Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

INFLUENCE OF MINERALS ON CONTROL MEASUREMENT OF AMMONIUM, NITRITE, NITRATE AND PHOSPHATE USING UV-SPECTROPHOTOMETER

(Pengaruh Mineral Terhadap Pengukuran Kawalan bagi Ammonium, Nitrit, Nitrat dan Fosfat Menggunakan Spektrofotometer UV)

Md. Salatul Islam Mozumder*, Shehab Shahreyar, Saiful Islam, Md. Delowar Hossain

Department of Chemical Engineering and Polymer Science,
Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh

**Corresponding author: salatul-cep@sust.edu*

Received: 12 February 2021; Accepted: 30 March 2021; Published: 25 April 2021

Abstract

Interference of different minerals on UV-spectrophotometric determination of ammonium nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and phosphate (PO_4^{3-}) was investigated in this study. Iron, magnesium and sodium acetate had significant interference in Nesslerization of NH_4^+-N which was needed to adopt standard protocol for the accurate determination of NH_4^+-N. However, adding 5 times higher Nessler reagent compared to standard method was used to overcome the limitations due to interference of minerals. In determination of NO_2^--N through sulphanilmide method, magnesium and iron did not show any significant affect. Presence of 5 g/L sodium acetate reduced the slope of calibration curve of NO_2^--N from 0.603 to 0.224. Presence of sodium, either in form of sodium acetate or sodium chloride, reduced the absorbance of sulphanilmide method for NO_2^--N determination. Increasing sodium acetate concentration from 2 to 10 g/L, the absorbance of 4 mg/L NO_2^--N was reduced by 5 times: 1.50 to 0.34. Identical absorbance for 4 mg/L NO_2^--N was found in the presence of 0.84 g/L NaCl ($\approx$ 0.33 g/L Na^+) and 2 g/L Na-acetate ($\approx$ 0.56 g/L Na^+) which indicated that chlorine also had interference on NO_2^--N measurement. The complex formed by nitration of salicylic acid was not interfered by the presence of potassium, phosphate, sodium, magnesium, iron and sodium acetate; almost the same slope (0.0095) was observed for both with and without presence of minerals. In addition, Ammonium molybdate method for the determination of PO_4^{3-} was a robust method since the measurement was not affected in the presence of minerals.

Keywords: UV-spectrophotometer, Nesslerization, sulphanilmide method, salicylic acid method, ammonium-molybdate method

Abstrak

Gangguan mineral berbeza dalam penentuan ammonium nitrogen (NH_4^+-N), nitrit-nitrogen (NO_2^--N), nitrat-nitrogen (NO_3^--N) dan fosfat (PO_4^{3-}) melalui spektrofotometrik-UV telah disiasat dalam kajian ini. Ferum, magnesium dan natrium asetat menghasilkan gangguan yang signifikan tindak balas Nessler bagi NH_4^+-N yang perlu di adaptasi dalam protokol piawai bagi penentuan tepat NH_4^+-N. Walau bagaimanapun, penambahan 5 kali ganda reagen Nessler berbanding kaedah piawai telah digunakan untuk mengatasi kelemahan iaitu gangguan dari mineral. Dalam penentuan NO_2^--N menggunakan kaedah sulfanilmida, magnesium dan ferum tidak menunjukkan kesan yang signifikan. Kehadiran 5 g/L natrium asetat mengurangkan cerun lengkung kalibrasi NO_2^--N dari julat 0.603 hingga 0.224. Kehadiran sodium, sama ada dalam bentuk natrium asetat atau natrium klorida, mengurangkan serapan bagi kaedah sulfanilmida dalam penentuan NO_2^--N. Peningkatan kepekatan natrium asetat dari julat 2 hingga 10 g/L, serapan 4 mg/L NO_2^--N menurun sebanyak 5 kali ganda: 1.50 hingga 0.34. Serapan sama bagi 4 mg/L NO_2^--N diperolehi dengan kehadiran 0.84 g/L NaCl (≈ 0.33 g/L Na^+) dan 2 g/L Na-asetat (≈ 0.56 g/L Na^+) telah menjelaskan klorin memberi gangguan kepada pengukuran NO_2^--N. Kompleks yang terhasil melalui penitratan oleh asid salisilik tidak diganggu oleh kehadiran kalium, fosfat, natrium, magnesium, ferum dan natrium asetat, kecerunan yang sama (0.0095) telah diperhatikan bagi kehadiran mineral atau sebaliknya. Selanjutnyam kaedah ammonium molibidat bagi penentuan PO_4^{3-} diketahui kaedah teguh kerana pengukuran tidak diganggu dengan kehadiran mineral.

Kata kunci: spektrofotometer UV, tindak balas Nessler, kaedah sulfanilmida, kaedah asid salisilik, kaedah ammonium-molibdat

Introduction

Trace elements in the surface water play a key role in the development of analytical method for the determination of water quality. Nutrient contents, specifically ammonium-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), and nitrate-nitrogen (NO_3^--N), are increasing in environmental water with the change of food habit and economic growth across the world. Albeit nutrients are essential trace elements in the surface water, concentrations of nutrients above certain levels can be hazardous to both life and earth [1]. Therefore, NH_4^+-N, NO_2^--N and NO_3^--N need to be analyzed and determined accurately for the proper treatment of wastewater considering the environmental and biological concern.

Phosphorus, commonly found as phosphate, is one of the abundant elements on the earth surface, while high concentrations of NH_4^+-N and PO_4^{3-} in the stream or lake avail massive algae blooming (eutrophication) which subsequently contributes to depleting the dissolved oxygen level in the water that is destroying aquatic life [2,3]. Furthermore, plants and animals use phosphate to conduct photosynthesis and respiration. Hence, plant growth rate is linked with increasing the phosphate concentrations [4]. For microorganisms and photosynthetic organisms in different environments, NH_4^+-N, NO_2^--N and NO_3^--N are available forms of N which need to be accurately measured for examining N cycling and their existence in terrestrial as well as in aquatic ecosystems [5].

With the improvement of environmental quality control and monitoring system in different countries, aqueous NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} are being controlled due to the strict regulation [6]. In addition, a number of in-plant processes is going to treat wastewater containing NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} following the recently advanced wastewater treatment technology where the ions need to be determined accurately and more easily. For instance, several determination methods, specifically spectrophotometric, fluorescence, chemiluminescence, and electrochemical detections, have been investigated by researchers for ammonium, nitrate, nitrite and phosphate ions [7]. Besides these, capillary zone electrophoresis, high performance liquid chromatography and ion chromatography methods have also been used for the aforementioned ions determination [8,9]. However, most of the above-mentioned methods reflect complexity either in determination steps or in pretreatment of samples. Hence, UV-spectrophotometric methods are most commonly used technique for the determination of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} due to its simplicity, easy measurement, low fixed and operating cost [10].

