On-site analysis of paraquat using a completely portable photometric detector operated with small, rechargeable batteries

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Keyword: Light-emitting diode; Paraquat; Portable photometric detector; Rice field, Sodium dithionite; Thailand

Abstract

This work describes a methodology that can be used to achieve on-site analysis of paraquat in water samples by using a miniaturized portable photometer consisting of a couple of light-emitting diodes (LEDs). Paraquat produces a colored radical via a redox reaction with sodium dithionite, which is unstable against oxygen in solution. The steps taken to stabilize the reagent solution included control of the pH and the addition of organic solvents, but the most effective was the formation of an oil layer. Together, these steps stabilized the reagent solution for two days. An increase in the duration of reagent stability, however, is necessary in order to transport the reagent for on-site applications in remote locales. For the time being, an excess amount of solid sodium dithionite can be added directly to sample solutions because the unreacted dithionite shows no influence on absorbance of the paraquat radical. Orange LEDs with a maximum emission wavelength of 609 nm were employed in the portable photometer to measure the absorbance of paraquat radical produced by a redox reaction that has an absorption maximum of 603 nm. The developed photometer showed excellent performance with a linear range of from 2.0 mg L^{-1} to 40.0 mg L^{-1} and a linear regression ($r^2 = 1$). The limits of detection and quantification were 0.5 mg L^{-1} and 1.5 mg L^{-1}, respectively, intra-day precision (n=3) and inter-day precision (n=5) were both less than 5%, and accuracy based on the percentage of sample recovery ranged from 89±0 to 105±0% (n=3). The proposed method was applied to the analysis of paraquat in water samples taken from rice fields. The results showed no paraquat in all thirteen samples, which could have been due to strong adsorption of paraquat by soil particles and/or to complications with the sampling conditions. To confirm the adsorption onto soil of paraquat contained in water, we constructed an artificial rice field where water containing paraquat was impounded above the soil layer. The results showed that paraquat in water gradually decreased within three days and could be measured in the soil on the fourth day. These results were confirmed by HPLC analysis, which underscores the utility of this portable photometer for the on-site monitoring of paraquat in water samples.

1. Introduction

Easy availability and reduced cost dictate that herbicides and pesticides will be employed both intentionally and accidentally in a country like Thailand where agricultural operations control 41% of the total land area [1]. Paraquat (1,1′-dimethyl-4,4′-dipyridinium) is a toxic chemical that is extensively used as a non-selective herbicide in Thailand because it facilitates control of weeds and grasses in many crops. Uses include pre-sowing as a grass killer in rice fields, as a pre-harvest desiccant in bean fields, and for inter-row weed elimination in sweet potato fields [2]. Paraquat is highly toxic to humans with an LD_{50} of approximately 3-5 mg kg^{-1} [3], and a small amount of oral ingestion can be fatal since there is no antidote. In fact, paraquat has exhibited energy-dependent accumulation into the lungs of mammalians including rats, dogs, monkeys, rabbits, and humans [4, 5]. Ingestion of this herbicide has morbidity and mortality rates (60%-80%) that are substantial due to multi-organ failure and pulmonary fibrosis with respiratory failure [6]. Many agricultural countries around the world have banned or restricted this herbicide, but Thailand has not. Therefore, a host of health problems and deaths continuously occur among Thai farmers and their families who use it in unsafe concentrations without adequate protective gear [7-9]. This fact suggests the importance of monitoring paraquat residue that pollutes the environment so that farmers can be notified and helped to prevent health risks posed by the residue.

Several conventional techniques have been utilized for paraquat investigation of environmental samples. These techniques include spectrophotometry [10], liquid chromatography [11], gas chromatography [12], and capillary electrophoresis [13] coupled with automatic systems or systems of ultra-high-performance detection. However, these techniques have problems that include high cost, large size, portability, excess amounts of time consumption, and/or complicated operation steps. Therefore, many publications have focused on overcoming these limitations, and the techniques they have introduced have become significantly popular.

One of the strategies to solve these problems has been the use of light-emitting diodes (LEDs) that have miniaturized analytical instruments and promoted their portability. LEDs possess unique properties that include low cost, small size, a broad range of emitted wavelengths, and a response that is stable and quick [14]. During the past few decades, many designs have been introduced for compact detection units using LEDs as a light source and/or as detectors with different wavelengths that range from UV to IR regions. For instance, Kim and co-workers employed a UV-LED emitting at 280 nm as an excitation source to monitor organic compounds in water [15]. Buah-Bassuah et al. used an LED with a 365 nm emission in fluorometry to study the chlorophyll content in the leaves of fruit [16]. Chuntib and Jakmunee utilized a red LED as a light source coupled with a flow system for paraquat determination in environmental water [17], but the system consisted of pumps, a PC, and a detector that diminished its portability. De Lima constructed

a portable photometer unit using two IR-LEDs (1,300 nm and 1,689 nm) as light sources that could be used to investigate aromatic hydrocarbons in water [18].

