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Urban Microclimate Impacts on *Aedes* Mosquitoes' Life Cycle: A concept paper

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Abstract
Aedes aegypti and *Aedes albopictus* are two types of *Aedes* spp. responsible for the spreading of dengue in urban settings. Over the years, dengue remained a significant public health concern due to its disease burden worldwide. Urban microclimate factors such as temperature, relative humidity, and rainfall have long been known to influence the increase or decrease of *Aedes* spp. density in urban settings due to their impact on the mosquitoes' life cycle. This concept paper intends to provide a conceptual framework for determining the impact of urban microclimate on the *Aedes* mosquito development life cycle in urban settings.

Keywords: Urban microclimate; *Aedes* spp; *Aedes* life cycle

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1.0 Introduction

Dengue burden has been steadily rising for the last half-century due to global travel and trade, urbanization, growth of population, and changes in climatic conditions that contribute to conducive conditions for *Aedes* and its dengue virus proliferation (Rocklöv & Tozan, 2019). Abd Majid et al. (2020) mentioned that rapid urban growth involving extensive land-use changes could induce mosquitoes' breeding sites. In other words, township development and infrastructure and the increase in human population will inevitably increase dengue cases in urban areas. Besides, the transmission risk of dengue was high in cities due to the high density of the *Aedes* mosquito population and existing infection within these areas (Wan Mohamad Ali et al., 2021). *Aedes* spp. such as *Aedes aegypti* and *Aedes albopictus* can be considered a concern to public health due to their ability to act as vectors to diseases such as dengue, Chikungunya, Zika, and other arboviruses (Scolari et al., 2019). *Aedes aegypti* is the primary vector of dengue and can be found in tropical and subtropical regions (Marinho et al., 2016), while *Aedes albopictus* acts as a secondary vector for dengue transmission (Teo et al., 2017).

This study aims to provide a conceptual framework for establishing the impact of microclimate on the *Aedes* mosquitoes' life cycle in urban city by using selected microclimate factors in urban areas at a smaller scale measured from field measurement rather than using remote sensing microclimate data. Objectives of this study include 1) To determine the microclimate characteristics in urban city and 2) To determine the impact of temperature and shading in urban city on *Aedes* mosquitoes' life cycle. This is done by collecting data on the actual microclimate conditions of urban areas and simulating the conditions in the laboratory to provide a better

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understanding of the impact of these microclimates throughout the *Aedes* life cycle. Additionally, changes in temperature in potential breeding water are observed (Dalpadado et al., 2022) together with the shading effect on the breeding site to understand its potential influence on the *Aedes* life cycle (de Jesús Crespo & Rogers, 2022; Sukiato et al., 2019).

2.0 Literature Review

Urban microclimate can significantly be influenced by urban design, affecting temperature, wind speed, relative humidity, and solar radiation in the urban area (Li et al., 2022). It has been known that climate factors such as temperature, rainfall, humidity, cloud, and wind influence dengue transmission (Hii et al., 2016). One reason for this is its impact on the behaviour and viability of the *Aedes* spp. (Edillo et al., 2022). In a review by Hii et al. (2016), based on articles in Malaysia related to climatic factors and dengue transmission, temperature, rainfall, and humidity were the main variables used in many studies. Temperature is a critical factor affecting *Aedes* spp, and due to its poikilotherms characteristic, having non-constant body temperature requires them to adopt various strategies to face thermal stress risk (Reinhold et al., 2018). The higher temperature in the urban areas has been related to more transmission of dengue, such as reported by Araujo et al. (2015) in the city of Sao Paulo, and the dengue incidence rate was found to be high in low vegetation areas with land surface temperature at 29 ± 2°C with a result from multiple cluster analysis indicate dengue cases were clustered more in areas with land surface temperature more than 32°C. A study by Marinho et al. (2016) found that throughout the life cycle of *Aedes aegypti*, the mosquito can survive in any temperature; however, susceptible to higher temperatures (39°C) which suppresses *Aedes* embryonic development causing larval death in several hours without hatching. Another study found that high temperatures resulted in smaller mosquito size, low larval density, and shorter development time to adulthood (Muturi et al., 2012).