Nowadays vials with reagents of different companies such as HACH, HANNA, BEKA Scientific GmbH, Lovibond etc. are widely used for the direct and automatic measurement of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} using spectrophotometer [11,12,13]. In spectrophotometric method, in-built calibration mostly accomplished with distilled water is embedded within the program of spectrophotometer for automatic determination. During the quantitative analysis of the sample following UV-spectrophotometric method, the sample shows error measurements when the samples contain different minerals which have interference on color forming complex. Therefore, real sample in spectrophotometer shows a different value from the actual one since the calibration of spectrophotometer has been accomplished with distilled water.

In this study, UV-spectrophotometric determination methods were adopted from the standard EPA method for real case scenario, i.e. in presence of minerals. In the experiment, the calibration curves were made with the standard method using distilled water as well as mineral medium for the determination of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} aiming to assess the effect of minerals on UV-spectrophotometric determination. The study intended to develop the accurate UV-spectrophotometric determination methods of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} ions concentration in the presence of common minerals like iron, magnesium, sodium acetate by overcoming the interference of these minerals on the determination.

Materials and Methods

Standard method of ammonium-nitrogen (NH_4^+-N) determination

The Nessler method is the widely applied analysis for the determination of NH_4^+-N in water using UV-spectrophotometer, specifically with the use of the concentration range 0.4 - 1.0 mg NH_4^+-N/L [14]. At the NH_4^+-N determination phase, yellow to brown color complex was produced through the Nessler ammonium reaction which strongly absorbed at 400 to 425 nm.

To prepare Nessler Reagent, 4.4 g $HgCl_2$ (Merck, India) was dissolved in 70 mL distill water and heated for complete dissolution. Besides the saturated solution of $HgCl_2$ Preparation, 11 gm KI (Sigma-Aldrich, Germany) was dissolved separately in the minimum amount of water to produce saturated solution of KI. Subsequently, saturated solution of KI was added drop wise to the saturated solution of $HgCl_2$ to form abundant precipitation of orange HgI_2. Maximum quantity of HgI_2 precipitate formation was observed at 1:2 molar ratio of the two reagents $HgCl_2$ and KI respectively. Further addition of KI led to the dissolution of orange precipitate of the HgI_2 by transforming into water soluble complex $K_2[HgI_4]$. At 1:4 molar ratio of the two reagents ($HgCl_2$ and KI), the total dissolution of HgI_2 precipitate and consequent yellowish color complex solution formation were observed. The yellowish color complex solution was taken in 250 mL beaker and mixed with 40 mL of 5 M NaOH solution (Merck, India) where NaOH concentration could be slightly higher than 5 M but not lower than 5 M. Finally, distilled water was added to make the solution 200 mL.

An amount 50 mL of standard (dissolving $(NH_4)_2SO_4$ (Merck, Germany)) or sample of NH_4^+-N solution with concentration of 0.4 - 8 mg/L was uniformly mixed with 1 mL of 0.5 g/L K-Na Tartarate (Rochelle salt solution) (Merck, India) and 1 mL of Nessler's reagent. The solution stagnant was kept for five minutes to complete the reaction; then absorbance was taken at 390 nm (where the maximum absorbance was found) using UV-spectrophotometer (SHIMADZU, UV-1800, Japan).

Standard method of nitrite-nitrogen (NO_2^--N) determination

In this method, sulphanilmide reagent (10 g/L) (Merck, India) (Reagent SA) was prepared by dissolving 5 g sulphanilamide in a mixture of 50 mL, 37% concentrated HCl and approximately 300 mL distilled water and then diluted to 500 mL. 0.2 mL of sulphanilmide reagent was mixed with 10 mL standard sample prepared by dissolving $NaNO_2$ (Merck, India) in distilled water and was allowed 8 minutes to complete the reaction. 0.3 mL of 1 g/L 1-naphthyl ethylenediamine dihydrochloride (Merck, India) solution (Reagent ED) was then mixed and kept for 10 minutes to complete the reaction before taking absorbance at 543 nm using UV-spectrophotometer. The stock solution of N-(1-naphthyl)-ethylenediamine dihydrochloride (Reagent ED) was stored in dark bottle as well as in refrigerated condition. It was needed to prepare fresh N-(1-naphthyl)-ethylenediamine dihydrochloride solution in every week or sooner if a strong brown coloration was observed.

Standard method of nitrate-nitrogen (NO_3^--N) determination

According to rapid determination method of nitrate-N, a complex molecule was formed by reacting with salicylic acid under highly acidic conditions and maximum absorbance was observed in basic (pH > 12) condition at 410 nm wavelength [15]. Salicylic acid-H_2SO_4 reagent was prepared by dissolving 5 g of salicylic acid (Merck, Germany) in 100 mL of conc. H_2SO_4 (Merck, India), while the reagent was important to store in a brown bottle at 4^0 C. 0.25 mL standard or sample was mixed thoroughly with 0.8 mL of 5% (w/v) salicylic acid-H_2SO_4 reagent. The mixture was kept for 20 minutes at room temperature to complete the reaction and then, 19 mL of 2 N NaOH was added to raise the pH above 12 that ensured the formation of color forming complex. Finally, the absorbance of sample was taken at 410 nm using UV-Spectrophotometer.

Standard method for phosphate (PO_4^{3-}) determination

To determine the PO_4^{3-} concentration, a combined reagent was prepared by mixing 500 mL of 2.5 M H_2SO_4, 50 mL of 2.75 g/L potassium antimony tartrate ($K(SbO)C_4H_4O_6.5H_2O$) solution, 150 mL of 40 g/L ammonium molybdate ($(NH_4)_6Mo_7O_6.4H_2O$) solution and 300 mL of 17.6 g/L ascorbic acid solution. A fresh mixture of this reagent mixture was needed to make every day. 13 mL combined reagent was mixed with 50 mL sample, and it shook thoroughly and made up to the mark 100 mL with distilled water. The solution was kept in rest for 30 minutes to develop color. After color formation in the sample solution, the absorbance of the sample solution was measured at 710 nm.

