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The quality of air at petroleum refining area in Bojonegoro, Indonesia: Morphological condition and chlorophyll level changes of *Muntingia calabura* L.

To cite this article: D A Kusumastuty *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **276** 012029

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The quality of air at petroleum refining area in Bojonegoro, Indonesia: Morphological condition and chlorophyll level changes of *Muntingia calabura* L.

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Abstract. Petroleum refinery activity practiced conventionally is indicated to result in a significant impact on the environmental changes. This research aimed at revealing the morphological condition and the chlorophyll level of *Muntingia calabura* L. growing around the petroleum refining area in Wonocolo, Bojonegoro, Indonesia. The data sampling covered eight areas; four of which are 50 meters away, while the four others are 100 meters away from the center location of the refinery. The chlorophyll levels were obtained from spectrophotometry technique. Based on the microscopic analysis, chlorosis and necrosis symptoms have been detected in all sampling locations. Based on the multivariate testing, the chlorophyll level has shown to be significantly different on distance factor ($F = 11.983$, $p < 0.005$), but there has been no significant difference on the point of the compass as well as interactional factors. Based on the univariate testing, the chlorophyll level on distance factor has shown to be significantly different, both on chlorophyll a level ($F = 18.867$, $p < 0.005$) and chlorophyll b level ($F = 25.085$, $p < 0.005$). The results have indicated that petroleum refining industry which is conducted conventionally in Bojonegoro contributes significant impact on the plant condition in its surrounding areas.

Keywords: Morphological, petroleum, Bojonegoro, chlorophyll, *Muntingia calabura* L

1. Introduction

Air quality determines the quality of life of all living beings on earth [1, 2]. Aside from containing essential components needed by the body, air is an abiotic component which interacts directly with the majority of terrestrial organisms. However, the decrease in air quality is a crucial problem which is faced by the world nowadays [3–5]. The decrease in air quality which has happened drastically in the past decades is caused by the dramatic rise of urbanization and industrialization [6, 7]. This sort of condition eventually results in negative impacts to the environment as well as the vegetation [8–12], human health [13–16], and global climate [16, 17]. Therefore, air quality is a problem that should not be underestimated by the world community.

Petroleum refining and processing are the contributors to air pollution which significantly affects the quality of air [18]. During the process of producing petroleum, the earth's atmosphere is contaminated by many kinds of dangerous substances, such as carbon monoxide, methane, nitrogen oxide, carbon dioxide, as well as an aerosol particulate matter which contains organic aerosol, and



even hydrogen sulfide [7, 18–22]. From the aforementioned emission, Sulphur dioxide and hydrocarbon are the primary pollutants [18]. Both main pollutants and other emissions are reported to bring negative impacts on living beings [22, 23]. If this problem is not addressed, then these conditions further aggravate the environmental conditions that exist on this Earth.

The negative impact of petroleum refining and processing will be worsened when the industrial process does not adhere to the standard operating procedures [9, 10]. Such condition can generally be found in illegal and conventional petroleum refineries [24–26]. During the refinery processes, oil refinery companies are not trying to minimize the emission of dangerous substances to the air. As a result, environmental damage negatively affecting all kinds of organisms in the refinery area will become greater [24]. Various studies and technological development have provided solutions to minimize the negative impacts of petroleum refining to the environment [27–29]. Therefore, the assessment of air quality in the petroleum refineries area needs to be conducted.

As sessile organisms, plants often respond to environmental conditions, from phenotypic to genotypic levels [30–38]. Plants also indicate condition changes when exposed to air pollution [39], [40]. Trees growing in industrial areas or near main roads will absorb pollutant on their leaf surface [41, 42]. Different types of responses can be shown by the trees in such condition, either in morphological, biochemical or even the psychological aspects of the plant [1]. Visible leaf damage, morphological anomalies, anatomical anomalies, and biochemical changes are the examples of response shown by plants growing in an area with low air quality [41, 43, 44]. As observed from those responses, the decrease of chlorophyll level accompanied by the yellowing and eventually blackening of the leaves is one impact which is readily visible [1, 8, 30]. These various changes could be a negative impact on the plant affected, but these conditions can be used as important information by researchers.