While most studies focus on temperature, rainfall, and humidity for the *Aedes* life cycle and dengue transmission, Sukiato et al. (2019) tried to determine the role of shading on immature stages of *Aedes* factors as an independent factor to the temperature where he found that larval and pupal mortality was higher at high temperature while shading was not significantly affecting hatchability of the mosquito. Shading can be provided by canopy cover over the breeding site of mosquitoes despite not significantly affecting the life cycle of mosquitoes (Sukiato et al., 2019). De Jesús Crespo & Rogers (2022) found that *Aedes aegypti* compared to *Aedes albopictus* prefers to lay their eggs under the shaded canopy in the high heat area, indicating that shading can be used as a predictive factor of *Aedes* spp segregation in the environment.

Nonetheless, understanding the microclimate impact on the *Aedes* mosquito life cycle in urban areas is crucial as urban areas provide a high number of potential breeding sites and human hosts for *Aedes* (Sukiato et al., 2019). Urban planning and development may influence disease control activities, especially for dengue and with the occurrence of urban heat islands in cities affecting relative humidity and rainfall which is crucial for *Aedes* survival, this disease prevalence may occur at an alarming rate (Akhtar et al., 2016). Findings on the life cycle of the *Aedes* mosquito are crucial in controlling transmission of dengue as they contributed to fundamental knowledge and understanding of local vector control activities, which can be applied to improve existing strategies by determination of specific times for larvicide and adulticide application done by relevant agencies in controlling *Aedes* mosquito population (Arévalo-Cortés et al., 2022).

3.0 Methodology

This study consists of two phases involving field measurement of microclimate factors and *Aedes* larvae collection in urban city and the experimental phase in the laboratory to simulate microclimate conditions in urban areas onto *Aedes* mosquitoes' life cycle (Fig 1). This will provide a better understanding of the urban microclimate and its potential implication on the *Aedes* life cycle in light of urban heat islands and global warming that worsen the microclimate situations in urban areas.

3.1 Field measurement of urban microclimate condition and Aedes mosquito larvae collection

3.1.1 Field measurement of microclimate condition

Field measurement of microclimate conditions allows for establishing required environmental readings to understand the microclimate of urban areas and an experimental study on the factors expected to influence the *Aedes* mosquito life cycle (Awang & Che Dom, 2020). There are two parts of measurement for this objective. The first part of field measurement involves the measurement of temperature, relative humidity, wind speed, and lighting in different sampling points located within one locality of an urban city with the highest number of dengue-reported cases based on the Ministry of Health, Malaysia database. The selected city will be determined before the field measurements, and 30 sampling locations will be within the study area.

Temperature, relative humidity, wind speed, and lighting will be measured based on hourly readings in all 30 locations during the field measurement to reflect the microclimate condition of the urban city. Previous study by Jin et al. (2018) included 27 sampling points in his study on the effect of urban morphology parameters on microclimate in Singapore. Temperature and relative humidity are measured using digitalized equipment or a data logger to record the temperature and relative humidity level in all sampling locations (Evans et al., 2019), while a lux meter will be used to record the shading/lighting of the water container left at the field for breeding water temperature measurement. These containers will be left under shaded areas and open areas under the sunlight around each sampling point. Hourly readings will be taken on lighting following the changes in sun position throughout the days. This is done to obtain information on general lighting/shading levels to correlate temperature differences in shaded and non-shaded containers, which may affect breeding water temperature. A reading for wind speed will be obtained through an anemometer. All equipment will be calibrated before the field measurement to ensure the accuracy of the measured parameters.

