GSJ: Volume 6, Issue 12, December 2018, Online: ISSN 2320-9186
www.globalscientificjournal.com

Purifying Inorganic Phosphate using Sawdust Activated Carbon in Solid Phase Extraction for Stable Oxygen Isotope ($\delta^{18}O_{PO4}$) Analysis: A Review on Different Methodological Approach

Sandy May F. Soriano*, Saiym Faustine M. San Jose, Kimberly Neal Nathalie R. Sasis, Karla Jane S. Tawing, Abigail P. Cid-Andres

Department of Physical Sciences, College of Science, Polytechnic University of the Philippines, Manila, Philippines
**Contact Information: sorianosandymay@gmail.com [S.M.Soriano]*

KeyWords
Isotope, Phosphate capture, Sawdust, Solid-Phase Extration, Stable Isotope analysis

ABSTRACT

Phosphate pollution in the ecosystem particularly in different bodies of water may lead to critical degree of contamination and potential ecological risks. As an approach to this problem, the role of solid phase extraction in purifying inorganic phosphate for stable isotope analysis is expected to have a significant outcome. In this review, analysis of isotopic composition of oxygen in phosphate ($\delta^{18}O_{PO4}$) is said to be a prominent research tool for examining phosphate in water and soil, however, this approach requires purified phosphate. There are several techniques in purifying phosphate, and one of the leading method is solid phase extraction that showed high efficiency percentage in purification of target analytes in many studies. Potential use of sawdust as an effective sorbent in solid phase extraction will be discussed in this paper. Moreover, challenges that are usually encountered in the analysis of $\delta^{18}O_{PO4}$ and its environmental applications will be tackled as well.

1. INTRODUCTION

Phosphorus is a highly reactive, nonmetallic element that is important as a constituent of the lithosphere and an essential component for every cellular material. Within water, abundance of phosphorus occurs in the form of orthophosphate ions (PO_4^{3-}) in which stand as the nutrient of water, but excess in phosphate concentration would lead to harmful algal blooms (HAB). Phosphate circulate through the lithosphere, hydrosphere, and biosphere by means of a biogeochemical process called Phosphorus cycle. In the natural ecosystem, phosphates are derived from the erosion or runoffs of rocks or land surfaces rich in the mentioned substance, afterwards conserved for later use as nutrients for plant growth. It is returned into the environment by production of inorganic phosphates from decaying plants and animal tissues. In agricultural systems, naturally occurring or environmentally available phosphates are combined with phosphates derived from fertilizers and travels all the way down the ground, passing through the unsaturated region and arriving at the storehouses of groundwater [24]. Content of phosphate in water often stands as the limiting factor for the growth of algae and aquatic weeds [48]. As mentioned before, HAB are kind of phenomena called eutrophication, it is the excess growth of algae and other microbes in the downstream water bodies due to gradual increase in phosphate concentration [24]. Several factors influence the concentration of adsorbed and dissolved phosphates in soil or sediments, and in groundwater; some of these are the electrical conductivity (EC), pH, temperature (T), and dissolved oxygen (DO). Also, there are types of minerals used by soils in adsorbing phosphates, therefore partly preventing contamination in the quality of groundwater [62].

Sample preparation is very important in performing analytical procedures. It improves the sample's limit of detection and isolates it from unwanted matrix components that can interfere the analysis [14]. Therefore, sample preparation has to be done carefully.

Solid-Phase Extraction or liquid-solid extraction is one of the high-performance method in sample preparation due to its ability to achieve higher recovery than other methods (D.A. Wells, 2000). It has been progressively used for extracting and determining concentration of different target components from liquid samples including phosphate. SPE was develop as an alternative to liquid-liquid extraction (LLE) and diminished multiple disadvantages such as usage of huge amount of solvents, long operation time, potential source of errors and high cost materials [52].

The most common design of this technique use polypropylene or sometimes a glass as a cartridge (membrane) to hold the sorption phase or resin. There is a wide choice of resin for SPE, it comes in different types depending on the target compound to be extracted. In Solid Phase Extraction, the analyte or the sample to be extracted is separated between a sorption phase (resin) and a liquid-liquid phase (sample). The sample must have a greater affinity to the sorbent than in the sample matrix. While the resin, must be a hydrophobic organic compound usually chemically coated with a powdered silica to attract hydrophobic compounds and extract it from the aqueous solution [12]. Generally, SPE is composed of four steps: preparation of column, loading of sample, post-wash of column and desorption of sample.

