CHAPTER 4

COLORIMETRIC-BASED SENSING FOR DETECTION AND QUANTIFICATION OF PHOSPHATE IN WATER AND SOIL ON A MOBILE DEVICE

In this chapter, the design of a smartphone colorimetric sensing is discussed to detect and analyse of phosphate level in water and soil medium. The introduction section illustrates the importance of monitoring phosphate in different media and the conventional techniques available for such purpose. The chapter further provides the introduction to colour spaces that has been used in the present sensing work. The practicability of the designed sensor has been assessed by estimating the phosphate level of field-collected water and soil samples via extracting the V-channel of HSV colour space of the captured sample's images and correlating it with the sample's phosphate content with the help of android applications. The accuracy of the device has been evaluated by comparing the results with the standard laboratory grade tool. Finally, the important sensoristic parameters of the designed smartphone-based colorimetric sensing tool have been thoroughly analysed and discussed.

4.1 Introduction

The primary macronutrients namely nitrogen, phosphorus, and potassium have a significant impact on agricultural productivity and quality. Among them phosphorus is an important element for the growth of plants. It has a huge impact on the productivity and health of terrestrials as well as aquatic live [1]. The phosphorus available in most native soils is usually present in various forms for plants to uptake.

It is often present in one of the three forms: orthophosphates, condensed phosphates (pyro-, meta- and poly-), and organic phosphorus [2]. Phosphate is an essential nutrient that provides energy to plants and drives most of the biochemical processes. However, in many agricultural fields, phosphorus-containing fertilizers are used in large amounts to compensate for the low phosphorus availability in the soil. The over-fertilization may lead to nutrient leaching, water pollution, and irreversible harm to aquatic lives [3, 4]. According to the World Health Organization (WHO), the permissible phosphorus concentration limit in drinking water is 0.1 mg/L [5], while the allowable phosphorus limits in natural and wastewater are 0.2 and 10 mg/L, respectively [6]. The monitoring of phosphorous levels in water and agricultural soil is therefore critical for maintaining the water quality and soil fertility. There are several methods to estimate phosphorous concentration such as spectroscopic, colorimetric, and electrochemical techniques, among which colorimetric analysis is widely employed. For colorimetric analysis different color spaces are being used to analyze the parameter of interest. A detailed background of the colorimetric analysis technique is discussed in the subsection below.

4.1.1   Introduction to colorimetric technique

The colorimetric technique is primarily based on and quantifying the amount of coloured compounds present in a solution [7]. Colorimetric analysis is a well-known analytical technique for determination of concentration of analytes present in a solution. A common analytical method mostly use for determining the concentration of analytes in a solution is colorimetric analysis. For the quantification of a wide range of biomolecules [8], harmful compounds for the environment [9], and macro organisms [10], colorimetric techniques are widely used. One of the simplest ways to detect parameter of interest in resource-constrained environments is the colorimetric detection viewed through our naked eye. Our visual reaction is stimulated by light, a region of the electromagnetic spectrum that ranges from 350 nm to 780 nm. Rod and cone cells, collectively known as photoreceptors, are found in the retina of the human eye [11]. The tristimulus response of these cone cells under varying brightness circumstances causes the human eye to perceive color. Although the human eye can easily distinguish between variations in color intensities, it also has some degree of uncertainty in color perception. Due to individual differences in how the human eye perceives color stimuli, visual approaches employed for colorimetric sensing are prone to errors. Additionally, the human eye's limited sensitivity to fractional color change and the absence of uniform sample lighting can result in considerable error in a variety of color-governed analytical and bioanalytical procedures.

