through zonal statistics.

Precipitation and Temperature

Total precipitation and maximum temperature data were obtained from the National Institute of Meteorology (*Instituto Nacional de Meterologia* – INMET). We identified 36 local weather stations in the state of Minas Gerais and mapped them with their corresponding latitude and longitude coordinates. The weather stations included the monthly precipitation in millimeter (mm) and maximum temperature in degrees Celsius from January 1, 2009 through December 31, 2013. We averaged the monthly precipitation in order to get the annual precipitation for the study period. The point locations of weather stations were added to ArcGIS and to be statistically analyzed.

We performed a kriging to interpolate unknown precipitation and temperature in other areas. The Kriging method uses a limited set of data points, in this case the weather stations, to interpolate the value of a variable over a specific spatial unit, precipitation and temperature in the state of Minas Gerais (Columbia University Mailman School of Public Health). We used the ordinary Kriging type that assumes that the data is stationary. The stationary assumptions states that the properties of the data are independent of the absolute location and direction in space (Henley, 2001). We excluded the missing values for weather stations that did not report precipitation for a year. We used the ordinary kriging type, hole effect to model the empirical semivariogram models. The interpolated annual precipitation and temperature were mapped for each year using ArcGIS 10.4. The average precipitation and temperature were extracted through zonal statistics per municipality. Temperature and precipitation were averaged over the 5-year period for the Poisson analysis.

Normalized Difference Vegetation Index (NDVI)

NDVI is an indicator of density of green land within a specific region based on infrared wavelengths. The NDVI data was extracted from ("Climate Engine," 2019) using the Landsat 8 Surface Reflectance (SR). Landsat Remote Sensing 8 SR is a product of NASA/USGS. Fifty-eight 30-meters granules were downloaded to cover the extent of the state of Minas Gerais from January 1, 2009 through December 31, 2013. The vegetation index granules were merged together through mosaic method to form one raster file clipped to the extent of the state of Minas Gerais. The mean NDVI was extracted through zonal statistics for each municipality.

Poisson Regression Model

We assessed the relationship of leprosy incidence rates with selected environmental predictors by using a model selection approach. Leprosy incidence over 5 years is the outcome of interest for Poisson regression analysis. The best model was selected based on the lowest Akaike information criterion (AIC) value. Multicollinearity among the predictors was evaluated from scatterplots and the correlation coefficient r values.

We performed a generalized estimating equations (GEE) Poisson regression models using SAS 9.4 software, where the logarithm of the number of new leprosy cases reported in each municipality was modelled as a function of leprosy incidence with the logarithm of each municipality population as an offset. We controlled for any differences among municipalities by using the random intercept to account for municipality level clustering. The incidence density ratio per 100,000 person-years was calculated to understand the correlation of leprosy incidence with independent variables in the model.

C. Results

Study Population

Minas Gerais consists of 853 municipalities with an average population estimate of 20 million (Figure 1). Population size varied based on municipalities, ranging from 807 to 2,452,617. From 2009-2013, a total of 7,794 leprosy cases were reported in Minas

Gerais. The number cases during the study period ranged from 0 to 141. Number of cases declined with each passing year. In 2009 there were 1,876 reported cases over the course of the year. In 2010 there were 1,629 cases, in 2011 there were 1,556 leprosy cases, followed by 1,481 cases in 2012, and 1,252 cases in 2013. The mean number of new cases per year over 5 years was 1.82 leprosy cases, with a standard deviation (SD) of 7.54 per municipality (Table 2).

From 2009-2013, the mean leprosy incidence rate was 7.11 per 100,000 inhabitants, with a SD of 18.39. The average annual incidence rate also decreased from 2009 to 2013, from 8.76 per 100,000 people to 5.04/100,000, respectively. The incidence rates across municipalities ranged from 0 to a maximum incidence of 384.04 cases/100,000 person-year. The distribution of the data was strongly right-skewed, according to the histogram generated in SAS 9.4. Figure 2 portrays the incidence rate over 5 years, with the mean incidence and standard deviation. The leprosy incidence rate and the smoothed rate for each year is described in Table 2.

Spatial Data Statistics

Global Autocorrelation Analysis

The results of the global spatial autocorrelation analysis for each year are presented in Table 3. The Global Moran's Index revealed a positive and statistically significant autocorrelation of leprosy incidence for each year. From 2009 to 2013, the Moran's I value under Monte Carlo simulation was 0.447 with a p-value of 0.002, and thus suggested spatial dependence of leprosy in Minas Gerais.

Local Autocorrelation Analysis

Results of the Local Moran's Index are displayed in a LISA cluster map for each year (Figure 3) and Table 4. Interactions are categorized as high leprosy incidence next to high leprosy incidence (high-high), and low incidence next to low incidence (low-low). Outliers are identified as high-low and low-high regions. All clusters noted in the cluster map are significant at $p = 0.05$. The number of municipalities that were categorized as high-high cluster cores were the highest (n=43) in 2011, followed by 2013 with 39 high-high municipalities. Likewise, the year with the highest number of low-low cluster cores were also in 2011. Across the years, most of the high-high municipalities considered cluster cores were commonly located in the northeastern part of the state. However, in 2011 some newly high-high significant municipalities were found on the western part of Minas Gerais. Most of the low-low municipalities were found in the southern part of the state.

Fifty-one municipalities were identified with a high-high clustering were primarily located in the Northwest and East of the state from 2009-2013 total incidence (Figure 4). Low-low clustering was identified in 122 municipalities scattered around Minas Gerais, primarily in the Southeast. We highlighted 11 low-high and 5 high-low outliers, where municipalities with low and high incidence occurred next to municipalities with high and low incidence respectively. Municipalities without significant clustering (n=664) were also portrayed in the map (Figure 4).

Hot-Spot Analysis

We performed a hot-spot analysis with the Getis-Ord Gi* statistic for each year (Figure 5). The number of hot-spot municipalities for leprosy incidence were the highest (n=65) in 2012 (Table 5). Similar to the local autocorrelation analysis, most of the hot-spots clusters were located in the Northwest and East of the state over the 5-year period, while the cold-spot were found in the southern of Minas Gerais. Sixty-two municipalities were considered a hot-spots and 135 were cold-spots with a 95% ($p < 0.05$) confidence over a 5-year period (Figure 6). These hot-spots suggested municipalities where higher incidence than expected was occurring.