Evaluation of effect of minerals on determination

To assess the effect of minerals on determination of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}, a combined mineral medium commonly used in bioprocess, biological nitrogen removal and phosphate recovery process, was considered in this study. The mineral medium, consisting of KH_2PO_4, K_2HPO_4, $MgSO_4$, $Na_3C_6H_5O_7$, $FeSO_4$ etc., together with NH_4-N, was significant and widely used for the proper growth of different organisms. The composition of the mineral medium used in this study is shown in Figure 1. The calibration curves of NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-} were prepared by following the standard method discussed above but, in this case, mineral medium was used instead of distilled water.

Beside these, the concentration of CH_3COONa, Mg and Fe were varied within the mineral medium to find out the effect on measurement. The stability of the color forming complex in presence of minerals was measured by keeping the color solution for an hour and measuring the absorbance in a regular interval.

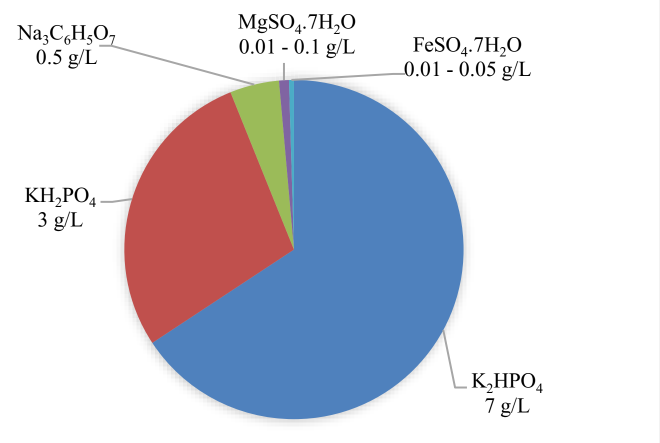


Figure 1. Mineral medium composition

Results and Discussion

NH_4^+-N determination in presence of mineral medium

Initially a calibration curve was made for NH_4^+-N using standard Nessler method. In this standard measurement, NH_4^+-N solution and reagents were prepared using distilled water. With varying NH_4^+-N concentrations from 0.4 to 8.0 mg/L, absorbance was measured by repeating each measurement for three times to ensure the accuracy. The produced calibration curve with standard deviation is shown in Figure 2a. A straight line relationship with slope 0.2152 and R^2 0.9993 ensured the accuracy of the measurement.

In the evaluation of interference of mineral medium (following the composition shown in Figure 1) on determination of NH_4^+-N, all the reagents were prepared with distilled water and the standard NH_4^+-N solution, but the dilutions were made using mineral medium. At the beginning of this measurement, absorbance was taken as per standard procedure; in the standard method, 1mL of Nessler solution and 1 mL of K-Na Tartarate were added in 50 mL sample (Figure 2b). It revealed that the low ammonium concentration (< 2 mg/L) did not produce sufficient color for the UV-spectrophotometric determination and subsequently the relation became non-linear. If water contained other color inducing substances mainly alkali ions like HS^-, Mg^{2+}, Fe^{2+} and Ca^{2+}. the Nessler method was not suitable for ammonium determination. These ions react with Nessler reagent and they should be removed through treatment with Rochelle solution or ethylenediamine-tetra-acetic acid (EDTA) or $ZnSO_4$, to overcome the interference of HS^-, Mg^{2+}, Fe^{2+} and Ca^{2+} [16]. In fact, stabilizer could be applied to remove hardness from water while it was collected from anaerobic digester for analyzing the NH_4^+-N [17]. Moreover, to increase the sensitivity of Nessler method the polyvinyl alcohol (PVA) could be added that increased the light absorbance [18,19].

In case of using 1 mL each reagent (as standard method), the Rochelle solution was not sufficient enough to remove the interfering substances. Due to the establishment of two equilibrium relationships; mineral medium with reagents and NH_4^+-N with reagents made an exponential relationship between absorbance and concentration. The interference for the reaction between mineral medium and reagents was overcome by increasing the amounts of reagents. The linearity of the relationship between NH_4^+-N concentration and absorbance increased with increasing amounts of reagents, specifically 3 to 5 mL each reagent in 50 mL sample (shown in Figure 2b). A linear relation passing through the origin was observed while 5 mL of Nessler and 5 mL of K-Na tartarate were used in 50 mL sample with minerals. To ensure the accuracy of the developed protocol for the determination of NH_4^+-N present in mineral medium, it was repeated for five times; the calibration curve with standard deviation is shown in Figure 2b. The slope of the calibration curve was found 0.207 with R^2 value 0.9917.

Effect of Fe and CH_3COONa on NH_4^+-N determination

In order to evaluate the effect of Fe concentration on UV-spectrophotometric determination of NH_4^+-N concentration, absorbance was taken using the same mineral medium following the composition shown in Figure 1 but changing Fe concentration (0.02 - 0.10 g/L). In the modified Nessler method, 5 mL of Nessler solution and 5 mL of 10 g/L K-Na Tartarate (Rochelle salt solution) solution in 50 mL of 6 mg/L NH_4^+-N solution were used. The stability of the color forming complex was evaluated by taking absorbance for the period of an hour (Figure 3a). Increasing Fe concentration from 0.02 g/L to 0.10 g/L, absorbance was decreased from 1.35 to 1.10. The brown color solution, which was produced by the reaction between ammonium and Nessler reagent, was stable during one-hour experimental period. Accurate analysis of NH_4^+-N could not be possible using original Nessler method due to strong interference of Fe^{2+} when Fe^{2+} content became more than 1.0 mg/L [16]. Fe^{2+} reacted with the Nessler reagents and formed a complex and turbid compound that increased the absorbance. However, five times higher Rochelle salt was used in this study that acted to minimize the interference of substances, like Fe, Mg and Ca which helped to measure NH_4^+-N accurately (within 3% variation).

Presence of sodium acetate had a significant effect on NH_4^+-N determination following Nessler method.

Addition of 2 g/L sodium acetate decreased the absorbance by 6 - 7%. However, when 10 g/L sodium acetate was used, an unstable color forming complex was formed. Initial absorbance was much lower compared to the presence of 2 g/L sodium acetate, but the color intensity as well as absorbance increased over time (shown in Figure 3b). Mechanism behind increasing the color intensity is still unknown. However, to measure the NH_4^+-N in presence of sodium acetate, a fixed time was essential to consider for both the calibration curve preparation and sample analysis.