4.1.2 Realization of the need of a smartphone-based colorimetric device

There are numerous methods for estimating the concentration of phosphorous in various sources. These include spectroscopic, colorimetric, and electrochemical techniques. All these approaches, in general, are laboratory-confined and involve equipment (spectrophotometers, flame photometers) that are costly, skill-intensive, and not suitable for autonomous operation in the field [12, 13]. Owing to the simplicity and ease-of-use, the colorimetric method is one of the popular and widely used approaches for such a purpose. However, the need for expensive, bulky, and sophisticated instruments limits their use for in-field applications. Electrochemical sensing is another widely used technique for the detection of chemical content in environmental samples. The majority of electrochemical sensors have been developed to detect nutrients, toxins, and pollutants in water and aqueous solutions [14–17]. Ion Selective Electrodes (ISE) method is also popular for rapid analysis of soil nutrients in unfiltered soil extract and naturally moist soils via electrochemical procedure [18]. The need for frequent calibrations of ISE is one of the major disadvantages of the technique as it requires additional soil extracts [19] and the measurement for accurate nutrient readings depends on the soil moisture. Li et al. [20] have reported the fabrication of a phosphate ion-selective electrode (ISE) using molybdenum and hydrogen phosphate ions $HPO_4^{2}-$ for quantitative analysis of phosphate in water. However, due to ageing effect, the electrodes can perform reliably up to three months and hence requires frequent change of the electrode. Besides, the electrodes have to rinse frequently for effective electrochemical sensing, which makes the process time-consuming. To avoid the limits of the above-mentioned laboratory-based tests and to reduce its size and cost, several groups have reported various smartphone based colorimetric methods for monitoring of different elements in water and soil. The smartphone-based colorimetric detection technique has emerged as a promising platform for sensing chemical and biological parameters as it provides an affordable, compact, and user-friendly sensing platform. Various groups across the globe have utilized the embedded sensors of the phone for sensing and imaging [21, 22], environmental [23], and agricultural [24, 25] parameters monitoring purposes. The ever-improving built-in camera of the phone with its advanced hardware and processing technologies enable researchers to develop alternative platforms that are truly reliable, hand-held, and user-friendly. Recently, several smartphone-based soil and water quality monitoring systems have been reported. In the reported work by Siddiqui et al. [26] a smartphone was used for soil sample preparation and detection of arsenic As(III) from the contaminated soil. The quantification of As(III) concentrations of soil samples was done by correlating the ratio of green to red values (G/R) that were extracted from the RGB

pixel values of the captured images with the target analyte's concentration. By determining the RGB values of the digital photo images of the soil samples taken by the camera phone, J. Jakmunee et al. [27] have developed a colorimetric analyzer to monitor available phosphorus in the soil. The RGB (Red, Green, and Blue) color index and HSV (Hue, Saturation, and Value) color models are primarily used for soil nutrients detection [28]. Considering the advantages of color spaces that are used for phone-based colorimetric analysis, present work discusses the utilization of V-space of HSV color space for the sensing of phosphate in the sample. The designed sensor converts the modulated signal in the form of color information which subsequently has been used to quantify the parameter of interest in the present study. By using two freely available android applications, the experimental data of the sensor have been analyzed within the phone itself thus, making the designed tool a truly standalone platform. The applicability of the proposed sensor has been evaluated by estimating the phosphate concentrations of different field-collected water and soil samples.

4.2 Materials and methods

4.2.1 Sample preparation

Prior to the commencement of the experimental investigations, all the glassware were washed with ethanol and distilled water to remove any possible contaminations from it. The standard ascorbic acid method has been used to detect phosphate concentration in different media. In this protocol, ammonium molybdate and potassium antimonyl tartrate react in the presence of sulfuric acid with orthophosphate to form a heteropoly acid-phosphomolybdic acid, which subsequently reduces to an intensely colored molybdenum blue solution upon treatment with ascorbic acid [29]. The intensity of the blue color is proportional to the phosphate concentration present in the sample. Based on this principle, the phosphate concentration can be quantified by cohorting it with the sample's chemically produced color. For the preparation of the phosphate stock solution, 21.96 mg of monopotassium phosphate (KH_2PO_4) has been dissolved in 100 ml of distilled water where 1 ml of the solution contains 219.6 μg of KH_2PO_4. 500 ml of standard phosphate solution has been prepared by diluting 60 ml of stock phosphate solution to 400 ml of distilled water where 1 ml of standard solution contains 6 μg/L of phosphate. Different concentrations of ten phosphate samples in the range 0.1–1 mg/L with a step increment of 0.1 mg/L and other nine samples with phosphate concentration in the range 1–5 mg/L with a step increment of 0.5 mg/L have been prepared from the stock sample by diluting it with distilled water. These standard phosphate samples were used to obtain the calibration curve for the designed sensor.