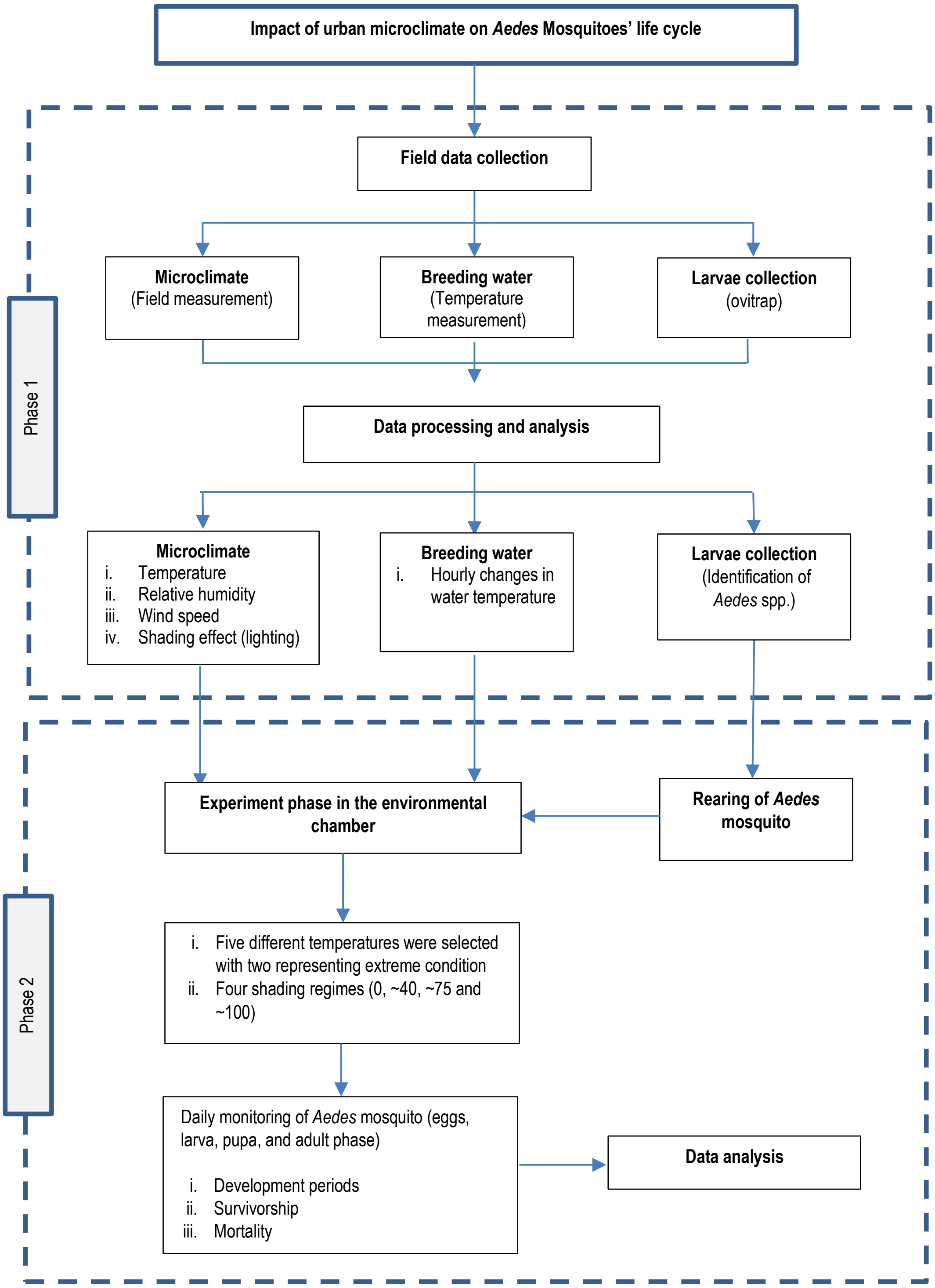

Fig 1: Framework in determining the impact of urban microclimate on *Aedes* mosquitoes' life cycle

The second part of field measurement is the hourly monitoring of changes in the temperature of breeding water for *Aedes* mosquitoes at the selected sampling points. Two containers filled with tap water will be allocated at each similar sampling point for measurements of microclimate conditions (temperature, humidity, and wind speed); one container will be placed in shaded areas, while another one will be placed in open areas under the sunlight. Hourly readings of breeding water temperature will be recorded. Measurement time will start from 6.00 am to 8.00 pm to cover the active period for biting in *Aedes* mosquitoes in contrast to the study by Awang & Che Dom (2020), which measures these parameters from 8.00 am to 6.00 pm. A thermometer will be used to measure the temperature changes in the water. Temperatures between the environment and breeding water will be compared to determine the correlation between changes in environmental temperature and water temperature, which may influence the development cycle of the *Aedes* mosquito.