The presence of changes in plants as a response to air pollution can be used to diagnose air pollution early [1, 44]. There have been plenty of researches which use plants as bio-indicator of air pollution [43–47]. Using plant as bio-monitor of air pollution can show a significant contribution to the environment [1]. However, the selection of plant as bio-indicator of air pollution needs to fulfill some prerequisites. One of them is that the plant should be relatively free from pest and disease impeding the fruit to grow maximally [40]. Departing from the above-mentioned notions, the plant can be utilized in testing the quality of air in the area of a petroleum refining and processing.

One of the areas in Indonesia rich in petroleum is the district of Wonocolo, Bojonegoro. In this area, there are many petroleum refining and processing activities which are conducted conventionally. The analysis of air quality in this area is of urgency, even so, the study which analyzes the causes of such problem. This current research aimed at analyzing the morphological and chlorophyll level changes of *M. calabura* L. in the area of a petroleum refinery in Wonocolo, Bojonegoro. *M. calabura* L. is selected because it meets with the criteria of an organism capable of indicating air pollution [40]. *M. calabura* L. is a plant which is readily available in Indonesia, not excluding in Wonocolo. Besides, this plant has been used as bio-indicator of air pollution in several previous studies [6].

2. Methods

This current research aimed at providing the descriptions on the morphological and chlorophyll level changes of *Muntingia calabura* L. The sampling location was in the area of petroleum refinery in Wonocolo, Bojonegoro, Indonesia. The data sampling was covering eight locations; four of which are 50 meters away from the center of refinery activities, while the four others are 100 meters away from the center. In detail, the position of the sampling location is presented in Figure 1. In this research, the samples were taken from the leaves of old *Muntingia* (the leaves are at least sixth from the end of the branch), unprotected leaves, and leaves from branches facing the old well of the petroleum refinery. The sampling was conducted in April 2018.