4.2.2 In-field sample collection and extraction procedure

Water samples from ten different sources of Sonitpur district, Assam, India, and twelve soil samples were acquired from the Department of Environmental Science of Tezpur University. The soil samples were air-dried and sieved to obtain a small (<2 mm) particle size for further use. Bray II solution for extraction of phosphorus from soil was prepared by mixing 20 ml of 0.5 mol/L HCl and 3 ml of 0.1 mol/L NH_4F and diluted to a volume of 100 ml with water to make the final concentrations 0.03 and 0.1 mol/L, respectively. Soil extracts were prepared by shaking 1 gm of soil sample with 7 ml of Bray II solution and left to stand for 10 min [30, 31]. The soil extract from each sample was filtered out with Whatman filter paper (grade2). Following this step, the photo images of these samples were captured by the designed sensor.

4.2.3 Spectrophotometric analysis of phosphate

A batch spectrophotometric procedure has been adopted to analyze the peak absorbance wavelength of the prepared phosphate samples. Five phosphate samples with concentrations 0.8, 1.0, 1.5, 3, and 5 mg/L have been prepared following the standard ascorbic acid method [29]. 10 ml of each standard phosphate sample solution was mixed with 1.6 ml of the combined reagent in test tubes. The mixed solutions were shaken and left to stand for 10 min. The samples gradually turned into blue color.
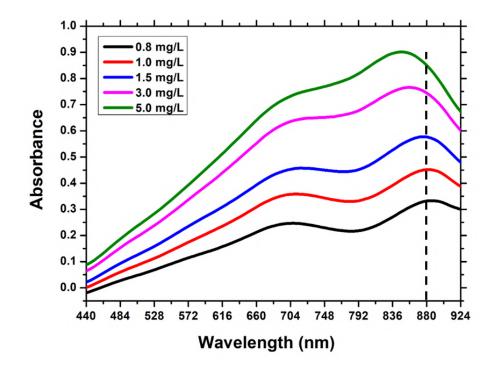

Figure 4.1: Absorbance spectra of reagent treated phosphate samples recorded with laboratory-grade spectrophotometer.

The absorbance spectra of these samples were recorded with a standard laboratory-grade spectrophotometer (UV-3600 Plus UV–Vis-NIR Spectrophotometer, Shimadzu). The characteristic spectra of the samples are shown in figure 4.1. The peak absorbance wavelength of the samples was observed at 880 nm wavelength. At this peak wavelength absorption condition, the phosphate concentration estimated by the standard spectrophotometric method has been compared with the results obtained from the designed smartphone sensor.

4.2.4 Design of the smartphone-based sensing system

In the present work, colorimetric based sensing has been adopted for detection of phosphate by analysing the captured images of the reagent treated coloured phosphate samples. In order to capture the image of the transmitted modulated signal from the cuvette, the camera position need to be aligned parallel with the cuvette position in the setup. The sensing system involves in the present work is a smartphone-based opto-mechanical set-up that consists of a 3D printed attachment. The design of the attachment has been developed on computer aided design software (ZW3D) and subsequently fabricated it in a 3D printer (Raise 3D N2). The set-up houses the optical components and can be attached easily to the smartphone as a plug and play device. It consists of an external white LED which has been powered from the phone's internal battery through a USB OTG cable. To collimate the light beam, a plano-convex lens (focal length of 11 mm, 7 mm diameter, and product id. 32–404, Edmund Optics) has been placed in the path of the optical source followed by a nylon sheet diffuser (thickness 5 mm) to avoid sharp illumination over the sample. The sample has been placed in a plastic cuvette (optical path length 12.5 mm) and is placed at a distance of 2 cm from the diffuser. The image of the transmitted modulated signal from the sample has been captured by the CMOS sensor (16 MP, 1920 × 1080 pixels) of the phone (Samsung Galaxy C9 Pro).

The schematic of the designed sensing system and the photo image of the prototype smartphone sensor are shown in figure 4.2(a) and 4.2(b), respectively. By using the diffuser in the optical set-up, one can avoid the V-value of the captured image of the sample from being saturated and thus, provides a fairly linear variation of the values with the change in phosphate concentration in the sample. The weight of the whole set-up, including the smartphone, is approximately 200 gm, making it very light-weight and field-portable sensing platform. To avoid any possible interference due to ambient light condition on the sensor's data, the set-up has been optically shielded.
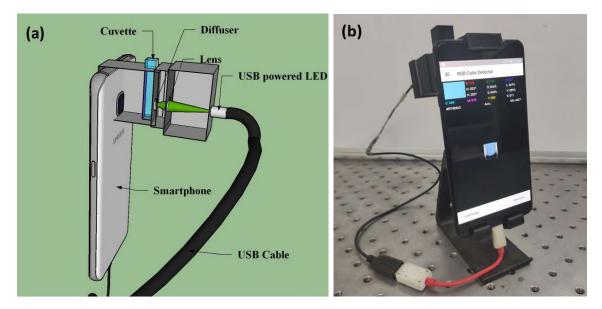
Figure 4.2: (a) Schematic diagram and (b) photograph of the designed sensor.