Environmental Predictors

Elevation

Higher elevation is commonly located in the middle and southern parts of the state, while lower elevation is normally found on the eastern part of the state (Figure 7a). The mean elevation for the state of Minas Gerais was 759.83 meters, ranging from as low as 198 meters to a maximum of 1,566 meters (Table 6). The lowest elevation was at 12 meters for the Aimorés municipality, which borders the state of Espírito Santo. The municipality with the highest average elevation was Alto Caparaó, with an approximate elevation of 2,827 meters above sea level. The mean elevation was normally distributed.

NDVI

Vegetation index ranges from -1 to +1, which means that +1 represents most dense vegetation while -1 indicates the least dense vegetation area. More vegetation was located along the eastern part of the state, where the Atlantic forest is found (Figure 7b). The mean vegetation index for the Minas Gerais was 0.59 units, with a SD of 0.07 ranging from 0.28 to 0.75 (Table 6).

Total Precipitation

The total annual precipitation distribution was left-skewed. The mean total precipitation was 114.21 millimeters, with a standard deviation of 25.07 (Table 6). Minimum precipitation ranged from 40.55 mm to 165.05 precipitation. Across all years, most of the rainfall was in the southern part of the state, with a decrease in precipitation during in 2012 (Figure 8). The highest level of precipitation occurred in 2009, followed by 2013, 2011, 2010, and 2012, respectively. There was a total decrease of precipitation from 2009 to 2012 of 26% of total precipitation.

Maximum Temperature

The average maximum temperature was 27.22 degrees Celsius, with a SD = 3.04 (Table 6). The distribution of temperature was left-skewed according to histogram created in SAS 9.4 software. The lowest maximum temperature was 13.53 degrees Celsius, with the highest maximum temperature at 31.95 degrees. From 2009 to 2013, the lowest maximum temperature occurred in the southern part of the state (Figure 9). The highest temperatures occurred in 2009 and the lowest temperature being reported in 2013, with a decrease of 19%, respectively.

Poisson Regression Model

After checking for multicollinearity and the lowest AIC value, the model with the lowest AIC only included NDVI and elevation. Since we are interested in the climatic variations of the environment, temperature and precipitation variables were kept in the model as the difference in the models based on the AIC was ≤ 2. Therefore, none of the variables were excluded from the model. The selected model was created at the aggregate level for leprosy incidence as the outcome with 4 main predictors: (1) mean maximum temperature in degrees Celsius, (2) mean total precipitation in millimeters (mm), (3) elevation (meters), and (4) vegetation index (near-infrared light).

After controlling for clustering among all municipalities in Minas Gerais, temperature (IDR=1.76, 95% CI: 1.64, 1.89, p= <0.0001) and precipitation (IDR=1.06, 95% CI: 1.01, 1.12, p= 0.0201) were positively correlated with leprosy incidence. Elevation (IDR=0.53, 95% CI: 0.51, 0.55, $p < 0.0001$) was negatively correlated with leprosy incidence. NDVI (IDR= 0.98, 95% CI: 0.95, 1.00, $p = 0.18$) was negatively correlated with leprosy incidence, yet not significant. (Table 7).

D. Discussion

Our findings illustrate the clustering of endemic regions in Minas Gerais located in the northeast and west regions from 2009 to 2013. Our study suggests that there is a relationship between leprosy and the environment, specifically, that higher temperatures and rainfall were found to be correlated with leprosy cases, while elevation and NDVI were found to be associated with lower disease rates. With the knowledge gaps in transmission routes, our study focused on the role of geographical factors that may affect disease transmission. Our results support the role of climate and geographic conditions in enabling a transmissible environmental reservoir of bacteria.

We found that higher temperatures were correlated with the leprosy cases. Evidence supports that increase or change in climatic temperatures and patterns has the potential of impacting many infectious diseases (Short, Caminade, & Thomas, 2017), especially vector-borne diseases such as zika, chikungunya, dengue, and yellow fever that are currently endemic in some regions in Brazil (Nava, Shimabukuro, Chmura, & Luz, 2017). Similarly, increased temperatures may suggest optimum survival of bacteria.

Previous studies found that *M. leprae* multiplies best at temperatures ranging from 27 – 30 degrees Celsius (Shepard, 1965). Climatic change can influence ecology of vectors directly, as well as urbanization and deforestation of areas that may indirectly impact the burden of disease. Even though leprosy transmission is poorly understood, it's imperative to consider the impact of increased temperature related to climate change on the incidence of leprosy.

Likewise, higher precipitation was correlated with leprosy incidence. Previous studies supported that *M. leprae* was found in water sources, such as dams, rivers, lakes, wells, and streams (Arraes et al., 2017) in the northeast of Brazil. In India, *M. leprae* bacilli was found in wells and sewers in areas considered endemic (Mohanty et al., 2016). Furthermore, the bacteria have been found in damp and wet soils, which probably impacts the longevity of bacteria in the environment (Turankar et al., 2012; Turankar et al., 2018). During the rainy season, humidity increases and makes the soil damp and wet, where the bacteria can survive.

Higher leprosy incidence was correlated with lower elevation and vegetation index. Elevation determines the water drainage system. Due to drainage systems, concentrations of water from rain or runoffs are commonly found in lower elevations. Elevated lands tend to have plants and vegetation that are affected by colder temperatures and demands less water, thus impacting plant growth. Plants at higher elevation have smaller leaves that affects the density of green plants, leading to a lower vegetation index (Borges et al., 2016). Also, NDVI was negatively correlated with leprosy, indicating that the higher the vegetation index, the lower the leprosy incidence. NDVI can be considered a marker of people living farther apart, because uninhabited areas tend to have higher

NDVI. Also, vegetation index was lower in urban areas, such as in the city Belo Horizonte. These findings highlight the importance to investigate the role of land use and deforestation on leprosy incidence. Since deforested lands have lower vegetation, it seems reasonable to explore it further.