For environmental applications in developing countries like Thailand and other locations in Southeast Asia, a portable and inexpensive detection unit is needed since agricultural areas tend to be remote locales where farmers have difficulty acquiring and using expensive and bulky instruments. Thus, an inexpensive portable device that could immediately provide easily interpreted results for farmers would be effective in helping them to prevent exposure to hazardous chemicals. Therefore, we have developed a completely portable photometric detection unit using paired LEDs as a light source and a light detector that can be operated by three rechargeable batteries in a closed box. To the best of our knowledge, this is the first report of paired LEDs in a detection unit that can be operated using only three dry-cell batteries as the power supply. The present photometer has provided promising results with good reproducibility and sensitivity in the determination of paraquat in both standard samples and spiked real samples. In terms of precision, accuracy, limits of detection, and limits of quantification, the performance of the photometer was investigated under optimized analytical conditions.

2. Materials and methods

2.1 PEDD detection system setup and instrumentation

Figures 1A and 1B display a photograph and the schematic diagram, respectively, of portable paired light-emitter detector diodes (PEDD) [19, 20] operated by rechargeable dry-cell batteries. The whole system requires only three 9 V dry cell batteries for operation. The total size of this portable device is approximate 18×20 cm, which is sufficiently small and convenient to allow portability and on-site application. Orange LEDs with a diameter of 5 mm (609 nm) served as both light source and detector. Some LEDs were purchased from DiCUNO JP Direct (Tokyo, Japan), and others were from Kaitodenshi acquired through Amazon, Japan. Constant voltage was supplied to the LED light source from an adjustable voltage station (Drok, Hong Kong) interfaced with rechargeable Li-Po batteries (~9 V, 800 mAh, Keenstone Ltd., CA, USA), which were purchased through Amazon, Japan. The specifications of the LEDs appear in Table S1 (Supplementary 1, Supplementary Materials). The LED detector was connected to an amplifier unit powered by two rechargeable batteries similar to those used for the LED light source. The PEDD detection system required two lenses to focus light (SODIAL lenses, 2.2 × 1.4 cm, 95% transmittance), and these were purchased through Amazon, Japan. A multimeter in DC voltage mode (TDE-14, Trusco Nakayama Co., Tokyo, Japan) was used to measure the photovoltaic power generated by the LED detector. The detection unit was fabricated in-house using aluminum plates and an electronic circuit that created an operational amplifier similar to that used in our previous work [21]. The total price for all components was approximately 10,000 Yen, which amounts to around 90 US dollars. A UV-Vis spectrophotometer (UV–

2400PC, Shimadzu, Kyoto, Japan) was used to measure the absorption spectrum of the paraquat radical and to study the stability of sodium dithionite. A spectrofluorometer (RF-5300 PC, Shimadzu, Kyoto, Japan) was used to measure the emission spectra of the LEDs.

2.2 Chemicals and reagents

All chemicals and reagents either were of analytical grade or were certified reference materials except for cooking oil that was purchased at a local market. Six herbicides including paraquat ($C_{12}H_{14}Cl_2N_2 \cdot xH_2O$), diquat ($C_{12}H_{12}Br_2N_2 \cdot H_2O$), atrazine ($C_8H_{14}ClN_5$), glyphosate solution ($(HO)_2P(O)CH_2NHCH_2CO_2H$), propanil ($C_9H_9Cl_2NO$) and 2,4-D ($Cl_2C_6H_3OCH_2CO_2H$), and sodium dithionite ($Na_2S_2O_4$) as a reducing agent were purchased from Sigma-Aldrich (MO, USA). Sodium hydroxide, methanol, acetonitrile, N,N-dimethylformamide (DMF), and phosphoric acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). Ethanol was from Nacalai Tesque, Inc. (Kyoto, Japan), chloroform was from Katayama Chemical (Osaka, Japan), dimethyl sulfoxide (DMSO) was from Kanto Chemical Co., Inc. (Tokyo, Japan), and sodium 1-heptanesulfunate was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The ultra-pure water system was from Millipore Direct-Q (Millipore Co. Ltd., Molsheim, France).

2.3 Preparation of stock solutions

A stock solution of paraquat (500 mg L^{-1}) was prepared by dissolving an appropriate amount in 50 mL of water with storage at 4 °C until use. Stock solutions of sodium dithionite were freshly prepared at a concentration of 10 mmol L^{-1} in a 100 mmol L^{-1} NaOH solution and in different solvents to study the stability. The solutions were stored in 30 mL glass bottles with N_2 purging. Stock solutions of NaOH were prepared at concentrations of 1 and 5 mol L^{-1} in water. Stock solutions (1,000 mg L^{-1}) of atrazine and propanil were prepared by dissolving them in MeOH and EtOH (50(v/v)%), respectively. Stock solutions (1,000 mg L^{-1}) of diquat and 2,4 D were prepared in water to a final volume of 25 mL. Stock solutions of herbicides and the commercially available glyphosate solution (1,000 mg L^{-1}) were employed for the interference study.