Effect of Mg concentration on NH_4^+-N determination

Water samples with Mg^{2+} concentration of 0.01 to 0.10 g/L were analyzed with modified Nessler method and the results were compared in Figure 3c. Initial absorbance at time, t = 0 was not changed significantly with changing Mg^{2+} concentration. While 0.01 g/L Mg^{2+} was present in mineral medium, absorbance for 5 mg/L NH_4^+-N was not deviated from its initial value. Increasing Mg^{2+} concentration, the absorbance was randomly deviated from its initial value. It was due to the formation of precipitate by the reaction of Mg^{2+} with Nessler reagents [20]. A high turbidity was formed at 0.10 g/L Mg^{2+} that increased the absorbance and later turbid compound was settled down that produced a clear solution as well as decreased the absorbance. Other researchers observed similar behavior using original Nessler method, following the findings of the researches; an accurate measurement of NH_4^+-N could not be possible in presence of Mg^{2+} [16,20]. 0.122 g/L Mg^{2+} ($\approx$ 0.5 g/L Mg^{2+} as $CaCO_3$) concentration augmented the absorbance more than double; consequently, large overestimation of NH_4^+-N concentrations was noticed. In fact, the absorbance varied proportionally to the Mg^{2+} concentration [16]. To overcome the interference of complex formation of Mg^{2+}, it was better to determine the absorbance immediately after mixing the reagents.

NO_2^--N determination in presence of minerals

To determine the NO_2^--N present in distilled water and in mineral medium following the composition shown in Figure 1, calibration curves were compared using sulphanilamide method in both conditions (Figure 4). To prepare the calibration curves, 10 mL of standard NO_2^--N solution either in distilled water or in mineral medium was mixed with 0.2 mL sulphanilamide (SA) and 0.3 mL 1-naphthyl ethylenediamine dihydrochloride (ED). Finally, the solution was kept stagnant for 10 minutes to complete the reaction. The results showed that mineral medium did not have any effect on the determination of NO_2^--N where the slope was identical for both the cases, specifically 0.6075 and 0.6032 (Figure 4a and b). Sodium acetate, which was used as carbon source in many bioprocesses, had a significant effect on NO_2^--N determination. Absorbance and slope of calibration curves decreased with the presence of sodium acetate (Figure 4c). It showed a straight-line relationship for 0 - 2 mg/L NO_2^--N concentration, while the maximum absorbance was around 0.5. Above 2 mg/L NO_2^--N concentration, the absorbance remained steady due to the limitation of reagent to produce more color forming complex. This limitation was confirmed by increasing the amount of reagents that ultimately increased the detection limit of NO_2^--N (Figure 5). The absorbance and slope of calibration curves increased with the increasing amount of reagents.

The ratio of sulphanilamide (SA) and 1-naphthyl ethylenediamine dihydrochloride (ED) used in NO_2^--N determination was crucial for the accuracy. Between the two reagents, 1-naphthyl ethylenediamine dihydrochloride was mainly responsible to develop the color forming complex through the reaction with reaction product of NO_2^--N and reagent SA. Increasing reagent ED from 1 to 2 mL in 10 mL sample (mineral medium and 5 g/L sodium acetate) and 1 mL reagent A, the slope increased from 0.38 to 1.72 (Figure 5c and d). The slope increased with the increasing amounts of reagents. Higher slope increased the accuracy of the measurement but decreased the detection limit or narrowed the concentration range for the determination. However, the amount of reagents used for the determination of NO_2^--N must be balanced between the slope of calibration curve and measurement limit.

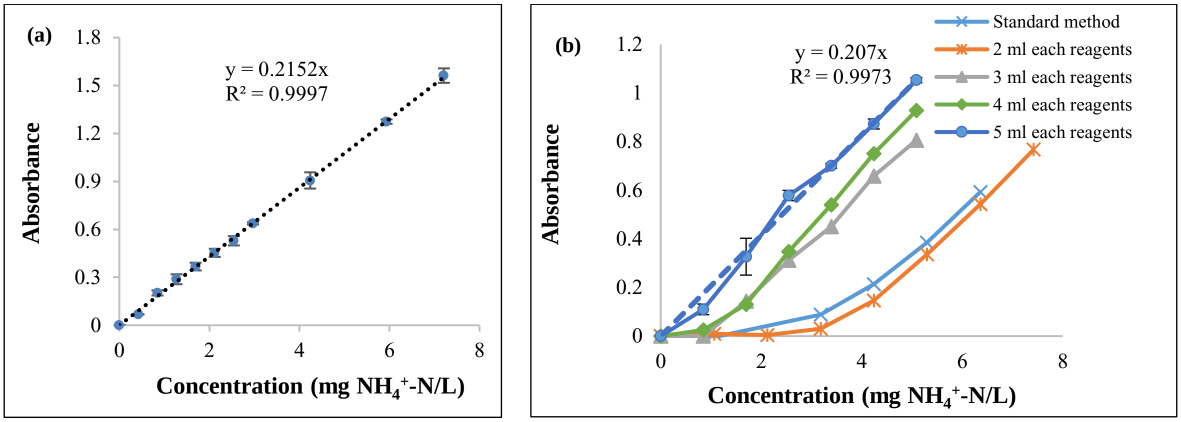


Figure 2. Relationship between the concentration and absorbance for NH_4^+-N determination: (a) using distilled water, and (b) using mineral medium with varying the amount of reagents