3.1.2 Collection of Aedes mosquito larvae

This stage will involve collecting wild strains of *Aedes* mosquito species using ovitrap devices (Fig. 2) (Arévalo-Cortés et al., 2022; Awang & Che Dom, 2020; Teo et al., 2017) placed in the selected locality in the city for microclimate measurements. Ovitraps will be placed in all 30 sampling points selected before the microclimate measurements. The type of breeding water and container will be standardized to prevent bias in the study. Larvae of *Aedes* mosquitoes collected from the field will be bred in the laboratory as part of the preparation for experimental study in the laboratory setting. The ovitrap used are black plastic containers containing an oviposition paddle filled with tap water at 5 to 6 cm in height, which will be placed in shaded and unshaded areas (Teo et al., 2017). During the research, ovitrap placed in the field will be collected after day four before being replaced with fresh ovitrap for subsequent collection (Teo et al., 2017). Collected larvae will be brought to the laboratory for identification and rearing for the experimental phase of the study.

Fig 2: Ovitrap for larvae collection

3.2 Experimental study on the impact of urban microclimate on Aedes mosquitoes' life cycle

3.2.1 Pre-experimental stage

Larvae collected from field study will be hatched in the laboratory before laboratory simulation involving different light regimes and temperatures. Larvae collected will be reared under controlled conditions of temperature, relative humidity, and photoperiod. Identification of the *Aedes* mosquito will be conducted to prevent mixed mosquito species for the study (Arévalo-Cortés et al., 2022). The method of rearing the larvae until they become mosquitoes, perform blood feeding, and lay eggs will follow the method by Arévalo-Cortés et al. (2022) and Edillo et al. (2022) for rearing and maintenance of the *Aedes aegypti* population.

Collected larvae rear under controlled conditions of temperature (28±1°C), relative humidity (80±5%), and photoperiod of 12 hrs light: 12 hrs dark as conducted by Arévalo-Cortés et al. (2022). Larvae of *Aedes* mosquitoes are segregated into plastic containers containing distilled water that replace every two days and covered with a fine-mesh cloth and fed daily using powdered fish food (Edillo et al., 2022). The feeding regime will increase based on mosquito stages (Arévalo-Cortés et al., 2022).

Adult mosquitoes are then identified (species and sex) (Edillo et al., 2022) and introduced to rearing cages (Arévalo-Cortés et al., 2022). Adult mosquitoes were given 10% of a sterile sucrose solution using a sterile cotton ball dispensed on a petri dish (Arévalo-Cortés et al., 2022). Mosquitoes were allowed to mate over 3-5 days, in which mated females were starved for 24 hours before being fed with chicken blood mixed with ethylenediaminetetraacetic acid (EDTA) (Edillo et al., 2022). Blood will be placed in a plastic bottle at the bottom depression, sealed with parafilm membrane, held at an upright position inside the cage, and covered with fine-mesh cloth. Luke's warm water is frequently added to the plastic bottle to simulate body heat and attract blood feeding (Edillo et al., 2022). Seventy-two hours after blood feeding, female mosquitoes will be provided with an oviposition container with filter paper as substrate and 100ml of water to oviposit (Arévalo-Cortés et al., 2022). The eggs produced at this stage will be used in the experimental stage to simulate the impact of different temperatures and lighting on *Aedes* mosquitoes' life cycle. The stages of the *Aedes* life cycle involved in the pre-experimental stage can be summarized in Fig 3.

3.2.2 Experimental stage

After the initial phase of collecting data on microclimate and collecting *Aedes* mosquito strains in the field, Similar conditions of temperature and lighting will be simulated in the laboratory setting to observe the developmental cycle of *Aedes* mosquitoes at different temperatures and lighting. An environmental chamber will be used to simulate the growth of *Aedes* mosquitoes at different temperatures and lighting to reflect the field conditions (Sukiato et al., 2019). The environmental chamber can regulate temperature and relative humidity to meet the objectives and prevent bias in the research.