Myriad social and economic variables have been identified as potential risk factors in contracting leprosy, such as poverty, education, inadequate sanitation, and illiteracy rates (Cabral-Miranda et al., 2014; Freitas, Duarte, & Garcia, 2014). Similarly, food shortage as an indicator of poverty was also associated with leprosy (Feenstra, Nahar, Pahan, Oskam, & Richardus, 2011). These factors likely all contribute to different aspects of transmission or host susceptibility, but our findings and prior studies suggest that the environment could be a real contributor to the spread. This is further supported by the published literature where evidence of *M. leprae* DNA and RNA has been found in soil, water, and free-living amoeba (Arraes et al., 2017; Turankar et al., 2016; Wheat et al., 2014).

One limitation of our study is the inconsistency in the calculated incidence rates. Leprosy is a rare disease with a long incubation period, thus underreporting is expected. Incidence rates calculated only take into consideration the detected new cases of disease from 2009 to 2013. Another limitation relates to the raw incidence rate in spatial analysis. The raw incidence rates can lead to statistical instability in showing the risk of a rare disease or when the population in a municipality is small. We were able to address this issue by spatial empirical Bayes (SEB) to smooth the incidence rate. Another important limitation is the difficulty in establishing temporarily on a cross-sectional study between the independent and dependent variable, where sometimes the independent variable may be a consequence of the dependent variable.

In the present study social and economic factors were not included in the analysis, although we tried to account for any differences among municipalities by using the random intercept to account for municipality level clustering. Previous studies have suggested that poverty, education illiteracy rates are correlated with leprosy. Therefore, these factors, may have confounded the results. However, sometimes an ecological study is important to identify areas where further work specific studies can be done. Future studies need to consider the influence of social and environmental factors that main impact the incidence of leprosy. Furthermore, future studies should focus on the influence of increased temperature and deforestation on leprosy.

This study was one of the first ever, and first in Brazil, to study the associations of climatic, elevation and vegetation cover with leprosy incidence addressing a critical knowledge gap regarding the role of the environment in the manifestation of leprosy cases in Minas Gerais. We emphasize the importance to certain geographical and climatic conditions that may influence leprosy transmission. We also suggest the importance of considering social and economic factors in area considered endemic. Lastly, there is enough evidence that catastrophic environmental changes will occur due to climate change that has the potential of intensifying health outcomes, especially transmission of infectious diseases, among vulnerable populations.

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Appendix

Table 1. *Data sources of environmental data used for the analysis.*

Parameter	Time Scale	Source
Elevation (meters)	Time invariant	United States Geological Survey (USGS), ASTER GDEM
Normalized Difference Vegetation Index (NDVI)	January 1, 2009-December 31, 2013	Climate Engine
Precipitation (millimeters)	Monthly and yearly (2009-2013)	National Institute of Meteorology (INMET)
Temperature (degrees Celsius)	Monthly and yearly (2009-2013)	National Institute of Meteorology (INMET)

Table 2. *Descriptive Characteristics of Hansen's Disease Cases and Incidence in Minas Gerais, 2009-2013 (N=7,794)*

Year	Cases N (Mean)	Raw Incidence Rate	Smoothed Incidence Rate
2009	1,876 (2.19)	8.76	11.36
2010	1,629 (1.90)	7.88	9.90
2011	1,556(1.83)	7.31	9.07
2012	1,481(1.72)	6.57	8.28
2013	1,252 (1.46)	5.05	6.76
Mean (SD)	1,555.2 (225.51)	7.11(18.40)	9.07(4.63)

Table 3. *Global spatial autocorrelation analysis of leprosy incidence rate in Minas Gerais.*

Year	Global Moran's I	Z score	p-value	Distribution Pattern
2009	0.221	10.959	0.002	Clustered
2010	0.362	17.886	0.002	Clustered
2011	0.267	13.232	0.002	Clustered
2012	0.245	12.120	0.002	Clustered
2013	0.297	14.687	0.002	Clustered
2009-2013	0.447	22.057	0.002	Clustered

Table 4. *Number of municipalities that were cluster cores detected by local indicator of spatial autocorrelation (LISA) of leprosy incidence rate in Minas Gerais*

	High-High	Low-Low	Low-High	High-Low	Not significant
2009	33	29	19	6	766
2010	37	35	18	4	759
2011	43	39	17	12	742
2012	39	28	26	13	747
2013	24	17	20	8	784
2009-2013	51	122	11	5	664

Table 5. *Number of municipalities considered hot- and cold-spot detected by Getis-Ord Gi*) of leprosy incidence per year*

	Hot-spot (95%)	Cold-spot (95%)	Not Significant
2009	52	112	689
2010	56	106	691
2011	59	127	667
2012	65	113	675
2013	46	150	657
2009-2013	62	133	658

Table 6. *Characteristics of environmental predictors*

	Mean	Min	Max	SD
Elevation	759.84	198.81	1,566.28	240.15
Temperature	27.22	13.53	31.95	3.04
Precipitation	114.21	40.55	165.05	25.07
NDVI	0.59	0.28	0.75	0.07

Table 7. *Raw Incidence Rate Poisson Regression Incidence Density Ratio (IDR)*

	Incidence Density Ratio	95% CI	p-value
Temperature	1.76	1.64, 1.89	<0.001*
Precipitation	1.06	1.01, 1.12	0.0201*
NDVI	0.98	0.95, 1.00	0.1763
Elevation	0.53	0.51, 0.55	<0.001*