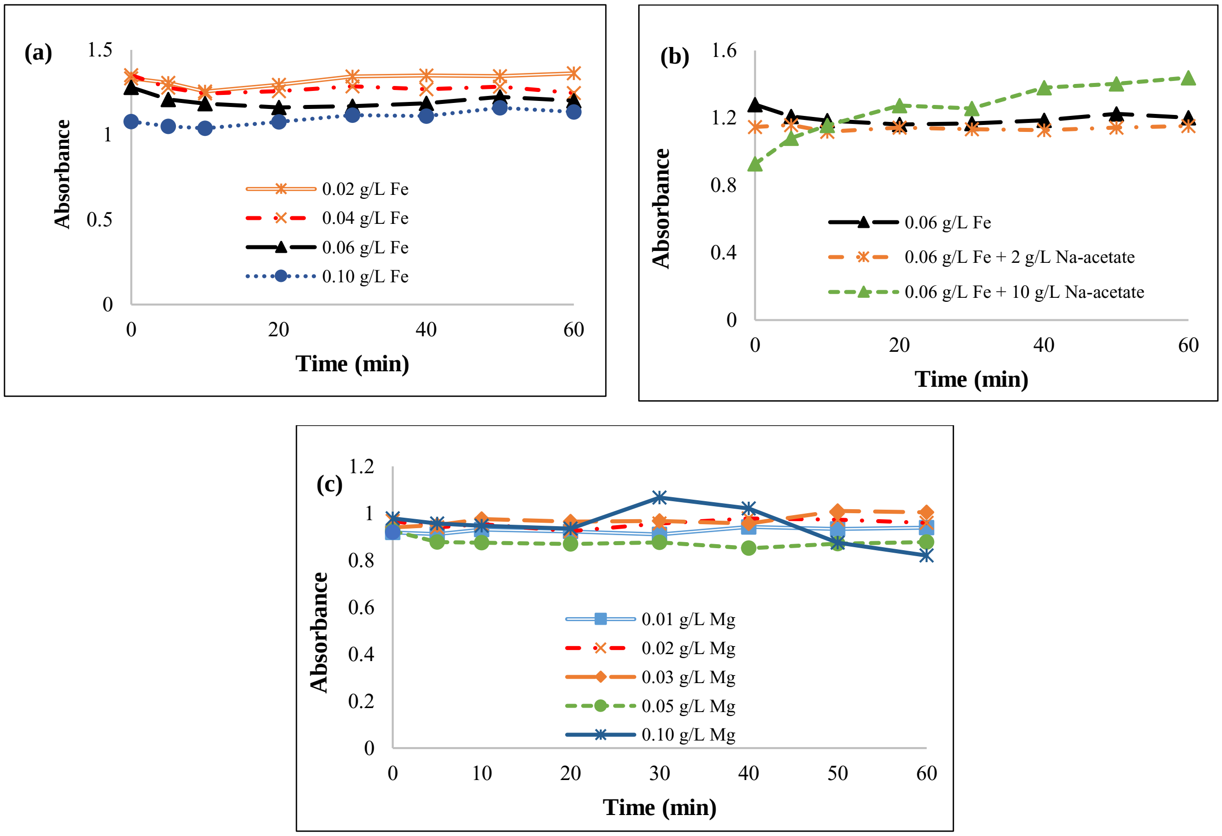


Figure 3. UV-Spectrophotometer absorbance of 5 mg/L NH_4^+-N in mineral medium as per Figure 1 but varying: (a) Fe^{2+} concentration, (b) sodium acetate, and (c) Mg^{2+} concentration

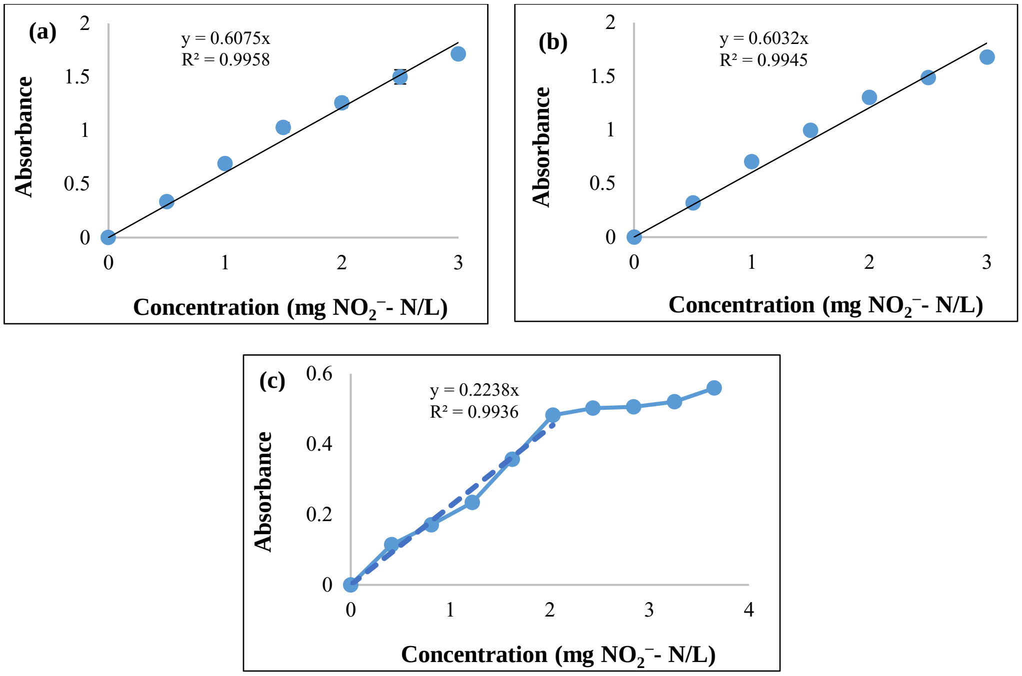


Figure 4. Calibration curves of NO_2^-- N using sulphanilamide method; standard was made with: (a) distilled water, (b) mineral medium, and (c) mineral medium and 5 g/L sodium acetate