There are different types of SPE namely, normal-phase, reverse-phase and ion-exchange. In normal-phase, the sample matrix (mobile phase) is mid- to non-polar, while the stationary phase and analyte of interest is polar. Reversed- phase, sample matrix is polar or moderately polar, the stationary phase is a non- polar and the target analyte typically mid- to non- polar. Reversed-phase is the most commonly used type of SPE. Ionic or ionizable compounds are usually the best compounds to be isolated in ion-exchange. This type of SPE is orthogonal compared to the normal and reverse-phase SPE [54].

SPE, having more advantages than the liquid-liquid extraction, it attracted a lot of researchers, thus, producing a lot of studies about improving SPE's resin or SPE method itself. Many previous researches have been talking about the adsorption property of some naturally occurring waste by-product such as sawdust, rice husk and coconut husk to be a potential adsorbent used in various adsorption techniques. And among these low-cost waste by-products, sawdust is said to be the most promising adsorbent for removing heavy metals, color-dyes and some unwanted materials from wastewater [2].

Sawdust, a lignocellulosic material, contains cellulose, hemicellulose, lignin and some functional groups namely hydroxyl, carboxyl, and phenolic compounds that makes the process of adsorption feasible [37]. One method to make the sawdust material a potential adsorbent is by converting the material into an activated carbon. Although, sawdust can be directly used as adsorbent without any treatment, studies have shown that depending on the activating agent used, activation of sawdust increases the surface area, total pore volume and possesses a texture with higher porosity characteristic [5].

Several applications or analytical analyses are used for purified and isolated compounds, be it a metallic, nonmetallic, organic, or inorganic, there are many analytical methods that can be used. However, these analytical techniques ranges from simply measuring the concentration - but is limited only to knowing the amount of compound in a certain sample, to a more sophisticated technique, that is knowing the isotopic ratio of an element in a compound to gather more information about the compound.

Isotopes of an element have nuclei that has the same number of protons but differ in number of neutrons. Isotopes can be classified as stable and unstable. Unstable isotopes are often referred to as radioactive isotopes. Stable isotopes, although they do not emit radiation, their properties can be used in wide variety of application including water and soil analysis and other environmental application. Stable isotopes are also used in forensics, health, and nutritional analyses.

This paper will discuss about phosphates in water and soil, different method of purifying phosphate, and its advancing environmental application. It will also tackle about basic adsorption chemistry, solid phase extraction, how SPE became more preferred method as oppose to LLE. Theoretical background regarding the analysis of stable isotope of oxygen in phosphate will also be discussed, and as well as the methodological challenges that usually arise when performing stable isotope analysis.

2. *Phosphate capture*

2.1. Occurrences of phosphate in water and soil

With the increasing and inevitable pollution in the ecosystems specially in the different bodies of water such as lakes, rivers, groundwaters etc., it may lead to critical degree of contamination and potential ecological risks [62]. One major nutrient of water that not even a single cell could live without is the phosphorus and its derivatives [45]. Phosphorus exists in many forms; Orthophosphates (reactive), condensed phosphates (pyro, meta, and polyphosphates) and organic phosphates (found in plants, animals and humans). Total Phosphorus in water are divided into two categories; the soluble phosphorus and suspended insoluble phosphorus. In which, under the soluble phosphorus includes soluble inorganic orthophosphates, hydrolyzable polyphosphates and organic phosphorus. While in the suspended inorganic phosphates includes insoluble inorganic phosphorous compounds, sorbed or fixed phosphorus and phosphorus in the microorganisms [48].

As it is well known that orthophosphate is the naturally occurring form of phosphorus, in water and soil sediments, it can be dissolved or adsorbed. Orthophosphates are adsorbed by soil in the cooperation of these types of minerals; clay and metal oxides [24]. However, there's a limit in the capacity of soil to adsorb the said nutrient that will remain the excess PO_4^{3-} dissolved in the water. Subsequently, the water would take the dissolved PO_4^{3-} down from the surface land and underneath the unsaturated layer of the ground, and keep it in the storehouses of groundwater called aquifer.