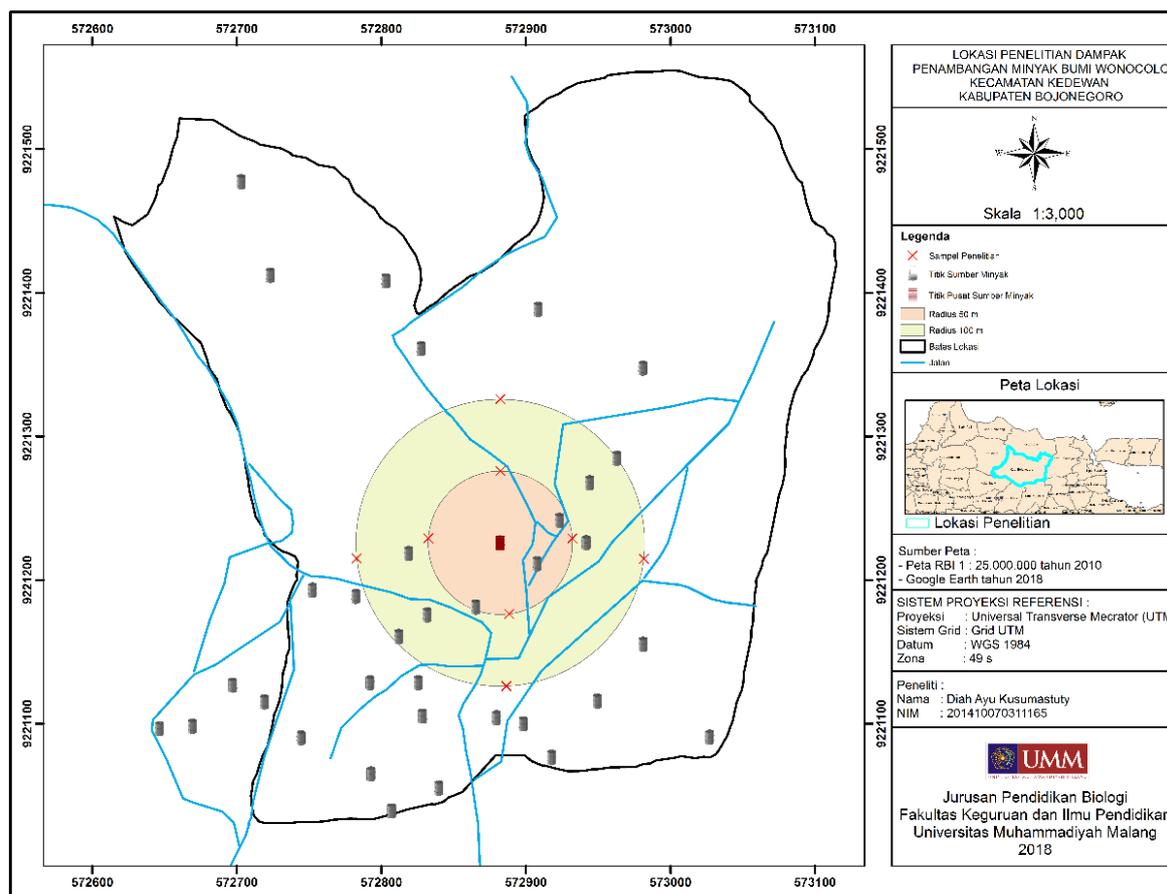


Figure 1. Maps of Petroleum Refinery in Wonocolo, Bojonegoro. The sign \times indicates the location of sampling; while sign \square represents the location of a petroleum refinery, and \blacksquare sign is the center location of petroleum source

There were two types of data collected in this research, namely the morphological changes of leaves and the concentrations of chlorophyll (Chl a and Chl b) in the leaves. The observation was conducted in the Biology Laboratory of University of Muhammadiyah Malang. The data of morphological changes of leaves were collected from microscopic observations. The changes of color were used as the parameter in the analysis of the leaves' morphological changes. Subsequently, the chlorophyll level data were measured by using Shimadzu UV-1800, UV-VIS Spectrophotometer type, CAT. No. 206-25400-58. The resolution of the spectrophotometer as high as 1 nm and the absorbance value measurement of λ 645 nm and λ 663 nm. The concentration of chlorophyll a and b were calculated by using the formula (1) and (2), subsequently, and stated in mg/ml [48].

$$12.7 D-663 - 2.69 D-645 \quad (1)$$

$$22.9 D-645 - 4.68 D-663 \quad (2)$$

Chlorophyll level data were analyzed using two-way multivariate analysis of covariance (two-way MANOVA), with independent variables 1) the distance of sampling location from the center of petroleum source, and 2) the location of the sampling based on the point of the compass. If the position factor of the sampling location or interaction factor concludes that there is a significant difference, the analysis is then followed by Least Significant Difference (LSD) test.

3. Results and Discussion

Based on the results of observation conducted in the petroleum refinery area in Wonocolo, Bojonegoro, the morphological changes of *M. calabura* L. leaves occurred in all stations. The morphological changes of leaves are presented in Figure 2 and 3. Based on Figure 2 and 3, necrosis and chlorosis symptoms have been present in all eight stations. From Figure 2 and 3, the symptoms of necrosis are shown by yellowing of the leaves, while the symptoms of chlorosis are shown by the appearance of black spots on the leaves. Furthermore, based on both figures, the leaves at Station 5 to 8 appear greener compared to the leaves at Station 1 to 4. Thus, the information obtained from both figures is that generally necrosis and chlorosis symptoms appear to be high in Station 1 to 4 (As previously explained, Station 1 to 4 is closer to the center of petroleum source than Station 5 to 8).

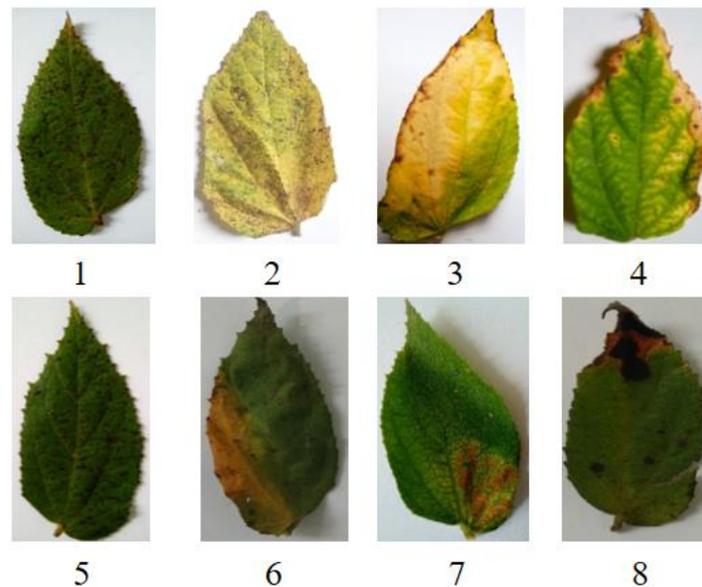


Figure 2. The condition of *M. calabura* L. leaf upper surface taken from eight different sampling locations in Wonocolo, Bojonegoro