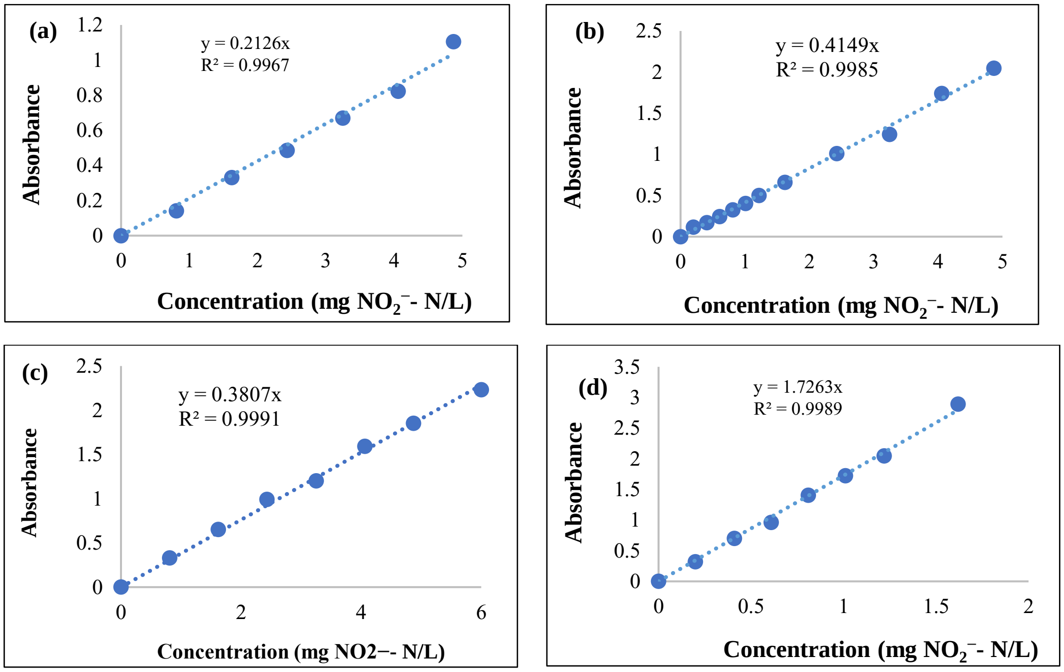


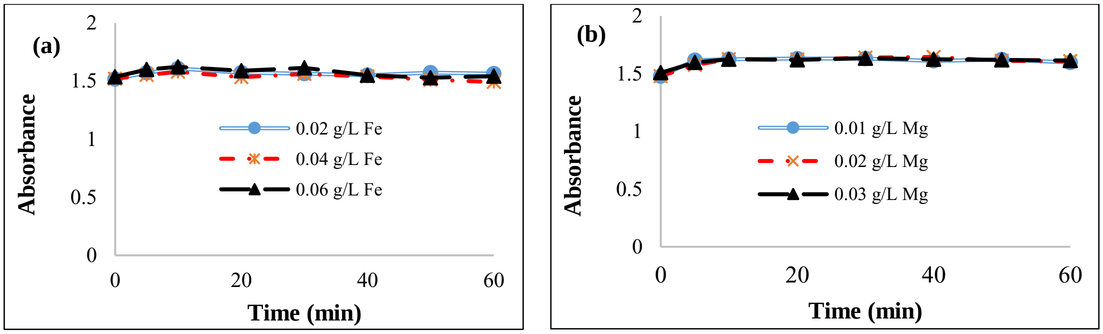
Figure 5. Calibration curves of NO_2^-- N using mineral medium and 5 g/L sodium acetate with varying amounts of color forming reagents: (a) 10 mL Sample + 0.3 mL SA + 0.4 mL ED, (b) 10 mL Sample + 0.5 mL SA + 0.75 mL ED; (c) 10 mL Sample + 1 mL SA + 1 mL ED, and (d) 10 mL Sample + 1 mL SA + 2 mL EB

Effect of Fe and Mg on NO_2^--N determination

To assess the Fe^{2+} interference in the measurement of NO_2^--N, absorbance was taken for 4 mg/L NO_2^--N dissolved in mineral medium, following the composition shown in Figure 1, but varying Fe^{2+} concentration from 0.02 g/L to 0.06 g/L. In this case, 0.5 mL sulphanilamide (SA) and freshly prepared 0.75 mL 1-naphthyl ethylenediamine dihydrochloride (ED) were used in 10 mL sample to develop the color forming complex. Finally, the results showed that Fe^{2+} did not have any interference on NO_2^--N determination (Figure 5a). In this regard, more than 95% reduction of color formation was noticed while Fe^{2+} was present in the determination of NO_2^--N [21]. Through a simultaneous addition of sulfanilamide and 1-naphthyl ethylenediamine to the sample that already contains iron, the limitations were minimized. Low tolerance of iron on determination of NO_2^--N was observed in azo dye method [22]. Following the evaluation of the iron interference (Fe^{2+} and Fe^{3+}) experiment, Fe^{2+} concentration below 20 mg/L and Fe^{3+} concentration below 100 mg/L did not make any interference to NO_2^--N determination (within 2% error) [23]. Almost similar result was observed in this study since no interference of Fe^{2+} up to 60 mg/L on NO_2^--N determination was noticed (Figure 6a). Similarly, Mg^{2+} concentration also did not have any effect on NO_2^--N determination (Figure 6b).

Effect of CH_3COONa on NO_2^--N determination

Absorbance was taken for 4 mg/L NO_2^--N in mineral medium following the composition shown in Figure 1 with varying sodium acetate concentration from 0 to 10 mg/L. The absorbance decreased with increasing sodium acetate concentration (Figure 6c). Sodium presence in sodium acetate was mainly responsible for the interaction and subsequent formation of color forming complex in sulphanilamide method. Interaction of sodium was ensured by decreasing absorbance using NaCl. Identical absorbance for 4 mg/L NO_2^--N was found in the presence of 0.84 g/L NaCl (≈ 0.33 g/L Na$^+$) and 2 g/L Na-acetate (≈ 0.56 g/L Na$^+$) which indicated that chlorine also had interference on NO_2^--N measurement. A permissible concentration of both sodium and chlorine was found 4 g/L in the measurement of NO_2^--N (with less than 2% error) [23]. But in this study, a very strong interference of sodium and chloride was observed even in a low concentration. However, in all cases (Figure 6a-c), initial absorbance at time, t = 0 min was little lower, a stable color forming complex formed within 10 minutes. For this reason, original sulphanilamide method guided to wait 10 minutes to complete the reaction before measuring the absorbance.



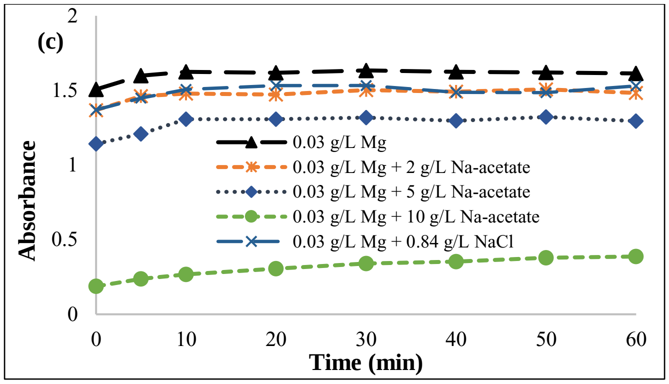


Figure 6. Effect of (a) iron (Fe^{2+}), (b) magnesium (Mg^{2+}), and (c) sodium acetate on NO_2^-- N determination using sulphanilamide method