2.4 Validation

Linear range, limits of detection (LOD), limits of quantification (LOQ), accuracy and intra- and inter-day precision were investigated to assess the analytical performance of the developed PEDD-based photometer. A stock solution of paraquat was diluted to 2.0, 5.0, 10.0, 20.0, and 40.0 mg L^{-1} with 100 mmol L^{-1} of NaOH (pH 13), and a small amount of sodium dithionite powder was added to the prepared standard solutions to construct a calibration curve for a paraquat radical. The LOD and LOQ are defined as $\frac{3.3\,S_{y/x}}{A}\sqrt{1+h_0+\frac{1}{I}}$ and $\frac{10\,S_{y/x}}{A}\sqrt{1+h_0+\frac{1}{I}}$, where $S_{y/x}$ is the residual standard deviation, A is the slope

of the univariate calibration graph, h_0 is the leverage for a blank sample, and I is the number of calibration samples, as suggested by Olivieri [22]. The definitions used for LOD and LOQ were recommended by the International Union of Pure and Applied Chemistry in 1995 [18]. Values for intra- and inter-day precision were reported in terms of the relative standard deviations (%RSD), which were evaluated by comparing the slopes of the calibration curves obtained in both the same day (n = 3) as well as on different days (n = 5), respectively. A sample recovery study demonstrated the accuracy of our developed method using the equation $\%\text{Recovery} = \frac{S_2 - S_1}{S_0} \times 100\%$, where S_0 is the concentration of the spiked standard (10 mg L^{-1} paraquat), S_1 is the concentration of paraquat found in a non-spiked sample, and S_2 is the concentration of paraquat found in the spiked sample.

2.5 Water collection and preparation

Water samples were collected from 3 locations consisting of 1) water from the Asahi River that supplies rice fields, Okayama, Japan (sample W1-W3); 2) water from a rice field in Kurashiki city, Okayama, Japan (sample W4-W8); and, 3); and, water from a rice field in Khuan Khanun, Phatthalung province, Thailand (sample W9-W13). The preparation of the water samples included filtration with a cellulose acetate syringe filter (pore size, 0.2 µm) followed by the addition of 40 µL of 5 mmol L^{-1} NaOH into 1,960 µL of the filtrates for pH adjustment (pH 13). After the pH adjustment, sodium dithionite was added into the solution for the determination of paraquat.

2.6 Extraction of paraquat from soil samples by digestion

The procedures for soil digestion were adapted from two methods reported by Roberts et al. [23] and T. Pérez-Ruíz and J. Fenoll [24]. First, a soil sample was heated at 100 °C for drying, and then 20 g of the soil was refluxed with H_2SO_4 (6 mol L^{-1}, 20 ml) using a mantle heater at a voltage of 80 V for 6 hours. The digested solution was filtered, followed by an adjustment of the pH to ~9 via the addition of NaOH tablets. The solution was filtered in order to remove precipitates that appeared after adjustment of the pH. The filtrate was passed through a cationic exchange column (HyperSep™ SCX Cartridges) to retain the paraquat. Finally, the paraquat was eluted from the column with saturated NH_4Cl (4 mL) followed by NaOH (2.5 mol L^{-1}, 2 mL). A 1 mL-aliquot of the extract was taken for HPLC analysis and the residual solution was employed for the analysis by our developed system after adjusting the pH to ~13 with 5 M NaOH. The yield of the extraction ranged from 56-67%, which was determined using soil samples spiked with a known amount of paraquat (refer to the details in Supplementary 2).

2.7 Determination of paraquat in an artificial rice field

An artificial rice field was constructed in a rectangular plastic box (size 11.5×14.5 cm) containing water (800 mL) on a soil layer (4 cm height) to allow the daily monitoring of the concentration of paraquat

sin both the water and soil. Initially, a standard solution of paraquat (100 mg L^{-1}, 200 ml) was spiked into the rice field. The concentration of the paraquat in the water was immediately determined after spiking and was assigned as the result for the "Day 1". Water samples were taken from the water layer for the test from Days 1 to 4 whereas a soil sample was tested on Day 4 when no paraquat was found in the water sample. The water samples were measured by our developed method after the preparation mentioned in Section 2.5 whereas the soil samples were extracted as mentioned in Section 2.6.

2.8 Determination of paraquat in water from rice fields via standard methods

High-performance liquid chromatography via UV-Vis detection was used as a standard method for determining paraquat concentrations. The chromatography system consisted of a 321 pump (Gilson, WI, USA) connected with a Rheodyne 7125 valve (20 µL sample loop) and a SPD-6AV UV-Vis detector (Shimazu, Kyoto, Japan). Paraquat was separated on a reversed-phase column (Inertsil^{TM}, ODS-2.5 µm, 4.6×150 mm, GL Sciences, Tokyo, Japan) using an isocratic elution of 20% MeOH containing 200 mmol L^{-1} phosphoric acid, 0.1 mol L^{-1} diethylamine, and 12 mmol L^{-1} sodium 1-heptanesulfonate, as reported by Hara et al. [25]. The paraquat was then detected via UV absorbance at 200 nm. The flow rate was set at 0.5 mL min^{-1} with ambient column temperature.