Leaves are more susceptible to air pollution compared to other parts of the plant, such as stem or root [39]. This condition is related to a complex physiological process which takes place in this organ [39, 49]. Therefore, industrial pollution from a petroleum refinery, which is the core concern of this research, can directly affect the condition of the leaves in the area. The yellowing of leaf shows the symptom of chlorosis; while brownish spots indicate the symptom of necrosis [49]. The results of this current research are in line with the previous studies which analyzed the effects of air pollution in other locations, such as in Quetta [42] and Kolkata [43]. The results of this study also proved that *M. calabura* L. leaves may become discolored when living in areas contaminated by pollutants in petroleum refinery area.

Chlorosis symptoms are caused by the decrease of chlorophyll level in the leaves [49]. The decrease in chlorophyll level will cause the green color of the leaf to wear off and change the color to yellowish shade. The decrease of chlorophyll level can slow down the process of photosynthesis of the plant [50]. This statement is in line with Yousafzai *et al.* who suggest that when the plant is exposed to an above normal level pollutant, the photosynthesis process will slow down or even become inactive [8]. Exposure to pollutant eventually causes scars on the leaves and causes tissue death in the area of scars. This condition is known as necrosis [49]. The chlorosis and necrosis events caused the color change of *M. calabura* L. which was observed in this study.

A more severe condition of chlorosis and necrosis in the leaves of *M. calabura* L which grow closer to the center of petroleum source is also in line with the analysis of chlorophyll concentration of

leaves from the plant. The data of chlorophyll a and b concentration from each station are presented in Figure 4 and 5. Based on Figure 5, leaves collected from station 1 to 4 have shown lower chlorophyll a concentration compared to leaves collected from those in Station 5 to 8. Based on Figure 6, the chlorophyll b concentration of leaves from Station 1 to 4 was also lower than the leaves from station 5 to 8.

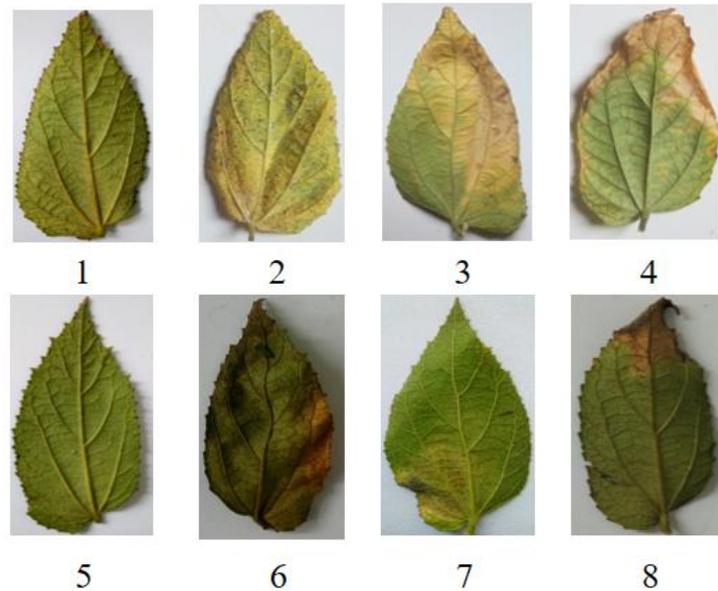


Figure 3. The condition of *M. calabura* L. leaf underside surface taken from eight different sampling locations in Wonocolo, Bojonegoro