4.2.5 Android applications used for analysis

Two freely available android applications, 'The RGB color detector' and 'StanXY' from the Google play store, have been used to detect and analyze the phosphate concentrations of the samples. The process flow of phosphate detection using these two android applications is described in figure 4.3. By using the 'RGB Color Detector' application, the color information of the captured images is first converted to RGB and then to HSV color spaces. The V channel of HSV color space can be derived from RGB channel [32] using the following relation,

$$V = \frac{\frac{1}{3} \times (R + G + B)}{255} \tag{4.1}$$

where, R, G and B represents red, green and blue channel in RGB color space, respectively. As can be seen from equation 4.1, the V channel values of HSV color space is better resolved than the RGB color model; thus, one can have higher sensitivity when V-channel values of the images are recorded. Whenever the shade of a color is changing, the three components, namely red, green, and blue channel of RGB color model changes, and average values of RGB recorded by the imaging sensor is used to correlate with the variation of a parameter of interest whereas in HSV model mostly the V (value) component changes. So, to recognize the change in pixels belonging to the shades of a color, the V (value) component of HSV color space has been extracted by using the 'RGB Color Detector' application. At first, for each standard phosphate sample, its V-channel values have been measured for five consecutive times, and the average value is recorded. Another free android application, 'StanXY' has been used to analyze the V component variations with the phosphate concentrations in the samples. Using the applications, a calibration curve is initially obtained

between the average V-channel values and the phosphate level concentrations of the standard samples and subsequently has been used to estimate the phosphate level of an unknown medium through incorporating the V-value in the calibration curve.
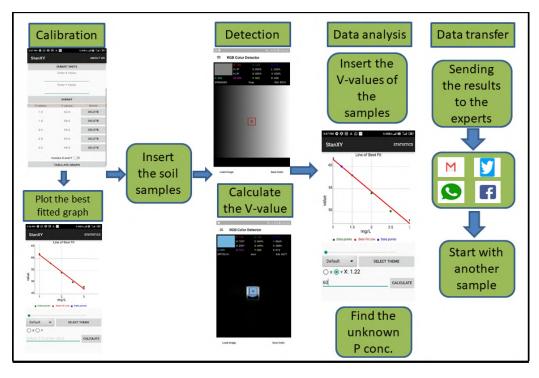

Figure 4.3: Process flow of phosphate detection using the two freely available android applications

4.3 Calibration of the device

Prior to investigate the phosphate concentration of unknown samples, the proposed sensor has been calibrated with 18 standard phosphate samples in the laboratory environment. Two different concentration ranges have been selected to study the characteristic of the designed sensor, a low range 0.1 to 1 mg/L with step increment of 0.1 mg/L and a high range 1 to 5 mg/L with step increment of 0.5 mg/L. Upon treatment of the synthesized reagents, the phosphate samples gradually turned into light blue color, and with the increasing phosphate concentration, the specific color became darker. Figure 4.4 shows the image of the samples acquired by the designed sensor. The calibration equation has been obtained through plotting a graph between the "Value" component of the HSV color model and the "concentration" of the phosphate samples, shown in figure 4.5.