In a study conducted by Liu Y. et al on 2018, phosphorus content in water and soil sediments are determined as Total Phosphorus (TP), Dissolved Phosphorus (DTP), Dissolved Inorganic Phosphorus (DIP) and Particulate Phosphorus (PP) using ammonium molybdate spectrophotometric method. On the other hand, varying phosphate concentration was used on other studies to ascertain its effect on Nitrogen (N), Arsenic (As), and other organic matters in water [22][58][62].

Innovated methods on purifying phosphates from water and wastewater would resolve current ecological and environmental issues in the quality and safety of water. Few of these involves filtration with the use of nanofilters, adsorption techniques using modified activated carbon and, bioreactors and columns filled with foam ceramics, sawdusts and other reusable wastes [1][30][40][56]. One example of these adsorption techniques is the Solid-Phase Extraction (SPE), this has become one of the most preferred method in the extraction of target compounds with the reason that it has a faster and convenient operation, easier manipulations, and reduced usage of organic solvents [33].

2.1.1. Sources of phosphates

2.1.1.1. Natural sources

There are several sources of phosphates in the natural environment. Eroded PO_4^{3-} from rocks is one, yet rocks rich in PO_4^{3-} came mostly from marine sediments (85%), and a little from igneous apatite deposits and guano [58]. Also, rural land runoff may contain sediments of approximately 500 to 2000 mg/L (ppm) of perchloric acid-oxidizable phosphorus that could append to the phosphate content in water. In United States, it was stated that animal wastes contribute an enormous amount of phosphate released in the environment, including the decomposition of plant, animal and human tissues. According to a study made by Holt, Timmons, and Latterell in 1970, the said animal wastes contains four times more amount of phosphates than it contains in the human wastes. Moreover, there was a report that indicate an annual range of 20-25% of phosphorus that was released in the Big Stone Lake are from cattle feeder lots.

2.1.1.2. Agricultural sources

Phosphorus as a primary nutrient for the growth of plants, it is added into the fertilizers used in farms and crops. Despite this fact, it was shown in a study that runoff from farm lands can contribute an appreciable amount of phosphorus to waters [48]. A study made by Timmons (1969), he investigated three methods of incorporating superphosphates into the surface soils concerning the concentration of the dissolved phosphorus in runoff waters. In which, he concluded that deeper incorporation of fertilizers into the surface soils gives substantially lower concentration of dissolved phosphorus in runoff waters. In that simple experimentation, it could be confirmed that fertilizer application on agricultural crops is another potential source of phosphates that can affect the quality of groundwater.

2.1.2. Phosphate migration

Migration of phosphorus from its sources to the hydrosphere was made possible due to erosion done by water in the form of land or surface runoffs, and rain precipitates. Surface-water runoff from rainstorms or excess irrigation is one primary way that dissolved PO_4^{3-} or sorbed PO_4^{3-} is being carried in most watersheds [24]. Metal Oxides and Clay with fine-grained iron oxides are bound to the soil, where in PO_4^{3-} binds and form metal salts. Iron oxides in soil may not be able to handle an enormous amount of PO_4^{3-}, hence, the adsorption capacity of soil would reach its limits. The excess PO_4^{3-} in this case, will dissolve and flow down the streambed. As it was stated by Domagaslki et al in 2012, that “Dissolved phosphorus can move vertically across the streambed interface in either direction, and in that process can alter the phosphorus concentration in streams and groundwater”. It is reported that the concentration of adsorbed PO_4^{3-} is inversely proportional with the concentration of dissolved phosphorus. In line with that, correlates the mobility of PO_4^{3-}, meaning the the higher mobility of adsorbed phosphates signifies lower mobility of dissolved phosphorus, and vice versa.

2.1.3. Parameters affecting phosphate concentration

Parameters affecting the concentration of adsorbed and dissolved phosphates in soil or sediments, and in groundwater are the pH, temperature, dissolved oxygen and electrical conductivity.

According to “Phosphorus and Groundwater: Establishing Links Between Agricultural Use and Transport to Streams”, it was announced that 1 out of 5 sites (Washington, California, Nebraska, Indiana and Maryland) where the acidity/basicity of the unsaturated-zone water and groundwater were tested, where in results implied that Maryland site have the greatest capacity to adsorb and retain PO_4^{3-} with pH of near neutral in the unsaturated-zone and with a slightly acidic groundwater. Concluding that, at the pH of neutral to slightly acidic, adsorption capacity of soils is at best.