**Statistically significant (p <0.05).*

Figure 1. *853 municipalities in Minas Gerais*

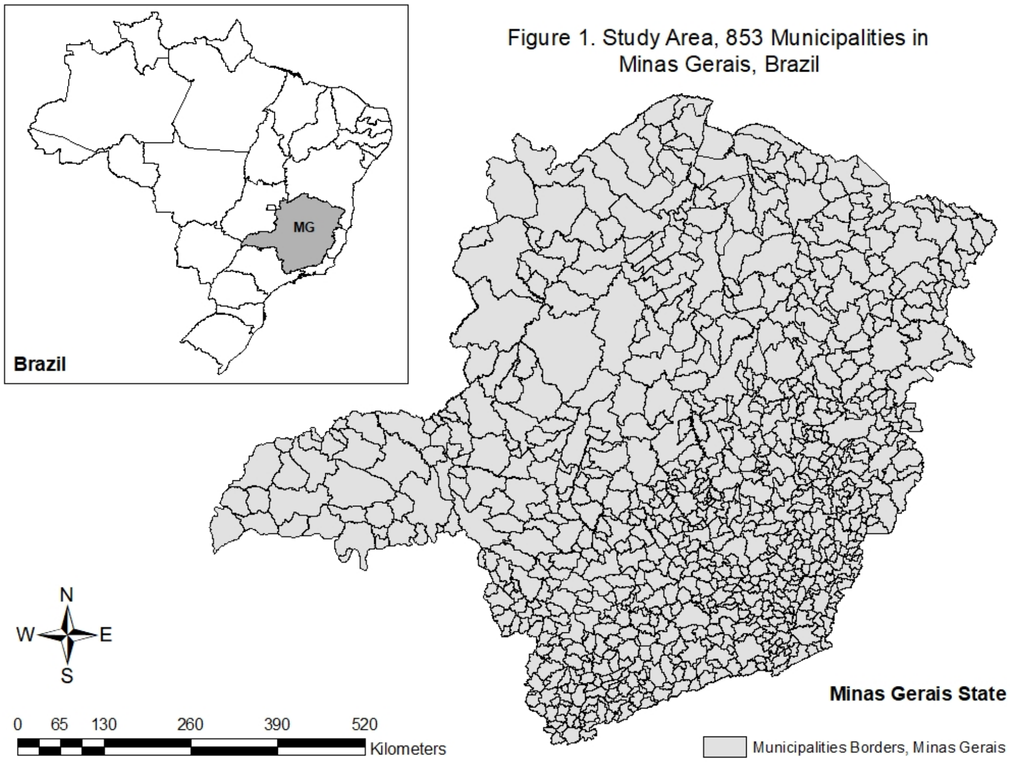

Figure 2. *Total leprosy incidence over 5-years with mean and standard deviational ellipse values displayed to show incidence distribution*

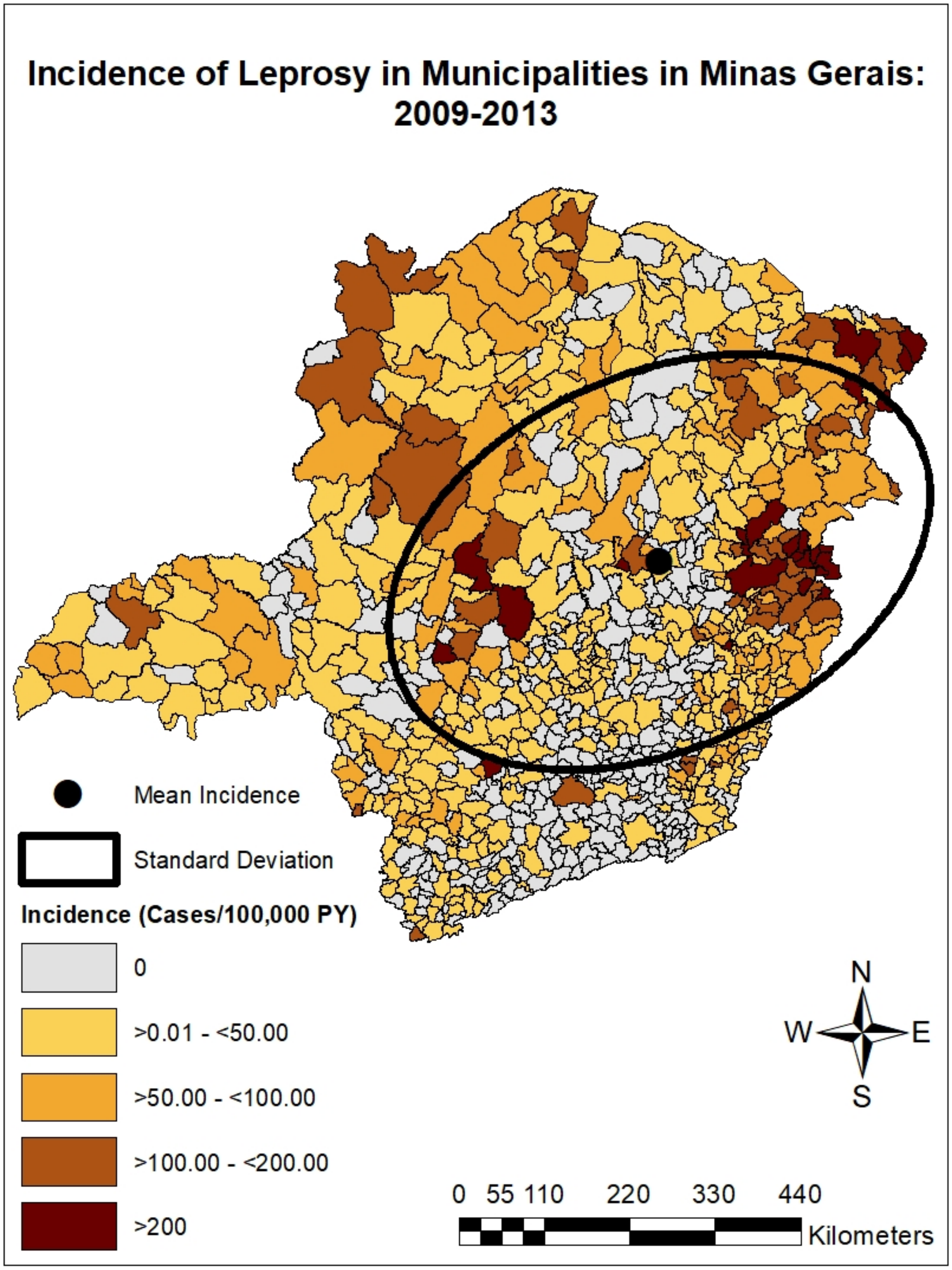

Figure 3. *Local indicators of spatial association (LISA) of incidence of leprosy/100,000 person-years for each year*