Four shading regimes will be established based on field data from the lowest lighting from shading to the brightest light intensity under the sunlight. Different lighting conditions will be created by covering the containers with different layers of muslin cloth, depending on the lighting level. The same techniques were used by Sukiato et al. (2019) in which shading was achieved by using muslin clothes to cover the container containing eggs of *Aedes aegypti*, and different layers were used to create different shading levels of 0%, ~40%, ~75% and ~100% representing 3100, 1860, 775, and 0 lux, respectively. Light lux measurement for the shading will be measured and recorded using Lux Meter to ensure the accuracy of lighting created by muslin cloth to reflect field conditions. These shading regimes will be replicated five times at different temperature levels (Sukiato et al., 2019) based on the field measurement of the microclimate temperature. Two of the temperatures set for the environmental study will reflect high temperatures during extreme conditions, while the other three represent normal temperatures in the urban city. Replication of shading regimes will be conducted at 37°C and 40°C (considered as extreme conditions) to represent the range of predicted temperatures in the tropics caused by climate change where *Aedes* mosquitoes can proliferate (Sukiato et al., 2019).

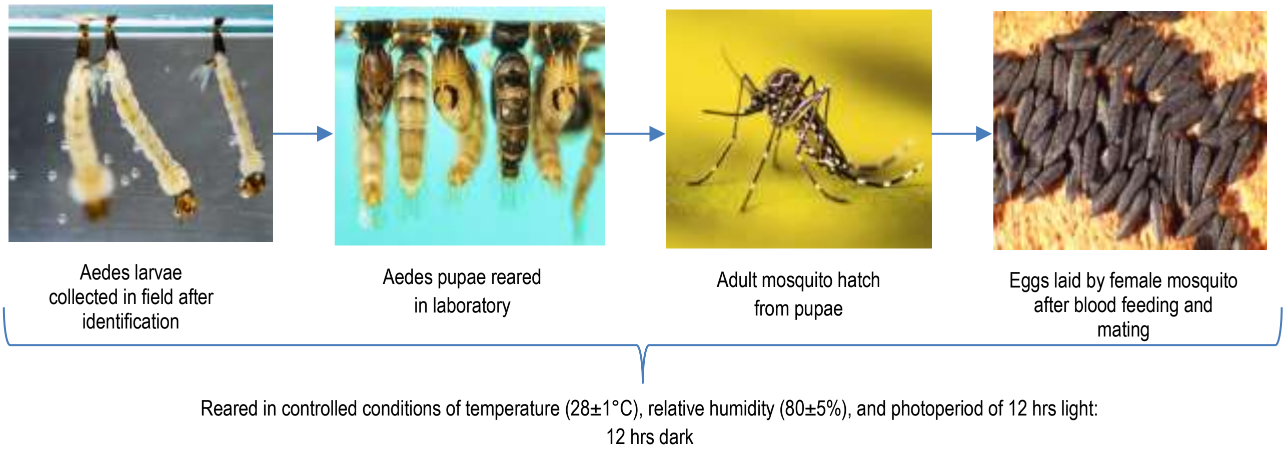

Fig 3: Stages of *Aedes* life cycle in the pre-experimental stage
Source: Centers for Disease Control and Prevention (2022)

An identical container will be used throughout the study to simulate the effects of temperature and lighting on the development cycle of the *Aedes* mosquito. Each container will be filled with 5 cm of tap water and left in the chamber for 24 hours to dechlorinate (Sukiato et al., 2019). A total of 30 eggs of *Aedes* mosquitoes reared from collected larvae from the field study will be allocated into breeding containers (Sukiato et al., 2019) for each shading regime for all test replications at different temperatures. Eggs will be transferred into the container from the filter paper onto 5.5-diameter petri dishes using the blunt end of a spatula (Sukiato et al., 2019) before being submerged into the container with 0.05 g of powdered fish food (Arévalo-Cortés et al., 2022; Sukiato et al., 2019). All containers will be covered with a mosquito net using a rubber band and labeled based on shading regimes and temperatures. Observations will be conducted on the development stages of *Aedes* mosquitoes from the embryonic phase to the adult phase until the end of the life cycle, yielding data on development periods, survivorship, and mortality for eggs, larvae, and pupae on a daily basis (Arévalo-Cortés et al., 2022; Awang & Che Dom, 2020; Sukiato et al., 2019). These observations will be done daily at a fixed time of the day.