Temperature of water partly affect the speed of growth of microbes like phytoplankton. High temperature in normal (May) and flood (August) seasons accelerates growth of phytoplankton compared to lower temperature of the dry season, with 18-25 °C and 9-14.7 °C of surface water temperature, respectively [62]. In short, the temperature is directly proportional to the speed of growth of microbes.

Iron oxides are maintained bound to the soil with the help of dissolved oxygen (DO). It is preventing from washing the metal oxides off of soil surface. As agreed by Domagalski and Johnson, the ability of iron oxides to adsorb and retain PO43- depends upon the presence of DO in the surrounding water and below-neutral pH (less than 7). Even though, DO helps on maintaining the metal oxides in soils, it might be the opposite in water, since phytoplankton consumes DO as part of their cellular respiration, high concentration of it in water would accelerate growth of microbes [62].

It was simply reported by Liu et al that annual electrical conductivity range is equal to 26.7-59.9 mS/m. Concluded that it has the same electrical conductance of tap waters.

2.1.4. Minerals in soil

As mentioned before, clay and metal oxides hold primarily responsible for PO_4^{3-} adsorption in soils. In addition, soils that are calcium carbonate rich could limit the mobility of PO_4^{3-} from going downstream the watersheds underground, because it would result to the formation of calcium phosphate [24]. Moreover, arsenic (As) correlates with PO_4^{3-} as its competitor in binding with metal oxides in soil. Forthwith could bring confusion if what compound from the two will be favored on being dissolved in the water or sorbed by the soil.

2.2. Pollutants in water

Over population of such compound in any bodies of water particularly ponds, lakes, rivers and groundwaters could categorize the compound as a pollutant, whether it’s a nutrient in water or any biochemical matter that is necessary for the aquatic life to survive.

2.2.1. Phosphate pollutant

Eutrophication is a phenomenal evident that in the excess of PO_4^{3-}, PO_4^{3-} become a pollutant in water. Increase in concentration of PO_4^{3-} may lead to degradation of water quality, depletion of Dissolved Oxygen, and formation of ammonia that could kill marine plants and animals.

With the common knowledge that the higher phosphate there is in water means the higher the risks of blooming pollution to water, signifies that microbes may rapidly grow and propagate, and could even exhibit an outbreak of HAB [62]SSSS. Although DO help by preventing metal oxides and other minerals from being washed off of the soil, with a gradual growth of microbes, DO in water are majorly consumed by the phytoplanktons and the like, instead of fishes, seaweeds, etc. Stated in a study made by Liu et al on 2018, nutrient structure (NS) of water was introduced and explained the rational relationship of nitrogen in the form of dissolved inorganic nitrogen (DIN) and phosphorus in the form of dissolved phosphorus (DTP).

$$NS = ([DIN])/([DTP]) \qquad \text{eq. (2.2.1)}$$

In DIN, it usually contains more than 90% of Nitrate Nitrogen (NO_3^-N) and less than 10% of Ammonia Nitrogen (NH_3^-N), yet changes in the temperature of water depending on the season could affect the ratio of NO_3^-N and NH_3^-N in DIN. It was elaborated that increase in temperature favor the reaction of nitrification to form NO_3^-N than NH_3^-N. Formation of ammonia in water are favored in lower temperature with high concentration of DIN paired with low concentration of DTP. In accordance to the basis of which from the two (N and P) are the limiting factor, value of NS in the mentioned situation would result to value of higher than 16, therefore, confirming that Phosphate does affect the production of ammonia in bodies of water.

Other naturally occurring components in water are consist of chemical and biological matter, like, sulfates (SO_4^{2-}), fluorides (F^-) and organic carbons (OC). Apart from the outcome with over population of PO_4^{3-} in water, OC could also strengthen the danger of microbial growth.