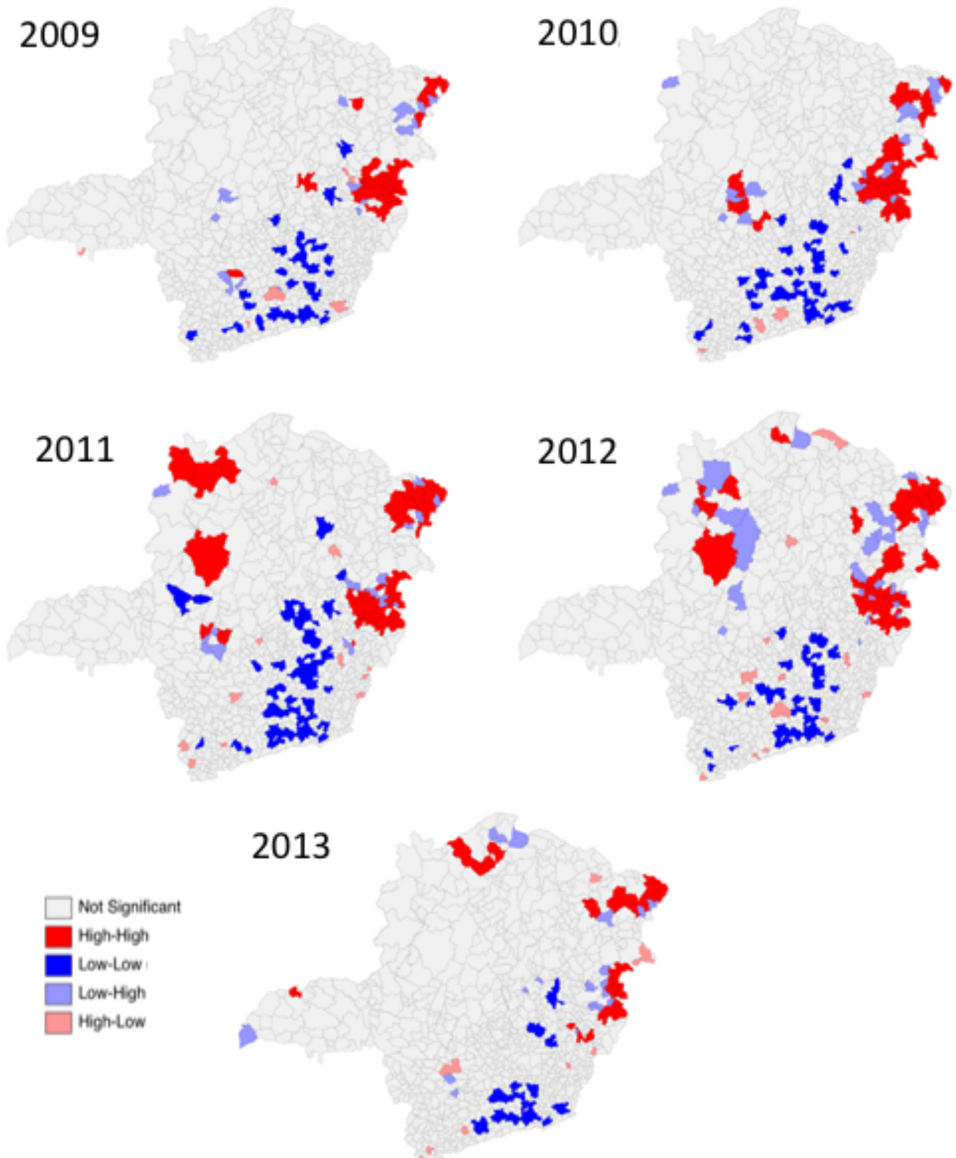

Figure 4. *Local indicators of spatial association (LISA) of total incidence of leprosy/100,000 person-years over 5 years (2009-2013)*

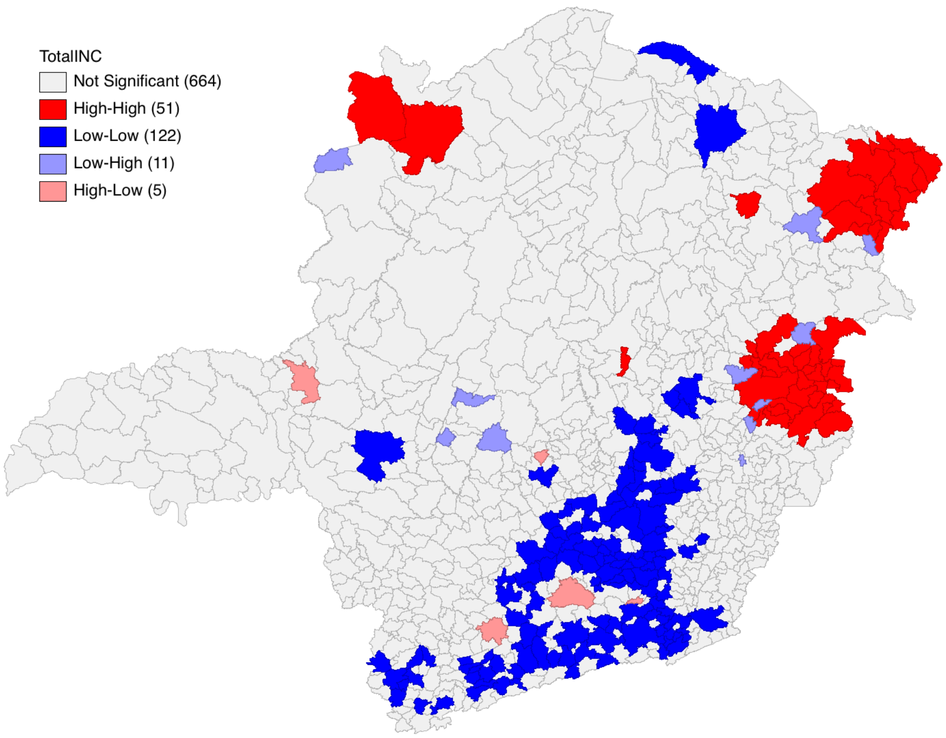

Figure 5. *Hot-spot and Cold-spot analysis (Getis Ord G*) of Leprosy Incidence for each year*