3. Results and discussion

3.1 Optimization of the reaction conditions

To complete the reduction of paraquat by sodium dithionite, a sufficient amount of reducing agent must be added to sample solutions. To find the optimum amount of sodium dithionite, the paraquat concentration was fixed at 38.89 µmol L^{-1} (10 mg L^{-1}) and a stock solution of sodium dithionite (20 mmol L^{-1}) was added to achieve concentrations of 20; 40; 200; 400; 600; 850; 950; 1,000; 1,950; 3,900; 7,800; and, 19,500 µmol L^{-1}, which represented molar ratios of 0.5, 1, 5, 10, 15, 22, 24, 26, 50, 100, 200, and 500, respectively. The relationship between the molar ratio and the absorbance of a paraquat radical, as measured by a conventional spectrophotometer is shown in Figure 2. Interestingly, the absorbance of a paraquat radical was suddenly increased up to a molar ratio of ~26 and then maintained a constant value to a molar ratio of 500. At a molar ratio of ~24, paraquat turned to a blue color, but the color immediately disappeared due to oxidation of the radical because of the depletion of the dithionite consumed by the atmospheric oxygen [26] during the mixing process. This result suggested that a paraquat radical without an excessive amount of sodium dithionite is easily decomposed by oxygen. Therefore, the excess sodium dithionite played an important role in obtaining a stable signal.

3.2 Optimization of the portable PEDD-based photometer

The developed PEDD-based photometer is completely portable and operates with no power cable, as shown in Figure 1. The parts of the photometer including the adjustable voltage station, in-house aluminum plate holder, two lenses, and amplification unit were arranged in an aluminum box. Only three rechargeable small dry-cell batteries (~9 V) were needed to operate all systems of the device, because the LEDs and the amplification unit require only low operation voltages. As mentioned in our previous work [21], rechargeable batteries play an important role in obtaining reproducible results. The emitted wavelengths of the LEDs for light source/detector and the operational voltage of the LED light source were investigated for the provision of good sensitivity and linearity.

An optimal LED was selected based on the overlap between the emission spectra of the LEDs and the absorption spectrum of a paraquat radical. The chosen version achieved its maximum wavelength at 603 nm, as shown in Figure S1 (Supplementary 3). Based on the results, the orange LED acquired from the DiCUNO company (λ_{max} = 609 nm) was the most suitable for both emitter and detector since the absorption maximum of a paraquat radical most closely approximated its emission wavelength. The LED detector is, in general, sensitive to light with the same, or higher, level of energy as that of its emission [27]. Therefore, to obtain better sensitivity, various LEDs that emit at wavelengths of 562 nm, 609 nm, 616 nm, and 648 nm were used as light detectors, and a fixed LED light source emitting at 609 nm was selected in this work. Although the red LED (λ_{max} = 648 nm) provided the best sensitivity, the linear range (1–20 mg L^{-1}) was narrower than the orange LED (λ_{max} = 609 nm) (2–40 mg L^{-1}).

The voltage applied to the LED emitter was varied at 1.8, 2.0, 2.2, 2.5, and 3.0 V by using an adjustable voltage device connected to one of the rechargeable batteries. When a high level of applied voltage provided intensity from the LED light that was sufficiently high to saturate the output signal of the LED detector, sensitivity was decreased. Conversely, a low level of applied voltage resulted in low intensity of the LED light that made it difficult to monitor changes in the photovoltaic signal, which affected the linearity characteristics (linear range and r^2), as shown in Table 1. To achieve a wider linear range and a good correlation coefficient (r^2 = 1), an applied voltage of 2.5 V was chosen for further study, although the sensitivity was slightly higher at 1.8 V.

3.3 Stability of sodium dithionite solution

3.3.1 Effect of Acidity

Sodium dithionite in solution was easily decomposed due to oxidation caused by the oxygen molecules dissolved in the solutions. Therefore, a reagent must be stabilized when applying the present device to the on-site analysis of paraquat. Many publications have reported that sodium dithionite is stable for only a few hours following exposure to moisture and O_2 [10, 17] that oxidizes sodium dithionite to hydrogen sulfite and hydrogen sulfate [28], as shown in Eq. (1).

$$Na_2S_2O_4 + O_2 + H_2O \longrightarrow NaHSO_4 + NaHSO_3 \quad (1)$$

Moreover, the rate of decomposition increases under acidic conditions, as mentioned in the Screening Information Dataset (SIDS) Initial Assessment Report [28]. Briefly, the decomposition processes under different acidities are shown in Eqs. (2) - (5).

- Strongly alkaline medium: $3Na_2S_2O_4 + 6NaOH \longrightarrow 5Na_2SO_3 + Na_2S + 3H_2O$ (2)
- Weakly alkaline to weakly acidic medium: $2Na_2S_2O_4 + H_2O \longrightarrow 2NaHSO_3 + Na_2S_2O_3$ (3)
- Acidic medium: $2H_2S_2O_4 \longrightarrow 3SO_2 + S + 2H_2O$ (4)
- Strongly acidic medium: $3\ H_2S_2O_4 \longrightarrow 5SO_2 + H_2S + 2H_2O$ (5)

Therefore, a strong alkaline condition (100 mmol L^{-1}, pH 13) was examined to prolong the stability of the sodium dithionite solution. As shown in Table 2, the alkaline condition enhanced the stability of sodium dithionite only for 4 hours, which is too short even for analysis at an equipped laboratory.