From the characteristic plots, it can be inferred that the V-value has been decreasing with the increase concentration of phosphate in the samples. Figure 4.5 shows the exponentially fitted data points, and a good exponential variation with a regression coefficient R^2 value of 0.995 has been observed. From this curve, a calibration
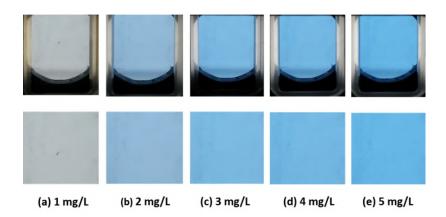
Figure 4.4: Photo images of the reagent treated phosphate samples captured by the designed smartphone sensor.
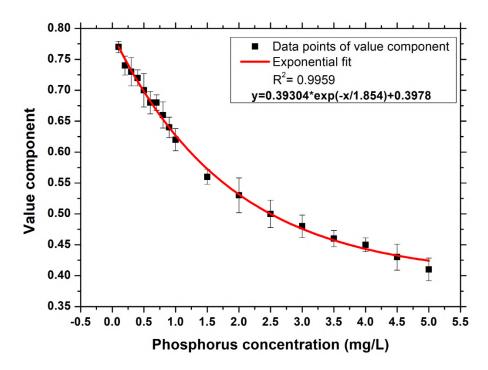

Figure 4.5: Calibration curve for the designed sensor obtained for phosphate concentration ranging from 0.1 to 5 mg/L.

equation has been obtained as given below

$$Phosphate\ concentration = (-1.854) \times log(\frac{value - 0.3978}{0.39304}) \tag{4.2}$$

The phosphate concentration of an unknown sample can be estimated using equation 4.2.

4.3.1 Android applications used for analysis

All the important sensoristic aspects of the developed sensor have been evaluated and are discussed as follows: **Sensitivity** of a sensor is defined as the change in output
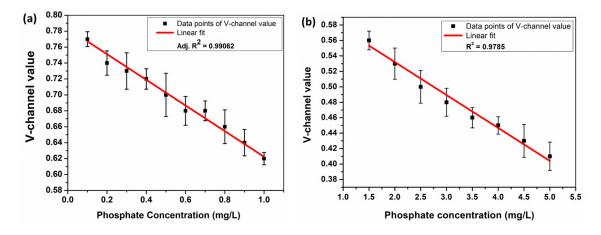
Figure 4.6: Linear fitted curve obtained with designed sensing tool for phosphate concentration range (a) from 0 to 1.0 mg/L and (b) from 1 mg/L to 5 mg/L.

for a unit change in the input [33]. It represents the slope of the calibration curve. Since the slope of the calibration curve is varying exponentially, we have considered two values of sensitivities for the proposed sensor for the two linear regions from 0 to 1 mg/L and 1 to 5 mg/L, shown in figure 4.6(a) and 4.6(b). The sensitivity of the sensor is calculated to be 0.161 AU. L /mg and 0.047 AU. L /mg in the concentration range 0-1 mg/L and 1-5 mg/L, respectively.

In order to compare the variations in both the H and V channels, the corresponding H and V channel values have been extracted from the images of the standard phosphate samples with concentrations ranging from 0 to 1.0 mg/L. The data obtained for H and V channel values have been plotted with the increased phosphate concentration. Figure 4.7(a) and 4.7(b) show the characteristic sensor responses while correlating the H and V channel values with the phosphate concentrations in the samples. The slope for H-channel is found to be 0.082 AU.L/mg while for V-channel this value has found to be 0.161 AU.L/mg.

Limit of detection (LoD) is another important figure of merit for any sensor. It is the lowest concentration of the analyte likely to be reliably detected from the analyte having zero concentration [33]. The limit of detection (LoD) of the sensor is calculated as per the given guidelines of the International Conference on Harmonization. The LoD of a sensing system can be estimated by

$$LoD = \frac{3.3\sigma}{S} \tag{4.3}$$

where σ is the residual standard deviation obtained from the calibration curve, and S is the sensitivity of the sensor. By putting the values of   and S in equation 4.3 for the two linear regions, the theoretical value of LoDs of the designed sensor is estimated to be 0.016 mg/L in the range 0-1 mg/L and 0.032 mg/L in the range 1-5 mg/L.
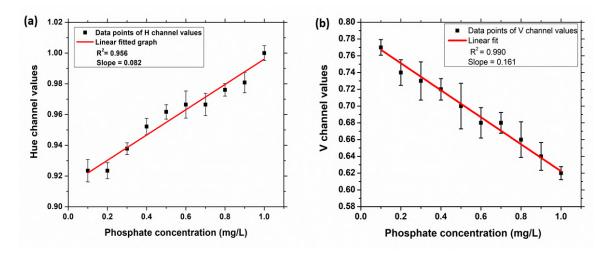
Figure 4.7: Linear fitted curve obtained with designed sensing tool for phosphate concentration in the range 0 to 1.0 mg/L for (a) H channel and (b) V-channel values..