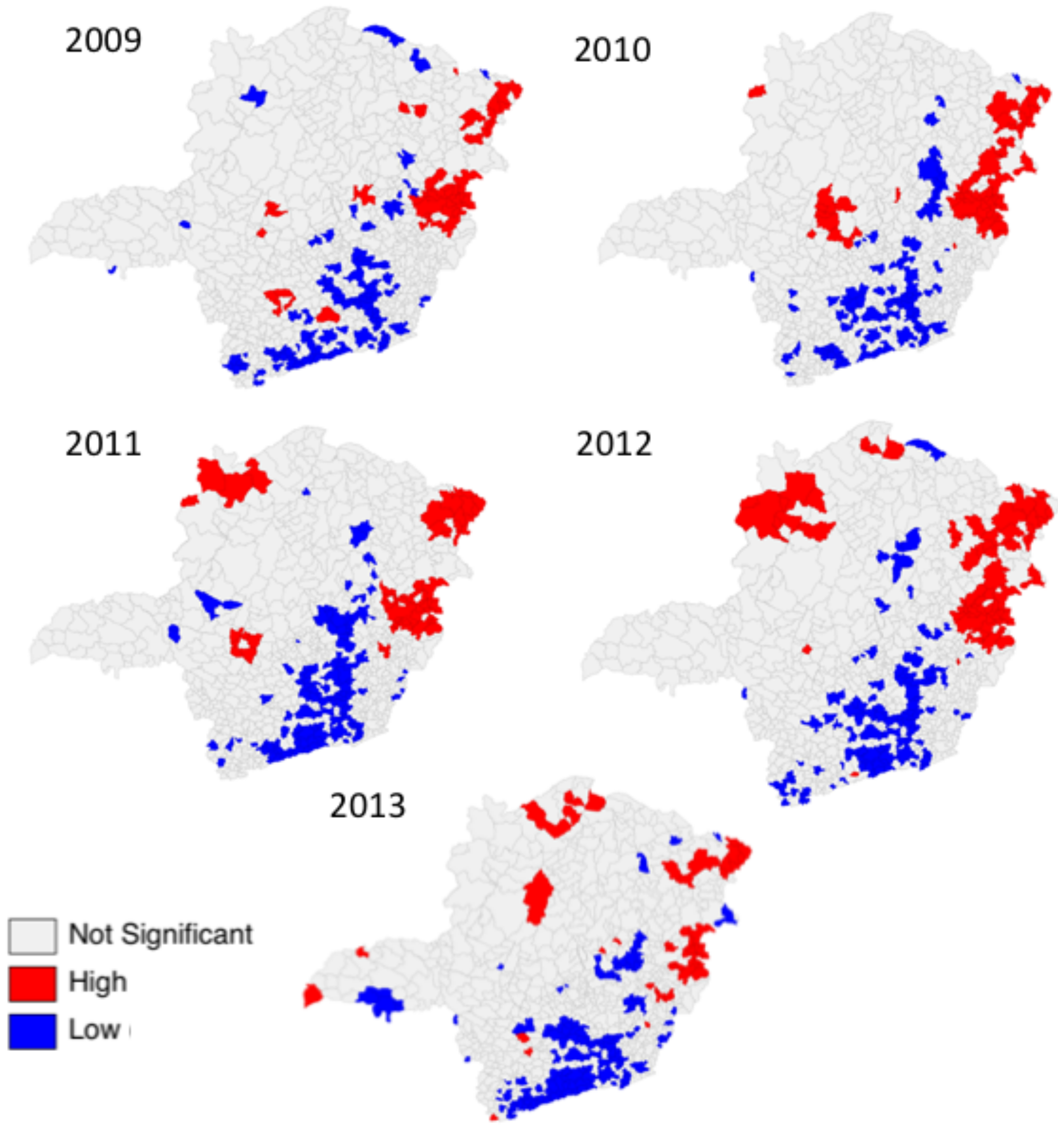

Figure 6. *Hot-spot and Cold-spot analysis (Getis Ord G*) of Leprosy Incidence over 5 years*

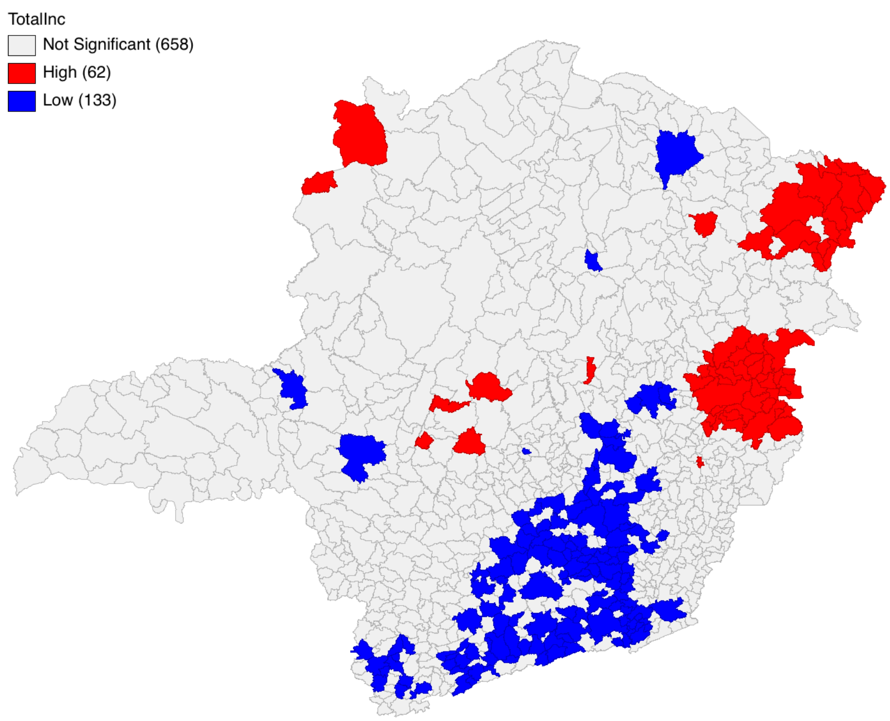

Figure 6: Hot-spot and Cold-spot analysis of total incidence of leprosy/100,000 person-years using the Getis-Ord Gi* statistic with queen contiguity using GeoDa 1.12.1.161.