3.3.2 Effect of organic solvent

Another parameter that possibly affects the stability of sodium dithionite solution is water content, as mentioned in reaction (1). From reaction (1), we hypothesized that water would enhance the decomposition of dithionite. Thus, organic solvents including methanol, ethanol, acetonitrile, DMF, and DMSO (20(v/v)%) in NaOH (100 mmol L^{-1}) were examined as a solvent to dissolve sodium dithionite. Since dithionite was less soluble in an organic solvent, mixtures of water and an organic solvent were employed. The stability of sodium dithionite was investigated by mixing paraquat at 10 mg L^{-1} (38.89 µmol L^{-1}) with sodium dithionite (1,950 µmol L^{-1}) dissolved in different solvents and measuring the absorbance of the paraquat radical after 20 min using a conventional spectrophotometer. Table 2 shows that MeOH (20(v/v)%) was the solvent that best prolonged the stability of the reduction agent, at almost 7 hours. Further increases in the MeOH content of up to 60(v/v)% tended to dissolve sodium dithionite. However, we found that the stability of sodium dithionite was poorer at 60(v/v)% than at 20(v/v)% MeOH (data not shown). Therefore, we concluded that the water content may not be a significant parameter of the dithionite decomposition.

3.3.3 Effect of cooking oil

We successfully prolonged the effectiveness of the reducing agent from a few hours to several hours using an organic solvent, but it was still too short to achieve on-site analysis. The main parameter that causes the decomposition of the reducing agent is O_2 from air. Hence, to block the dissolution of O_2, a cooking vegetable oil available in a local market was added on the top layer of the dithionite solution. The dithionite solution was stored in a micropipette tip as shown in Figure S2 (Supplementary 4, Supplementary Material). The blocking of O_2 by an oil layer significantly improved the stability for as long as 2 days. The

improvement was brought about by a rate of O_2 diffusivity into oil (~10^{-10} m^2 s^{-1}) that is ten times slower than into water (~10^{-9} m^2 s^{-1}), as reported by Chaix et al. [29]. The stabilization of the reagent solution for 2 days was long enough for daily analysis in a laboratory, but further stabilization was still necessary for analysis in a remote area of a developing country such as Thailand.

3.3.4 Use of powder

As shown in Figure 2, excess amounts of sodium dithionite showed no influence on absorbance by the paraquat radical. This fact is advantageous for application to on-site analysis, because precise addition of the reagent is unnecessary. According to the results in Figure 2, the addition of the reagent at a molar ratio of more than 50 leads to a stable absorbance. Finally, we decided to add only the sodium dithionite powder directly into the sample solutions since the solid state of sodium dithionite is much more stable than the solution, and this form also is more amenable to on-site applications.

3.4 Analytical performance

The developed portable device was validated based on the parameters of linearity, LOD, LOQ, and precision (intra- and inter-day) according to the articles by Olivieri and Shrivastava et al. [22, 30]. The calibration curves were constructed by plotting absorbance calculated from the voltages for the blank and standard samples against the concentrations of paraquat. The linear range of the measured paraquat was 2.0 – 40 mg L^{-1} with good correlation coefficients of $r^2 > 0.999$ with values for LOD and LOQ of 0.5 and 1.5 mg L^{-1}, respectively. The precision obtained from %RSD of the slope of calibration curves was less than 1% for intra-day and less than 2% for inter-day measurements, which is lower than the acceptable value of 5% RSD. Therefore, the developed photometer showed good reproducible signals even on different days.

3.5 Interference study

The most popular herbicides used in Thailand are atrazine, propanil, diquat, 2,4-D, and glyphosate, and these were selected as possible interferences. These herbicides were individually mixed with 5 mg L^{-1} of a paraquat solution, with the exception of glyphosate, which was added into 2 mg L^{-1} of a paraquat solution due to the low concentration of a commercially available glyphosate standard solution. The interference study and reported concentrations of the herbicides in the environment samples are summarized in Table 3. The results show that only diquat interfered with the redox reaction due to a chemical structure that is similar to that of paraquat.

3.6 Investigation of paraquat in water samples from rice fields

All thirteen water samples collected in Japan (W1-W8) and Thailand (W9-W13) were prepared as mentioned in Section 2.5 before analysis using the developed portable device. Table 4 shows that no water samples contained paraquat even in the samples from Thailand, although the samples were collected from

fields where heavy utilization of paraquat was reported. The possible reasons for the results are as follows: 1) strong adsorption of paraquat by the soil [31]; 2) mineralization of paraquat by soil microorganisms [23]; 3) dilution of paraquat due to heavy rain in the days before the sample collection; and, 4) the length of time between spraying and sample collection, because the spraying of paraquat was in June-September while the samples were collected in October. These factors could possibly reduce the concentration of paraquat in the water of the fields to undetectable levels.

The proposed method was validated by sample recovery tests using water samples spiked with 10 ppm of standard paraquat followed by filtration with cellulose acetate membrane (0.2 μm pore size). The percentage of recovery ranged from 82.7±2.6 to 98.0±0.0%, which is acceptable for obtaining reliable values. In addition, the results from the proposed method were compared with the paraquat concentrations in water samples with and without a spike obtained by HPLC-UV detection. The results from HPLC also found no paraquat in all thirteen samples. Paraquat concentrations in the spiked samples were comparable in samples tested by both the portable photometer and HPLC, as shown in Figure S3 (Supplementary 5). These results prove that the accuracy of the proposed method is appropriate for application to on-site paraquat investigations of water samples.