3. Solid Phase Extraction

Solid phase extraction (SPE) is a widely used sample preparation technique in analytical laboratories because of its rapidness and selectivity [8][23]. This method is commonly used for extracting and isolating target analytes, usually from a mobile phase. It is usually utilized to clean up a sample before analysis or performing other analytical methods to quantify the presence of analyte in a sample. The primary goals of SPE are trace enrichment (concentration), matrix simplification (sample clean up) and medium exchange (shifting from sample matrix to a different solvent). It was initially developed as a replacement to liquid-liquid extraction (LLE) to reduce errors and other disadvantages. In the present time, SPE is now the most common sampling technique in many areas of chemistry, like pharmaceutical, clinical, environmental, food and industrial chemistry [8]

3.1 Solid Phase Extraction (SPE) as a Replacement for Liquid-Liquid Extraction (LLE)

For many reason, solid phase extraction is the most preferred sample preparation technique than other analytical method, such as Liquid-liquid extraction. SPE is a cost-effective technique, often achieving higher recovery of analyte than other methods because of its selectivity [14]. Conventional LLE is labor intensive, consumes long operation time, difficult to automate, and is frequently plagued by practical problems. One of those problem is the formation of emulsion between the aqueous and organic layers preventing phase separation. In addition, it consumes a relatively large volumes of high-purity solvents that requires a disposal of high cost materials. By contrast, there is a lot of advantages in using the SPE method from a low intrinsic costs, shorter operation time, low solvent consumption and simpler processing procedures. SPE is also easier to automate using robotics, centrifugal or special-purpose sequential or parallel flow processing units that simultaneously extract and prepare samples for separation [8][13][52].

Many analyst had used solid phase extraction and liquid-liquid extraction in their studies to compare each performances. According to the study of J.L Bonnefous et. al. about the determination of diltiazem and its metabolite plasma, solid phase extraction had resulted to a higher recovery with 90% of compounds, whereas for liquid-liquid extraction, mean recovery ranged from 62% to 82%. They conclude that SPE is more convenient, rapid and sensitive because of its improvement in limit of detection than LLE and represents a useful analytical tool for the monitoring of diltiazem and its metabolites in clinical investigation [23]. Another comparison of SPE and LLE by M. P. Juhascik and A. J. Jenkins for alkaline drugs, focused to determine the feasibility of switching from an existing LLE to SPE. In a blood sample, the limits of detection (LOD) of 122 drugs and metabolites were determined using the SPE method and compared to the LOD's by LLE. Both method established that there were 41 drugs that has LOD's in blood; SPE had a lower LOD for 16 (39%), while LLE had a lower LOD for 8 drugs (19.5%), and for the remaining drugs LOD's were comparable. The SPE method had detected several drugs that LLE couldn't detect most notably, morphine and benzoylecgonine [38].

3.2 Retention Mechanisms in Solid Phase Extraction

To know the appropriate type of SPE or the right sorbent to use in analysis, an analyst must understand the mechanism(s) of interaction between the sorbent and the target analyte. The properties of both solute and the sorbent whether it is polar, non-polar or ionic must be considered. The most common retention mechanisms in SPE are based on Van Der Waals or dispersion forces (non-polar or hydrophobic interactions), dipole-dipole forces (polar interactions), dipole-induced dipole interactions, hydrogen bonding, pi-pi interaction and electrostatic attraction [54].

According to Supelco solid phase extraction comes with general theory interactions: Normal phase, Reversed phase and Ionic exchange

3.2.1 Normal Phase

This type of separation involves a mid- to non-polar sample matrix (mobile phase), polar stationary phase and a polar analyte of interest (e.g. acetone, chlorinated solvents and hexane). Under normal phase condition the retention of an analyte is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. Among others, these include retention mechanism such as hydrogen bonding, л – л interactions, dipole-dipole interactions, and dipole-induced dipole interactions. To elute an adsorbed compound from a normal phase SPE tube or disk, solvent must be more polar than the sample's original matrix to disrupt the forces that bind the compound to the packing [54].

3.2.2 Reversed Phase

Typically involves a polar or moderately polar sample matrix (mobile phase) and a non-polar stationary phase. The analyte of interest typically mid- to nonpolar. The retention applied in this mechanism is nonpolar-nonpolar attractive forces or commonly called the Van Der Waals forces. Due to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface, retention of organic analytes from solutions like water onto these SPE materials happened. A compound adsorbed by these mechanisms is eluted by a nonpolar solvent to disrupt the binding mechanism [54].