NO_3^--N determination in presence of mineral medium

The method that was used to measure NO_3^--N in this study is well-known as salicylic-acid method [24]. Calibration curves were prepared with the use of the standard solution with distilled water and also with the use of the mineral medium, following the composition shown in Figure 1, with 5 g/L sodium acetate. While the stock solution and dilution were conducted using mineral medium, all the reagents were prepared with distilled water. Both calibration curves showed a straight line relationship with R^2 value above 98% and almost similar slopes, specifically 0.0094 and 0.0095 (Figure 7(ia) and 7(ib)). It indicated that mineral medium did not have any significant interference on NO_3^--N measurement in UV-spectrophotometer. Similarly, presence of ammonium-N, nitrite-N and chloride did not interfere on determination of NO_3^--N [24]. Albeit interference of iron (iron > 10 mg/L) was found on the colorimetric determination of NO_3-N using a standard method that involved reduction of NO_3^--N to NO_2^--N using cooperized cadmium followed by NO_2^--N determination by sulfanilamide method [21]. Sulfanilamide method for NO_2^--N determination has interference with some minerals. However, in this study, NO_3^--N was directly determined without reduction of NO_3^--N to NO_2^--N and found no interference of minerals.

PO_4^{3-} determination in presence of minerals

The calibration curve for the determination of PO_4^{3-} was prepared using either distilled water or mineral medium following the composition shown in Figure 1. The results were shown in Figure 7(iia) and 7(iib) where both the calibration curves showed a straight line with R^2 value above 99%. The slope of these straight-lines was close to each other, for instance 0.4315 and 0.4242, which indicated that minerals present in medium did not have any interference on PO_4^{3-} measurement for the use of ammonium molybdate method. Following the analysis of soil sample, metal ions such as Cr, Al, Fe, Hg, Pb, Cd, Zn, Ni, Co, Mn, Ca, Mg and etc. did not show any interference in phosphate determination [25]. A high tolerance of foreign ions in the UV-Spectrophotometric determination of PO_4^{3-} with ammonium molybdate solution was found, and it was coincided with this study [26].

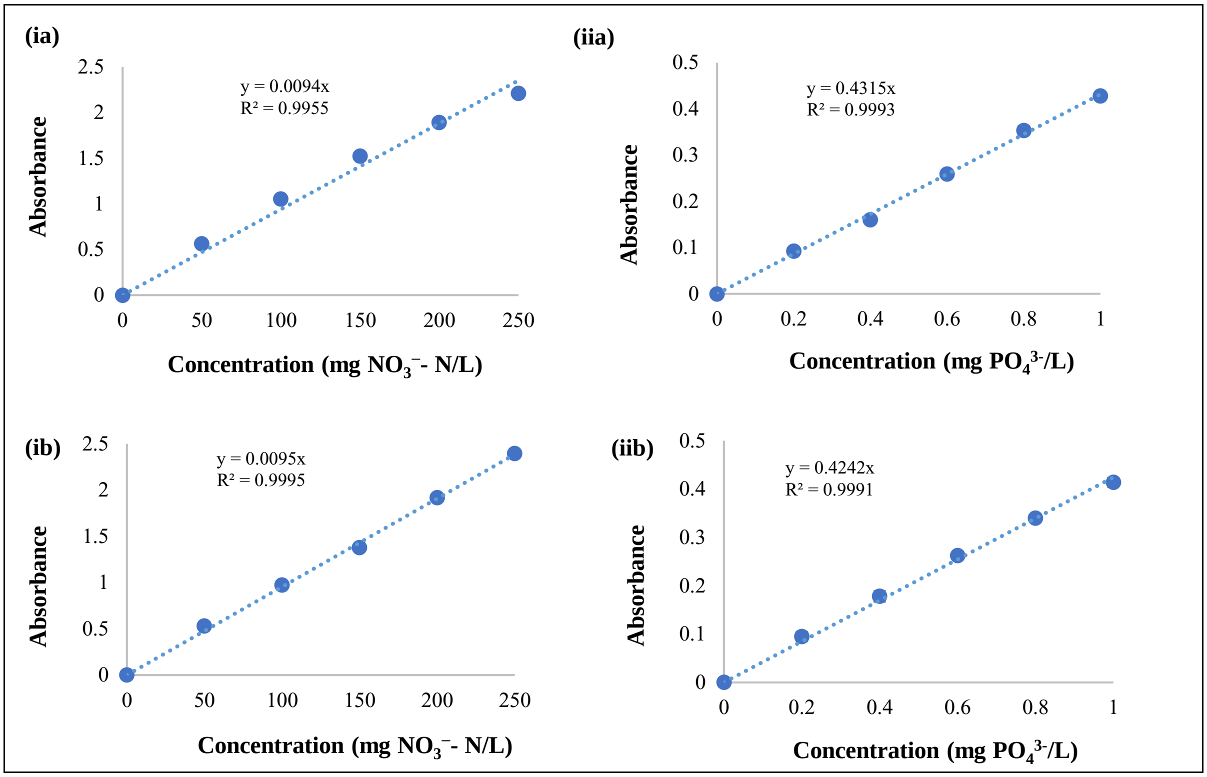


Figure 7. Calibration curves of (i) NO_3^--N and (ii) PO_4^{3-} where the standard was made with: (a) distilled water, and (b) mineral medium and 5 g/L sodium acetate

Conclusion

Mineral medium had a significant interference on original Nessler method of NH_4^+-N measurement which need to be adopted with the standard protocol for an accurate measurement of ammonium in water. On NH_4^+-N measurement, presence of Fe^{2+} reduced the absorbance but overestimated the NH_4^+-N concentration in Nessler method, whereas Mg^{2+} generated precipitation through the reaction with Nessler reagents which interfered on measurement. To overcome the interference of sodium acetate and Mg^{2+} in Nessler method, it would be better to take the absorbance immediately after mixing the reagents. However, for accurate measurement of NH_4^+-N in real sample, presence of other cations has to be measured where the calibration curve will be in the presence of those cations. On NO_2^--N determination, iron and magnesium did not have a significant effect, while sodium and chlorine showed a significant interference where a clear decrease of absorbance was observed due to the presence of sodium either in sodium acetate or other form. 1-naphthyl ethylenediamine was mainly responsible for the development of the color forming complex through reaction with the product of NO_2^--N and sulphanilamide. During the measurement of NO_2^--N, the concentration of sodium in the medium has to be measured initially and then the calibration curve will be made with the same sodium concentration. For the determination of NO_3^--N, salicylic acid method was more robust compared to copperised cadmium reduction method. Moreover, iron, magnesium, and sodium as sodium acetate did not have a significant effect on NO_3^--N and PO_4^{3-} determination.