Figure 7. *Elevation and Normalized Difference Vegetation Index for Minas Gerais*

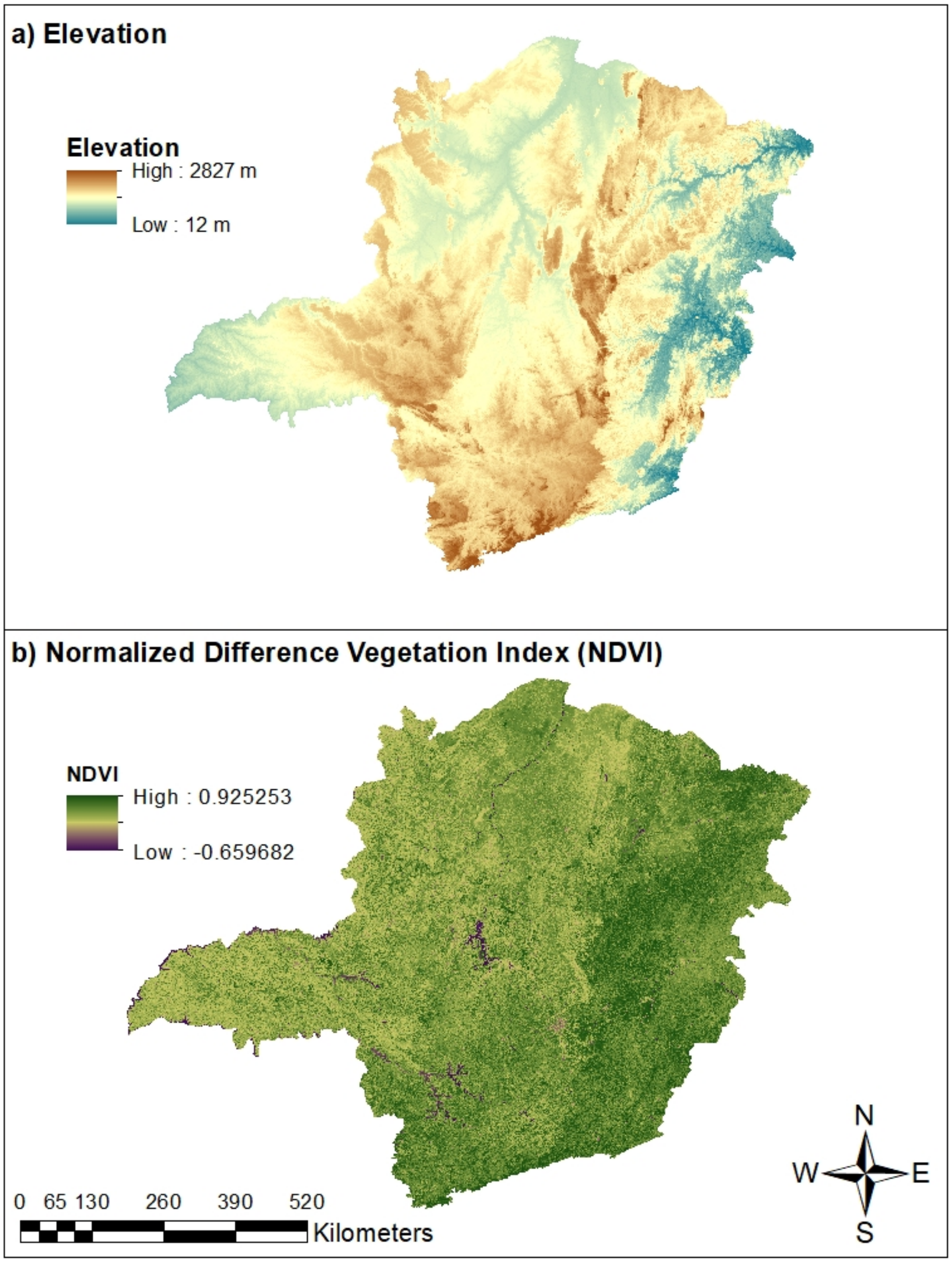

Figure 7. Elevation and NDVI in Minas Gerais.

Figure 8. *Total Annual Precipitation (mm) over 5-year study period in Minas Gerais*