3.2.3 Ionic Exchange

This type of SPE can be used for compounds that are charged when in a solution. The sorbent has an ionically charged surface of opposite charge to the sample ions. Aqueous buffer is used as a mobile phase, where both pH and ionic strength are used to control elution time. A negatively charged (anion) compounds can be isolated on a quaternary amine bonded silica with Cl- counter-ion or aminopropyl bonded silica cartridges. Positively charged (cation) compounds can be isolated by using sulfonic acid bonded silica with Na+ counter-ion and carboxylic acid bonded silica with Na+ counter-ion cartridges. The electrostatic attraction of the charged functional group on the compound to the charged group that is bonded in the silica surface, is where the retention mechanism based. To retain the compound by ion exchange from an aqueous solution, the pH must be one at which both the compound of interest and the functional group on the bonded silica are charged. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and, thus, the longer it will take to elute [54].

3.3 Sampling Formats in SPE

Solid-phase extraction during the last 25-plus years, has become one of the most common sample clean-up and concentration technique used by analytical chemists. Single disposable columns and cartridges has been a traditional format for SPE. Its advantages are that multiple samples can be extracted, only low volume of solvents are used and procedures can be easily automated. The technology has been improved with the introduction of more selective solid sorbent chemistries to improved performance and automation possibilities. The range of sampling formats has been expanded from simple packed syringes to cartridges, discs, SPE pipette tips and 96-well plates. These developments have facilitated automated off- and on-line sample processing [13][47][21].

3.3.1 Cartridge

The usual design of a classic SPE cartridge is a small propylene or glass container with sorption phase. This is still the most popular format of SPE with typically between 20-mm frits between 20-μm frits. Disadvantage with SPE cartridge is that sample processing rates are slow because of their cross-sectional area, they become blocked by particles and adsorbed matrix [47][36].

3.3.2 Discs

The parameters of interest in selecting a disc for a particular application are size, sorbent chemistry and sample capacity. SPE discs are available in various styles and sizes. The disc was a packing-impregnated polytetrafluoroethylene (PTFE) flexible disc that had approximately 8-μm particles that superficially resemble to a filter paper discs. This format had a much greater cross-sectional area than the cartridges. Subsequently, analyst can use higher flow-rates for large volumes of low-concentration sample, as might be encountered in analyses [8][47].

3.3.3 SPE Pipette Tips

The pipette tip-SPE (PT-SPE) is a miniaturized format of SPE and has become an important tool for concentration and purification because of its excellent selectivity and sensitivity. The significant advantages of this method includes higher speed, reduces solvent consumption because of the small bed volume and sorbent mass in the pipette tip, and enhanced ease of operation [43]. In additional it could be used with multichannel pipetters, and the liquid could be bidirectional. Several companies began to manufacture pipette tips, each with a different twist [47].

3.3.4 96-Well SPE plates

The 96-Well SPE Plates is a new format of SPE with 8 x 12 well spaced 9.0mm from centre-to-centre. It is based on the standard microtiter plate format. Each of the 96-Well plates has a small 1 or 2 mL SPE column with 10-100 mg of packing material. As in an SPE cartridge, the packing material is placed between the bottom frit (membrane) and a top frit. This plate allows 96 samples to be extracted in approximately one hour or less. Processing 96 sample simultaneously reduces handling errors and limits labor-input [47].

4. Sawdust as an adsorbent

In solid phase extraction, resin plays an important role in isolating and extracting target compounds. However, the popular format of a typical SPE setup as well as the sorbents used are known to be very expensive. For this reason, maximizing the use of waste by-products is an efficient way to produce a low-cost SPE setup, at the same time reducing wastes that is abundant in the environment. There are several agricultural waste by-products that are used as adsorbent for removal of dyes and heavy metals in limnological waters under study. And one of the most promising material used for various studies is the wood sawdust.

Wood sawdust is a naturally occurring waste by-product that is observably potential for the adsorption of heavy metals or non-metallic compounds. However, several methods have been used to treat wood sawdust to produce a more effective adsorbent. One method is the activation of sawdust by treating the sample with activating agents such as phosphoric, sulfuric and boric acid. Although converting the sawdust to form activated carbon is widely used technique for adsorption, various studies have established other method which include the modification of sawdust. The mentioned impregnation techniques can increase the capacity of sorbent to adsorb.

In this section, related studies concerning the adsorption capacity of sawdust proving that it is a potential adsorbent was discussed and reviewed.