Acknowledgement

The authors gratefully acknowledge the Bangladesh Bureau of Educational Information and Statistics (BANBEIS), Bangladesh for financial support to conduct this research (Project ID: SD2017530) and Dr. Mohammad Shafiqul Islam (Associate Professor, Department of English, Shahjalal University of Science and Technology) for the Proofread of this manuscript.

References

1. Rezende, D., Nishi, L., Coldebella, P. F., Silva, M. F., Vieira, M. F., Vieira, A. M. S., Bergamasco, R. and Fagundes-Klen, M. R. (2016). Groundwater nitrate contamination: assessment and treatment using moringa oleifera lam. Seed extract and activated carbon filtration. *The Canadian Journal of Chemical Engineering,* 94: 725-732.
2. Ramos, A. C., Regan, S., McGinn, P. J. and Champagne, P. (2019). Feasibility of a microalgal wastewater treatment for the removal of nutrients under non-sterile conditions and carbon limitation. *The Canadian Journal of Chemical Engineering,* 97: 1289-1298.
3. Foulon, E., Rousseau, A. N., Benoy, G. and North, R. L. (2020). A global scan of how the issue of nutrient loading and harmful algal blooms is being addressed by governments, non-governmental organizations, and volunteers. *Water Quality Research Journal,* 55 (1): 1-23.
4. Yaqoop, M., Nabi, A. and Worsfold, P. J. (2004). Determination of nanomolar concentrations of phosphate in freshwaters using flow injection with luminol chemiluminescence detection. *Analytica Chimica Acta,* 510: 213-218.
5. Gamon, F., Tomaszewski, M. and Ziembińska-Buczyńska, A. (2019). Ecotoxicological study of landfill leachate treated in the ANAMMOX process. *Water Quality Research Journal,* 54 (3): 230-241.
6. Brezinski, K., Gorczyca, B. and Sadrnourmohammadi, M. (2019). Ion-exchange for trihalomethane control in potable water treatment - a municipal water treatment case study in Rainy River, Ontario, Canada. *Water Quality Research Journal,* 54(2): 142-160.
7. Moorcroft, M. J., Davis, J. and Compton, R. G. (2001). Detection and determination of nitrate and nitrite: a review. *Talanta,* 54: 785-803.
8. Fukushi, K., Tada, K., Takeda, S., Wakida, S., Yamane, M., Higashi, K. and Hiiro, K. (1999). Simultaneous determination of nitrate and nitrite ions in seawater by capillary zone electrophoresis using artificial seawater as the carrier solution. *Journal of Chromatography A*, 838: 303-311.
9. Okemgbo, A. A., Hill, H. H. and Siems, W. F. (1999). Reverse polarity capillary zone electrophoretic analysis of nitrate and nitrite in natural water samples. *Analytical Chemistry,* 71: 2725-2731.
10. American Public Health Association (2005). Standard methods for the examination of water and wastewater. Access from https://www.standardmethods.org/
11. He, X., Sun, Q., Xu, T., Dai, M. and Wei, D. (2019). Removal of nitrogen by heterotrophic nitrification–aerobic denitrification of a novel halotolerant bacterium Pseudomonas mendocina TJPU04. *Bioprocess Biosystems Engineering,* 42: 853-866.
12. Mozumder, M. S. I., De Wever, H., Volcke, E. I. P. and Garcia-Gonzalez, L. (2014). A robust fed-batch feeding strategy independent of the carbon source for optimal polyhydroxybutyrate production. *Process Biochemistry,* 49: 365-373.
13. Zhao, J., Wang, X., Li, X., Jia, S., Wang, Q. and Peng, Y. (2019). Improvement of partial nitrification endogenous denitrification and phosphorus removal system: balancing competition between phosphorus and glycogen accumulating organisms to enhance nitrogen removal without initiating phosphorus removal deterioration. *Bioresource Technology,* 281: 382-391.
14. Crosby, N. T. (1968). Determination of ammonia by the Nessler method in waters containing hydrazine. *Analyst,* 93: 406-408.
15. Cataldo, D. A., Haroon, M., Schrader, L. E. and Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant-tissue by nitration of salicylic-acid. *Communications in Soil Science and Plant Analysis,* 6(1): 71-80.
16. Jeong, H., Park, J. and Kim, H. (2013). Determination of NH_4^+ in environmental water with interfering substances using the modified nessler method. *Journal of Chemistry,* 2013: 1-9.
17. Boopathy, R. (2003). Use of anaerobic soil slurry reactors for the removal of petroleum hydrocarbons in soil. *International Biodeterioration and Biodegradation,* 52: 161-166.

18. Hach, C. C., Brayton, S. V. and Kopelove, A. B. (1985). A powerful kjeldahl nitrogen method using peroxymonosulfuric acid. *Journal of Agricultural & Food Chemistry,* 33: 1117-1123.
19. Gulsoy, G., Tayanc, M. and Erturk, F. (1999). Chemical analyses of the major ions in the precipitation of Istanbul, Turkey. *Environmental Pollution,* 105: 273-280.
20. Foyn, E. (1950). Ammonia determination in sea water. *ICES Journal of Marine Science,* 16: 175-178.
21. Colman, B. P. (2010). Understanding and eliminating iron interference in colorimetric nitrate and nitrite analysis. *Environmental Monitoring and Assessment,* 165: 633-641.
22. Norwitz, G. and Keliher, P. N. (1985). Study of interferences in the spectrophotometric determination of nitrite using composite diazotisation - coupling reagents. *Analyst,* 110: 689-694.
23. Tarafder, P. K. and Rathore, D. P. S. (1988). Spectrophotometric determination of nitrite in water, *Analyst,* 113: 1073-1076.
24. Cataldo, D. A., Maroon, M., Schrader, L. E. and Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis,* 6(1): 71-80.
25. Ganesh, S., Khan, F., Ahmed, M. K., Velavenda, P., Pandey, N. K. and Mudali, U. K. (2012). Spectrophotometric determination of trace amounts of phosphate in water and soil. *Water Science and Technology,* 66(12): 2653-2658.
26. Mahadevaiah, Kumar, M. S. Y., Galil, M. S. A., Suresha, M. S., Sathish, M. A. and Nagendrappaa, G. (2007). A simple spectrophotometric determination of phosphate in sugarcane juices, water and detergent samples. *E-Journal of Chemistry,* 4(4): 467-473.