3.7 Investigation of paraquat in water and soil samples from the artificial rice field

To verify that our methodology can be applied to paraquat analysis in rice fields, an artificial rice field was constructed as mentioned in Section 2.7. The concentrations of paraquat in the water samples during Days 1 to 4 were measured by our device and by HPLC, as shown in Figure 3. The paraquat content in the water was dramatically decreased from 22.2 mg L^{-1} to 2.1 mg L^{-1} within 3 days and no paraquat was found on Day 4. Hence, paraquat was extracted from the soil sample on Day 4 to confirm the adsorption of paraquat onto the soil. The soil sample was taken from the surface of the soil layer because the paraquat would have tended to localize on the surface of the soil layer [32]. The result showed that the soil sample contained paraquat at the concentration of 0.014±0 mg g^{-1} on Day 4 when the paraquat had completely disappeared from the water. These facts indicate that the device permits the on-site analysis of paraquat in water samples and provides a simple extraction method for soil samples when the paraquat content in soil also must be monitored in the field.

As seen in Figure 3, the results of the PEDD photometer were comparable with those of HPLC in terms of the obtained concentration and reproducibility. These results suggest that the PEDD photometer is reliable in the measurement of paraquat in both water and extracts from the soil. Therefore, the PEDD photometer would be applicable to the monitoring of paraquat in the field without the need of an extra power supply.

4. Conclusions

A completely portable photometer operated using only three rechargeable dry-cell batteries was developed and applied to the analysis of paraquat. Sodium dithionite is a reductant reagent that was needed to produce a colored paraquat radical, but it proved unstable under atmospheric conditions in solution. Therefore, the reagent solution required stabilization before it could be applied to on-site analysis. An adjustment of pH and the addition of an organic solvent enhanced the stability of the reagent solution for several hours. A simple and inexpensive alternative method that involved the formation of an oil layer on top of the reagent solution extended the stability to two days by reducing the oxygen diffusion rate. Further stabilization was necessary for on-site analysis, however, since the reagent must be transported to remote locations. Finally, a solid form of the reagent was directly added to the sample solutions, because sodium dithionite is more stable in the solid state and absorbance of the paraquat radical was not influenced by an excess amount of the reagent. The proposed portable photometer showed good analytical performance in terms of linearity, precision (intra- and inter-day), LOD, LOQ, and accuracy (% recovery). The proposed method is reliable and suitable for on-site paraquat determination, which was certified by the results of HPLC.

5. Acknowledgements

This research was supported by JSPS KAKENHI Grant Numbers JP17H05465, JP19H04675, and JP20H02766. SS received funding from a Japanese government scholarship (Monbukagakusho: MEXT). The authors would like to thank Mr. Motonari Kobayashi (The Division of Instrumental Analysis, Department of Instrumental Analysis & Cryogenics, Advanced Science Research Center, Okayama University) for his gracious and excellent help in fabricating the holder for the photometer. We thank Mr. Sahapong Somwong for his suggestions concerning the electronic device and circuitry. Sample collection in Kurashiki, Okayama, Japan and in Khuan Khanun, Phatthalung, Thailand succeeded with help from Mr. Tetsuya Fujita, Mr. Aummorn Sukwin, Mr. Adual Keawkongtham, and from the Katkhaw, Ritboon and Matitham families.

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418 **Table 1** Linearity characteristics at different levels of voltage applied to the LED light source

Applied voltage (V)	Linear equation*	Correlation coefficient (r^2)	Linear range (mg L^{-1})
1.8	A = 0.0032C – 0.0047	0.9700	0.5 – 30
2.0	A = 0.0023C – 0.0015	0.9950	0.5 – 40
2.2	A = 0.0019C – 0.0005	0.9997	0.5 – 40
2.5	A = 0.0018C – 0.00003	1.0000	0.5 – 40
3.0	A = 0.0018C + 0.0005	0.9999	0.5 – 40

419 *A = Absorbance, C = Concentrations of paraquat in units of mg L^{-1}

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424 **Table 2** Storage time of sodium dithionite in different solvents and the absorbance of paraquat radical at
425 603 nm

Solvent	Storage time	Absorbance
NaOH (100 mmol L^{-1})	4 hours	0.49±0.00 (0.71%)
MeOH (20% v/v)	6 hours 40 minutes	0.50±0.00 (0.80%)
EtOH (20% v/v)	6 hours	0.50±0.00 (0.72%)
ACN (20% v/v)	4 hours 40 minutes	0.49±0.01 (1.7%)
DMF (20% v/v)	5 hours 40 minutes	0.49±0.01 (1.5%)
DMSO (20% v/v)	5 hours 40 minutes	0.50±0.00 (0.61%)

426

Table 3 Study of interference

Herbicide	Limited concentration in several sample [a]	Tolerated limit	
		Concentration (%Recovery ± S.D.)[c]	Ratio to the paraquat concentration
Atrazine	150 µg L^{-1} in ground water	400 mg L^{-1} (97±5)	80
Propanil	0.5 mg L^{-1} in river water	More than 500 mg L^{-1} (97±4)	More than 100
Diquat	0.07 mg L^{-1} in drinking water	6.5 mg L^{-1} (106±5)	1.3
2,4 D	45 mg L^{-1} in river water	More than 500 mg L^{-1} (100±0)	More than 100
Glyphosate [b]	4.8 mg L^{-1} in river water	More than 200 mg L^{-1} (100±0)	More than 100