The degree of agreement between a test result and the established reference value or actual value of the property being measured is known as **accuracy**. Accuracy can be calculated using the %bias given by [34].

$$\%bias = \frac{Known\ P\ conc.-Mean\ P\ conc.}{Known\ P\ conc.} \times 100 \qquad (4.4)$$

Precision is a term used to describe the degree of agreement or scatter between a set of measurements made under specific conditions using repeated samples of the same homogenous material. There are three types of accuracy: intermediate precision, reproducibility, and repeatability. In this work, the repeatability of the precision has been assessed, which represents the precision over a short period of time under the same operating conditions. It can be assessed using % residual standard deviation (% RSD), and it is represented as [34]

$$\%RSD = \frac{Standard\ deviation}{Mean} \times 100 \qquad (4.5)$$

To determine the accuracy and precision of the designed sensor, we considered five standard phosphate samples with concentrations 0.5 mg/L, 1 mg/L, 1.5 mg/L, 3 mg/L, and 5 mg/L so that it covers the low, medium, and high concentration of phosphate within the calibration range. The phosphate concentrations of these samples were initially verified with the standard spectrophotometer and then measured with the designed sensor in the same ambient conditions. After calculating the %bias and %RSD of the five standard phosphate samples using the above formulas, the mean bias accuracy value was found to be 0.83%, while the mean RSD was 1.75%. The low value of %bias and %RSD suggest that the designed smartphone sensor can reliably monitor phosphate concentration with good degree of precision.

With the designed sensor the analysis of 12 samples can be performed within 15 minutes. Hence, the sample throughput of the proposed method is thus approximately 48 samples per hour.

4.3.2 Performance evaluation of the sensor characteristics in presence of interfering elements

In the final step of the present work, the performance of the designed sensor has been evaluated by measuring sensor responses in the presence of other interfering elements. Six salt samples, namely Zinc chloride ($ZnCl_2$), Sodium chloride (NaCl), Magnesium chloride ($MgCl_2$), Iron chloride ($FeCl_3$), Manganese chloride ($MnCl_2$), and Copper chloride ($CuCl_2$), have been considered as possible interfering elements for the present study. 1 mM of each salt solution has been added separately to 1 mg/L standard phosphate solution. Furthermore, a mixture of all these solutions has been added to the standard phosphate solution to see the response of the designed sensing system. While adding the salt solution to the phosphate sample, no precipitation formation was observed. A total of eight samples, including the reference sample with 1 mg/L phosphate concentration, have been considered to study the response of the smartphone sensor.
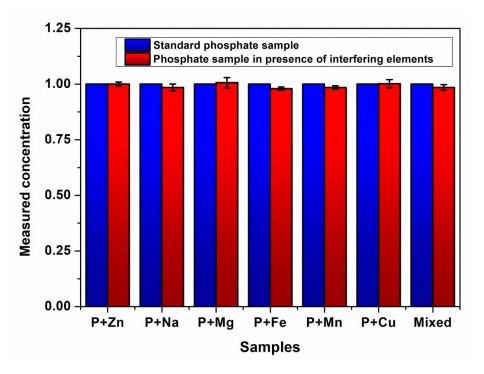

Figure 4.8: Bar graph representation of measurement of phosphate concentration in standard samples in the presence of considered interfering elements obtained with the designed smartphone sensor.

Figure 4.8 illustrates the bar graph representation of the phosphate concentration

measurements of the prepared samples recorded by the designed smartphone sensor. The figure clearly shows that the response of the sensor has not been perturbed by the presence of other elements in the test samples. A variation as low as 1% in the presence of iron and sodium in the phosphate sample has been noticed, attributed to the change of color of the phosphate samples in the presence of these two elements. Again, to test the significance of the difference in measuring phosphate concentration by the designed sensor in the presence of other elements, t-test analysis has been performed. Here also, an insignificant difference, with t (6) = 2.06, p = 0.09 has been observed. These experimental results indicate that phosphate concentration can be reliably monitored even in the presence of the interfering elements in the test sample with the proposed sensing system.