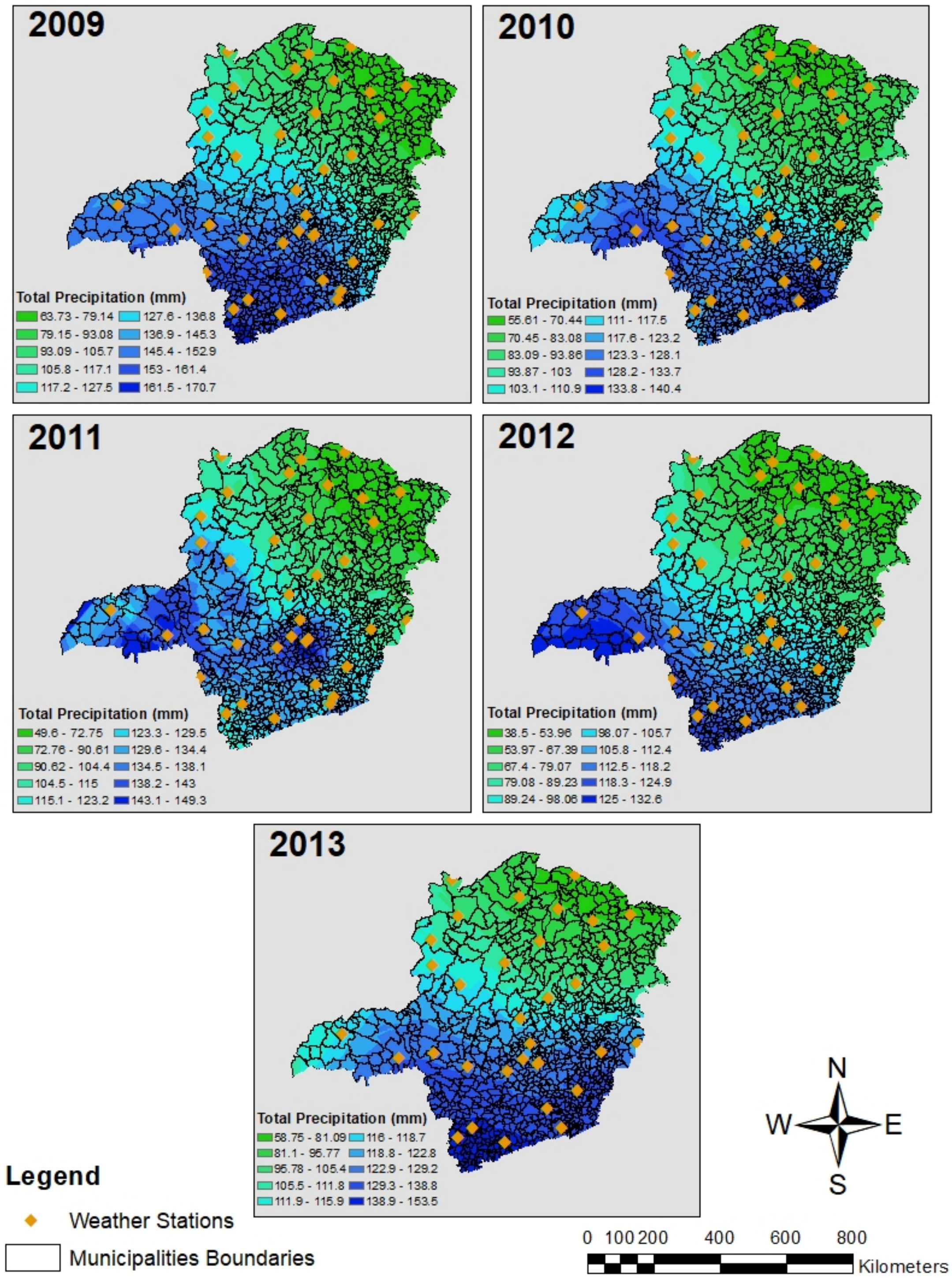

Figure 8. Total precipitation (mm) in Minas Gerais from 2009 to 2013.

Figure 9. *Maximum Temperature (Celsius) over 5-year study period in Minas Gerais*

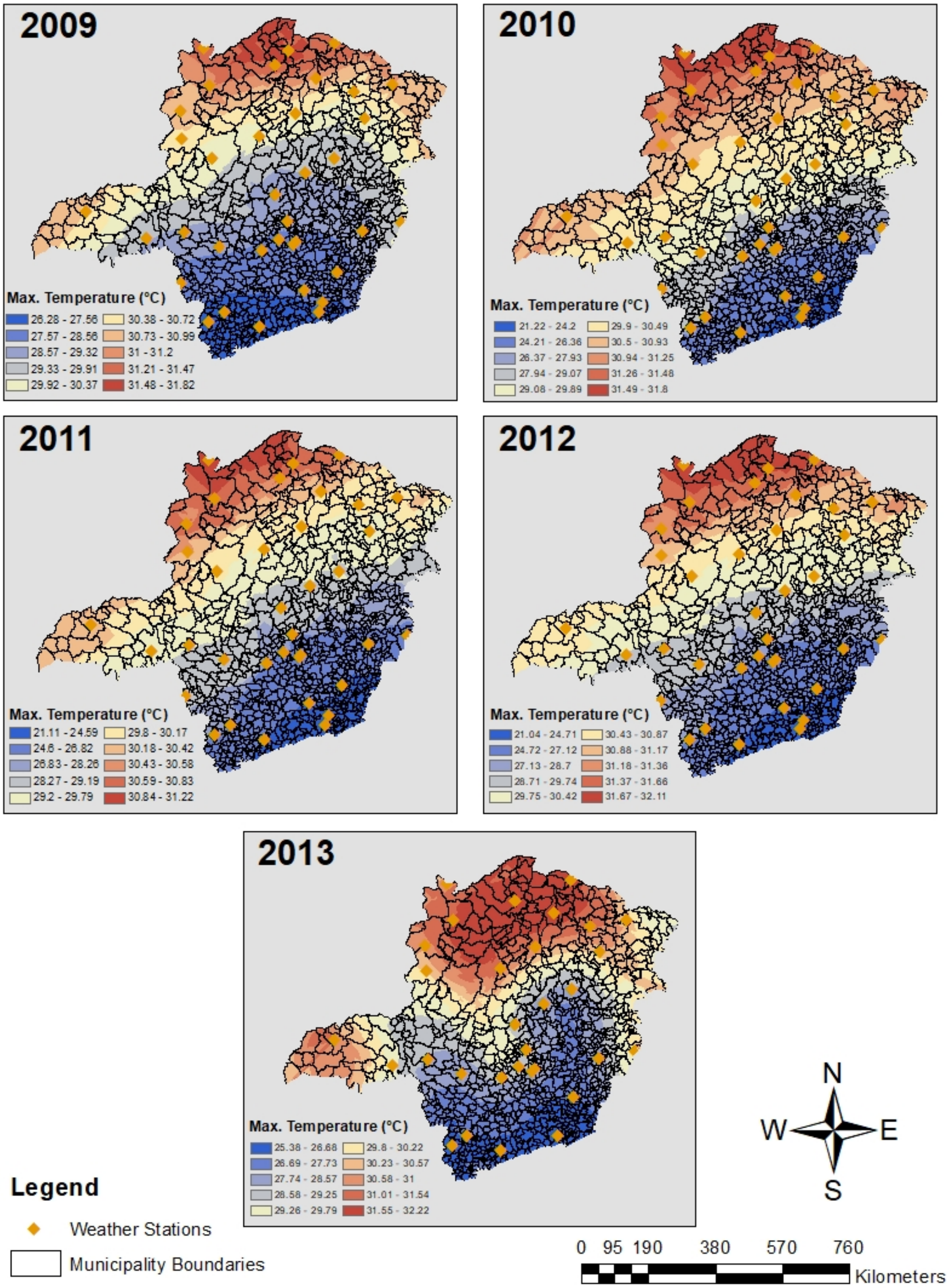

Figure 9. Maximum temperature (Celsius) in Minas Gerais from 2009 to 2013.