[a] The limitation of concentration of the herbicides in the environment sample were reported in the reference [33-35]

[b] Paraquat concentration was fixed at 2 mg L^{-1}

[c] Percentage recovery of paraquat after adding interference at a tolerated concentration

Table 4 Investigation of paraquat and recovery study in water samples

Sample	Paraquat concentration ± S.D. (%RSD) (mg L^{-1})			Percentage recovery± S.D.
	Amount found in non-spiked sample	Standard spike	Amount found in spiked sample	
W1	< LOQ	10	9.7±0.2 (2.5%)	96±2
W2	< LOQ	10	9.4±0.0 (0.0%)	93±0
W3	< LOQ	10	9.5±0.2 (2.5%)	95±2
W4	< LOQ	10	9.4±0.0 (0.0%)	94±0
W5	< LOQ	10	9.4+0.0 (0.0%)	94±0
W6	< LOQ	10	8.9±0.0 (0.0%)	89±0
W7	< LOQ	10	9.9±0.0 (0.0%)	98±0
W8	< LOQ	10	9.7±0.3 (2.6%)	97±3
W9	< LOQ	10	10.5±0.0 (0.0%)	105±0
W10	< LOQ	10	9.4±0.0 (0.0%)	94±0
W11	< LOQ	10	9.4±0.0 (0.0%)	94±0
W12	< LOQ	10	9.9±0.3 (3.1%)	97±3
W13	< LOQ	10	10.0±0.0 (0.0%)	100±0

434 **Figure Legends**

435 **Figure 1** Photograph (A) and schematic diagram (B) of the portable paired light-emitter detector didoes
436 (PEDD) detection device operated by rechargeable dry cell batteries connected with a multimeter

437

438 **Figure 2** Absorbance of paraquat radical at different mole ratios between sodium dithionite and paraquat.
439 Wavelength, 603 nm; the concentration of paraquat, 10 mg mL^{-1}.

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441 **Figure 3** Paraquat content in water and soil samples obtained from the artificial rice field. White and gray
442 bars indicate the level of paraquat analyzed by the PEDD photometer and HPLC, respectively. Error bars
443 indicate standard deviations (n=3). The results on Days 1 to 3 were obtained from the water samples
444 whereas the result on Day 4 is from the soil sample.

445

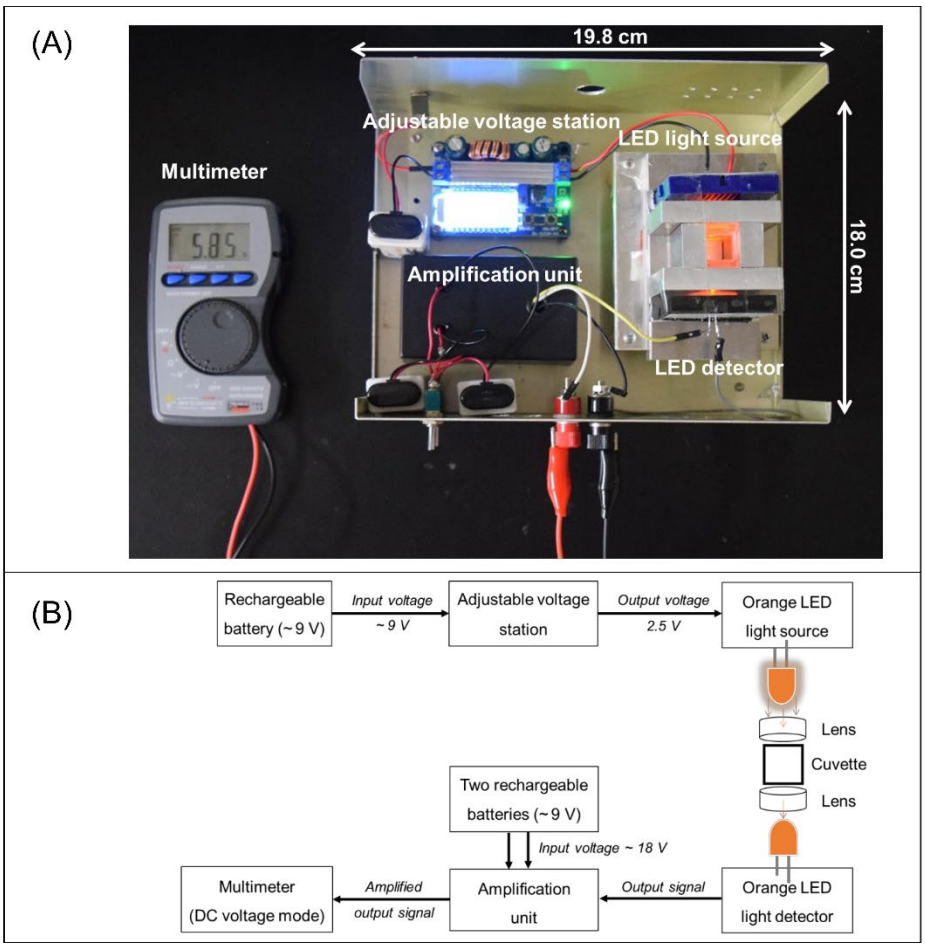
(A)
19.8 cm
Adjustable voltage station
LED light source
Multimeter
18.0 cm
Amplification unit
LED detector
(B)
Rechargeable battery (~ 9 V)
Input voltage ~ 9 V
Adjustable voltage station
Output voltage 2.5 V
Orange LED light source
Lens
Cuvette
Lens
Two rechargeable batteries (~ 9 V)
Input voltage ~ 18 V
Multimeter (DC voltage mode)
Amplified output signal
Amplification unit
Output signal
Orange LED light detector