3.2.3 Post-experimental stage

Recorded data from both field and experimental studies will be analyzed using the Statistical Package for Social Science (SPSS) version 26, in which the effects of temperature and lighting will be associated with the development cycle of *Aedes* mosquitoes in terms of development periods, survivorship, and mortality for eggs, larvae, and pupae. All data will be tabulated in Microsoft Excel version 2019 before data analysis. Temperature, humidity, wind speed, lighting, and breeding water temperature will be analyzed descriptively into mean, standard deviation, minimum, and maximum readings using SPSS. Similarly, in the experimental stage, descriptive analysis will be conducted on survivorship, developmental periods, and mortality of eggs, larvae, pupae, and adult mosquitoes. The correlation will be tested between air temperature and breeding water temperature and between lighting/shading and breeding water temperature from field measurements using Pearson Correlation. Tests such as one-way ANOVA, regression tools, and t-tests can be used to fulfil the targeted objective (Awang & Che Dom, 2020) on the impact of different temperatures and shading on the *Aedes* life cycle using the survivorship, developmental periods, and mortality of eggs, larvae, pupae, and adult mosquitoes.

4.0 Conclusion

This paper intends to provide a conceptual framework for determining the impact of urban microclimate on *Aedes* mosquitoes' life cycle. This research is expected to produce knowledge on the impact of local microclimate conditions of the urban area on the life cycle of *Aedes* mosquitoes in terms of their survivability, development periods, and mortality at each stage of their life cycle. As dengue is a public health concern in many countries, including Malaysia, the finding is crucial for relevant agencies such as public health agencies and local authorities in planning strategies to combat dengue transmission in urban areas. Moreover, urbanization in many countries causes urban heat islands while climate change and global warming increase the frequency as well as the intensity of heat waves affecting microclimate, particularly temperature levels in urban cities. Thus, it is vital to understand how these phenomena, which cause temperature elevation in urban areas, can affect the *Aedes* mosquito survivability, hence, may influence future dengue transmission in the cities and its surrounding areas.

Currently, this study focuses on one locality with the highest number of dengue cases, which produced limited findings relevant to this area with specific microclimate characteristics and does not reflect the other cities or countries with differences in geographical locations, city sizes, vegetation levels and designs that may contribute to difference microclimate characteristics, particularly temperature level. Thus, further research is suggested to be conducted in various settings in different geographical locations and microclimate characteristics. Future research may include factors such as food availability to *Aedes* mosquitoes in urban areas which support its viability and habitat utilization in addition to microclimate characteristics to identify further other contributing factors in the urban environment that support *Aedes* mosquito's survival and dengue transmission in urban areas despite the increasing temperature in urban cities. These studies may also include the difference in *Aedes* mosquitoes' viability to spread dengue in urban and rural settings with different microclimate characteristics and the implication of extreme weather events such as heat waves to the mosquitoes' life cycle.

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Paper Contribution to Related Field of Study

This research will contribute to a better understanding of the impact of urban microclimate on *Aedes* mosquitoes' life cycle to allow future planning and design of built environments in urban settings and dengue control strategies and measures. Health authorities may use the findings from this study in predicting dengue transmission during extreme events such as heat waves and to face the danger of global warming and climate change regarding communicable disease transmission. Local authorities can benefit from this study in terms of disease prevention and preparedness as well as the creation of safe and healthy cities which targets to ensure cities and human residents are resilient, safe, and sustainable in line with Sustainable Development Goals (SDGs), especially with the potential increase of communicable disease transmission such as dengue in urban cities due to climate change and urbanization.

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