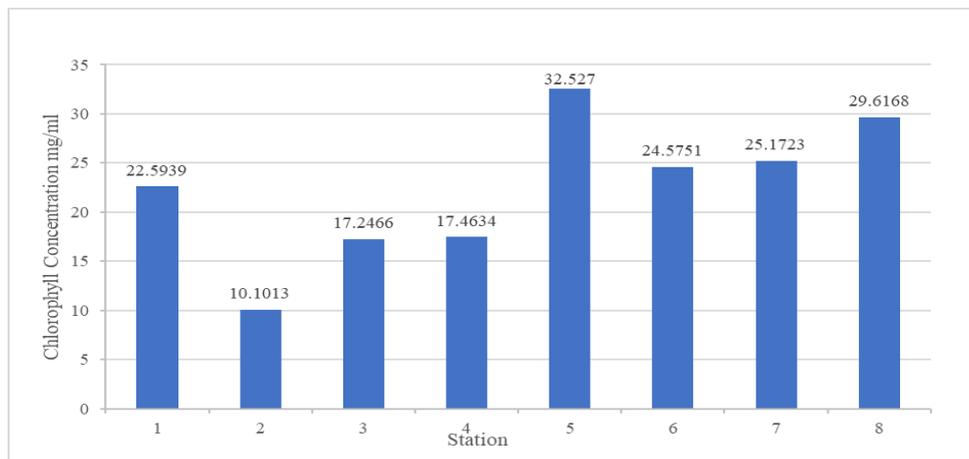


Figure 4. The comparison of chlorophyll a level in *M. calabura* L. from eight different stations in Wonocolo

Subsequently, the chlorophyll level data were tested utilizing two-way MANOVA. The results of the multivariate test are presented in Table 1; while the results of the univariate test are presented in Table 2. Based on the multivariate test results, the differences of chlorophyll levels are present merely due to distance factor [$F(2,15) = 11.983, p = 0.001; \eta^2 = 0.615$]. Meanwhile, the differences on chlorophyll levels are not present in the point of compass factor [$F(6,30) = 1.240, p = 0.314; \eta^2 = 0.199$] nor interaction factor [$F(6,30) = 1.336, p = 0.272; \eta^2 = 0.211$]. Furthermore, based on the univariate testing, it can be seen that the differences of chlorophyll levels in distance factor are

present, both in chlorophyll a [$F(1,16) = 18.867, p = 0.001; \eta^2 = 0.541$] and chlorophyll b [$F(1,16) = 25.085, p < 0.001; \eta^2 = 0.611$]. The results of this analysis have shown that the factor significantly affecting the chlorophyll levels in the leaves of *M. calabura* L. is the distance of the tree from the center of petroleum source; while the position of the tree based on the four points of the compass has not affected the chlorophyll levels of the leaves.

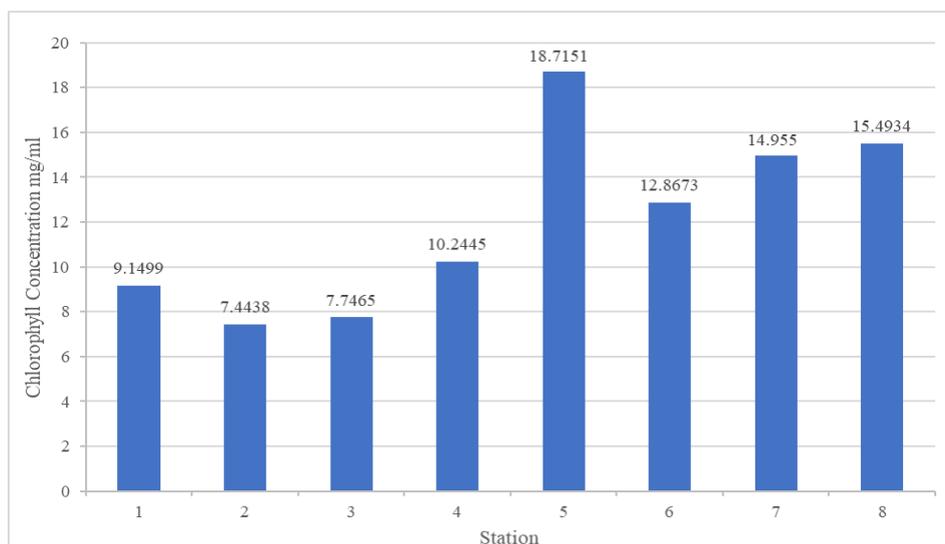


Figure 5. The comparison of chlorophyll b levels in *M. calabura* L. from eight different stations in Wonocolo