Figure 1

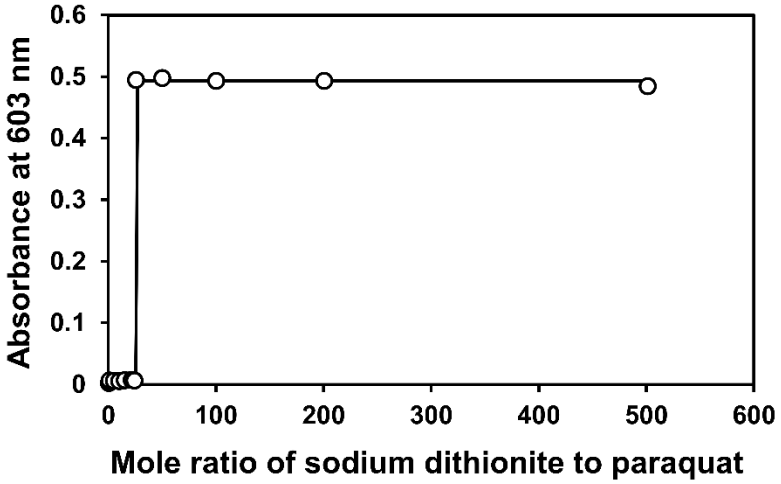
Absorbance at 603 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Mole ratio of sodium dithionite to paraquat

Figure 2

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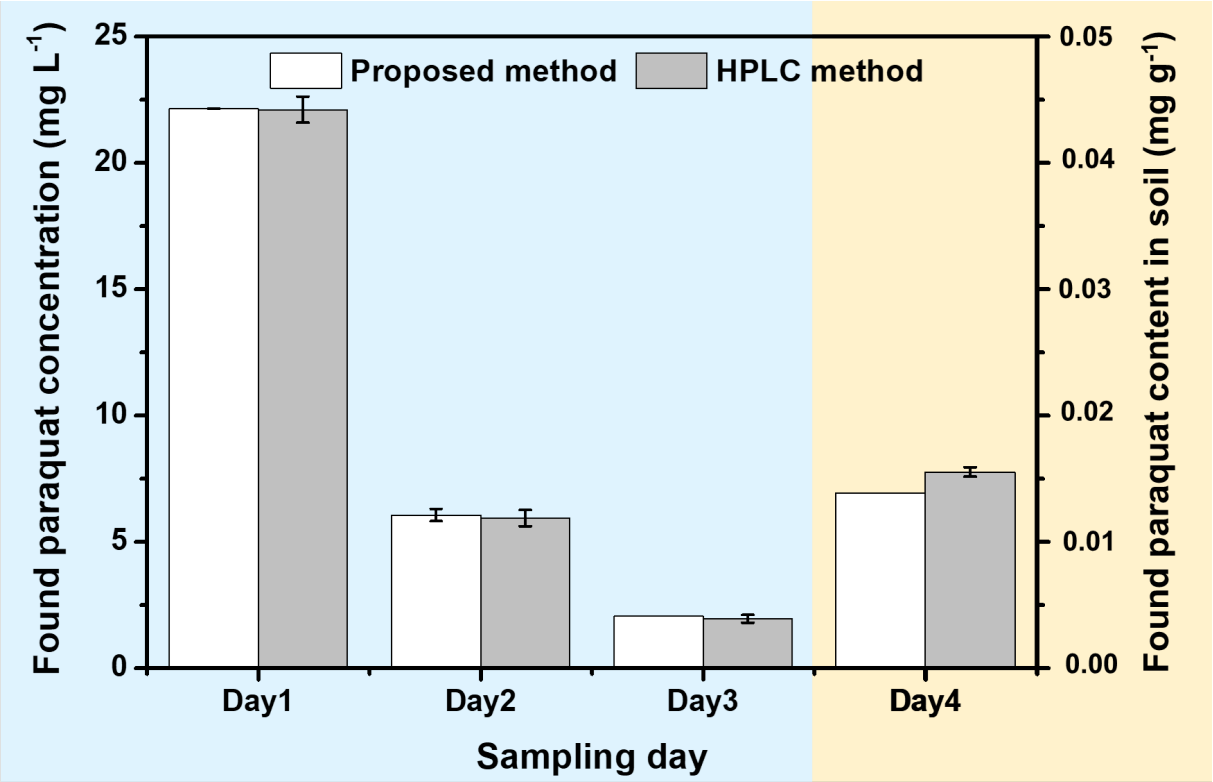

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465 Figure 3

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