4.3.3 Evaluation of sensing performance for field-collected samples

The feasibility of the designed sensor has been evaluated for in-field applications. Ten water samples from different regions of Sonitpur district, Assam, have been collected to detect the phosphate concentration in these samples. Also, twelve more soil samples have been collected from different farmlands (described in section 4.2.2) have been prepared in the laboratory to investigate the usability of the designed tool for real field applications. Figures 4.9(a) and 4.9(b) illustrate the bar graph representation of the phosphate measurements of both field-collected water and soil samples recorded by the designed sensor and the standard spectrophotometer. The figures clearly show that the experimental results obtained from both the tools are nearly the same. The error bars indicate the standard deviations yield in the measurements of the samples for five consecutive times. Compared to standard spectrophotometer data, the maximum variation in phosphate measurements yielded by our designed sensor was found to be less than ± 2%, which again suggests high reliability of the designed sensing system. To test the hypothesis that no significant variation between data obtained from the smartphone sensor and the standard spectrophotometric tool, an independent sample t-test for both the sets of fields-collected water and soil samples has been performed [35]. For the water samples, the difference in data obtained with smartphone sensor and spectrophotometer was found to be insignificant, t (18) = 0.073, p = 0.47 (1 tail). Similarly, for the soil samples, the difference in data obtained with smartphone sensor and spectrophotometer was found to be insignificant, t (22) = 0.07, p = 0.47 (1 tail). These low t-test values for both field-collected water and soil sample indicate that there is no significant variation between the phosphate concentration measurements by the standard spectrophotometer and the designed smartphone sensor.
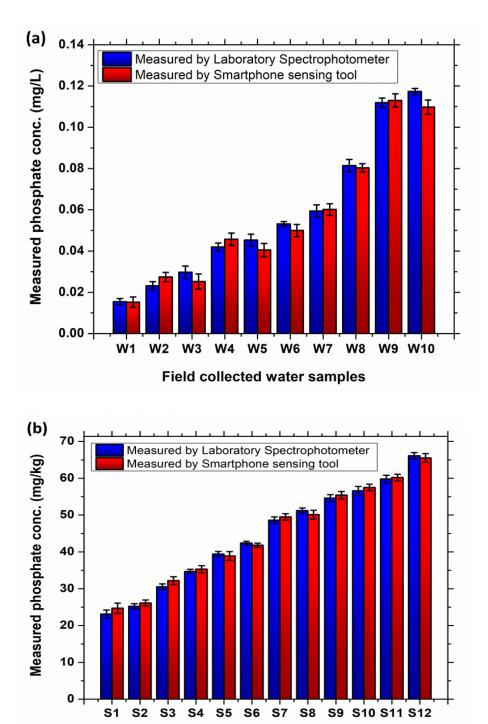
Figure 4.9: Bar graph representation of a comparison of phosphate measurements of the field-collected (a) water and (b) soil samples by the standard spectrophotometer and by the designed smartphone sensor.

For in-field testing, the soil samples are needed to be air-dried and sieved to obtain a small particle size (< 2 mm). If the soil is dry enough, then the process of air-drying can be avoided, and the soil samples can be prepared in the field itself by sieving only. If the soil is wet, the sample needs to be dried first, and subsequent steps can be performed upon proper drying of the soil sample. If the soil sample is wet enough, then it has to be brought to the laboratory for proper drying. For dry soil, one can prepare the soil samples in the field itself for the sensing of phosphate levels in soil by the designed sensor.

4.4 Summary

In summary, this chapter demonstrates the usability of a smartphone for the measurement of phosphate concentration in water and soil media. By coupling a compact 3D printed set-up that houses the optical components and the sample holder to the camera of the phone, the phone has been converted into a colorimetric sensor. The designed sensor is optically shielded, that helps to improve the precision and accuracy of the measurements. The use of the phone's internal battery to power the optical source obviates the need for an external power supply and subsequently makes the sensor low-weight, compact and self-sustained. The designed sensing tool delivers accurate measurements of phosphate levels in different media, and its performance was observed to be at par with the standard spectrophotometer. Apart from the handheld feature, the sensing device offers several advantages like user-friendliness, low-cost, and has the ability to share the in-field data with the central laboratory instantly. The use of the smartphone application offers another advantage of analyzing the sensing data within the phone itself, thus, making it convenient for in-field applications. It is envisioned that with the proposed sensing system, a common citizen can easily measure the phosphate concentration of water or soil media without the requirement of any laboratory equipment.

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