Table 1. The result of the multivariate test on the effect of distance and point of compass factors on the chlorophyll levels of *M. calabura* L. in Wonocolo

Sources	Hypothesis df	Error df	F	Sig.	η^2
Distance	2	15	11.983	0.001	0.615
Point of Compass	6	30	1.240	0.314	0.199
Distance*Point of Compass	6	30	1.336	0.272	0.211

Table 2. The result of the univariate test on the effect of distance factor to the chlorophyll levels in the leaves of *M. calabura* L. in Wonocolo

Dependent variables	Hypothesis df	Error df	F	Sig.	η^2
Chlorophyll a	1	16	18.867	0.001	0.541
Chlorophyll b	1	16	25.085	<0.001	0.611

The results have described that the decrease of chlorophyll a and b levels of plants which grow in the area where the air is polluted are consistent with the findings of the previous studies. Those studies used not only *M. calabura* L. [6] but also using other plants, such as *Pentas lanceolate* Forssk. Also *Cassia siamea* Lam. [44], *Tanacetum vulgare* L. [30], and *Mangifera indica* [8, 45, 46]. The extreme decrease of chlorophyll levels on the leaves of trees growing near the center of petroleum source has been caused by the increase of pollutants in the location. Some types of pollutants which are reported to cause the decrease of chlorophyll levels are carbon monoxide, nitrogen oxide, and Sulphur dioxide [51–53]. Therefore, it is possible, the air in Wonocolo has been polluted by these gases.

Several researchers have proposed the causes of the decrease of chlorophyll levels of plants which are exposed to air pollution. First, chlorophyll is susceptible to degradation to become phaeophytin due to the loss of magnesium ions. This process might happen due to the extreme level of Sulphur

dioxide which causes the substitution of the ions into two H atoms [54]. Secondly, the degradation of chlorophyll b to become chlorophyllide can also be caused by the increase of Sulphur dioxide concentration capable of releasing phytol cluster of the chlorophyll [54, 55]. Another contributing factor which causes the decrease of chlorophyll level when the tree is exposed to air pollution is the inhibition of air diffusion due to pollutant particle deposition at the stomata which hinders the chlorophyll synthesis [53, 54]. Besides, when the level of pollutant is high, chlorophyllase activities will also rise. Chlorophyllase is an enzyme that catalyzes the hydrolysis of chlorophyll into chlorophyllide [56]. As a result, more chloroplasts will degrade.

Chlorophyll is the main component in plant metabolism [57] and can be used as a plant health parameter [58]. This component is in charge as the main photoreceptor in the process of photosynthesis [59], a process which is aided by sunlight which turns carbon dioxide and water into carbohydrate and water [49]. The decrease in chlorophyll pigment causes a decrease in plant productivity [58]. This condition leads to the depletion of energy, growth inhibition, and even death [1, 6, 40, 49]. Based on the findings of this current research, it can be concluded that the decrease of air quality in an area where petroleum refining and processing are conducted conventionally contributes to the decrease in chlorophyll level on plants. Conventional petroleum refining and processing often neglect the management and operational standards. This condition can harm the environment, including the vegetation living in it. Under such condition, the harmful pollutants released to the air become uncontrollable.

4. Conclusion

In this research, morphological condition and chlorophyll a and b levels in the leaves of *M. calabura* L. which grow around the petroleum refinery in Wonocolo have been analyzed. The findings show that the leaves collected from eight different stations have indicated the symptoms of chlorosis and necrosis. Furthermore, the two-way MANOVA testing results inform that the chlorophyll levels of leaves of plants growing closer to the center of petroleum source have shown to be significantly lower than those growing further away [$F(2,15) = 11.983, p = 0.001; \eta^2 = 0.615$]. The results from the univariate testing confirm that there has been a significant difference of chlorophyll levels between plants growing 50 meters away from the center of petrol source and plants growing 100 meters away, both in chlorophyll a [$F(1,16) = 18.867, p = 0.001; \eta^2 = 0.541$] and chlorophyll b [$F(1,16) = 25.085, p < 0.001; \eta^2 = 0.611$].

Acknowledgments

Authors are thankful to University of Muhammadiyah Malang for providing the lab facilities.

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