4.1. Activated carbon derived from sawdust

Activated carbon is widely used for various applications such as air purification, water purification, air filters in respirator, etc. The ability of activated carbon to purify and filter is because of its structure that contains micropores, mesopores and macropores. Also, it has a large internal surface and high degree porosity that is the reason why it is used in the recent years [34]. This property of activated carbon leads to its good performance as an adsorbent.

Variety of sources are used to produce activated carbon. However, expensiveness and regeneration difficulty of commercially available activated carbon hinders its widespread use. For this reason, many researches has developed and used agricultural waste as precursor, specifically, sawdust in substitute to produce activated carbon. The activation of carbon involves two important steps namely, the carbonization and activation. In carbonization process, the lignocellulosic components of sawdust will be decomposed to eliminate the non-carbon element of the raw material by thermal decomposition that is divided into four phases depending on the temperature of each stage. Whereas, the pore beyond the material is starting to develop. In activation, either physical or chemical treatment is used. Physical activation is conducted at high temperature and in the absence of oxygen in an inert atmosphere. Production of activated carbon by chemical treatment on the other hand, is conducted at low temperature and is impregnated with activating agents including potassium hydroxide (KOH), sodium bicarbonate ($NaHCO3$), zinc chloride ($ZnCl2$), phosphoric ($H3PO4$) and sulfuric acid ($H2SO4$). These activating agents act as dehydrating or oxidizing agent which also decomposes lignocellulosic materials and enhances the percent yield of activated carbon produced as well as its adsorption capacity.

Attia et al. (2015) presented the effect of activating agents prepared in three series of sawdust activated carbon wherein a common horizontal furnace was used for different activation process. In series A, direct carbonization at 700°C under the influence of nitrogen flow was conducted for 2hrs and was then oven dried at 110°C for 24hrs. On the other hand, the produced char in series B has been impregnated with KOH at 1:1 ratio. The dried solid mixture then was employed to carbonize at 600°C for 2hrs and was soaked in HNO3 for 24hrs to remove alkali residual in the carbon pores. Series C then was prepared by impregnating the produced char with 50% (v/v) H3PO4 overnight and carbonized at 600°C for 2hrs, and was further washed by 1% NaHCO3 to eliminate acid residual. In the study, the surface morphologies of the tested samples were presented by scanning electron microscope (SEM) and based on data, although activation by H3PO4 produced activated carbon with the highest surface area, activation by KOH yields also a highly defined pores and cavities. Whereas, this implies that in activation of carbon, activating agent is an important factor to improve the porosity and surface area of the material.

In general, utilization of wood sawdust is proven to be a potential substitute for commercialized activated carbon. Also, this material provide a practical and sustainable source for development of a better quality low-cost activated carbon that would be of great help in treating waste and limnological waters.

4.2. Characterization of sawdust adsorbent

Sawdust as an adsorbent is used for water treatment such as removing organic and inorganic compounds. It consists of abundant lignin, cellulose, hemicellulose, and functional groups including carboxyl, phenol, amide and hydroxyl compounds which are responsible for the adsorption process of the structure [37]. And to enhance the adsorption capability and efficiency of sawdust, chemical pre-treatment is further needed [2]. In section 1.1, it is observed that sawdust waste by-product is an appealing material to substitute the commercially activated carbon. Recent studies proved the hypothesis about the adsorption capacity of sawdust activated carbon by conducting an analysis. Fourier transfer infrared (FTIR) and scanning electron microscope (SEM) are examples of those instruments that are needed in investigating the composition and surface morphologies of the sample.

Benyoucef et al. (2012) tested the suitability of modified Aleppo pine sawdust with urea for removal of phosphate ions from water. The surface morphologies of unmodified and chemically modified sawdust were determined using SEM. And the images revealed that the chemically modified sawdust created more pores than in unmodified one. Due to higher porosity developed, this means that the chemically modified sawdust also yields a more accessible surface area thus, a greater adsorption capacity occurred at the equilibrium. Khattri et al. (2011) presented the characterization of sagaun sawdust by IR spectroscopy to determine the functional groups present in the surface of the adsorbent. The IR spectra however, shown the absorption band at 1639 cm-1 and 1512 cm-1 indicating the presence of aromatic ring. Also, the presence of strong bands of Si–O linkage was observed at 1033 cm-1 and 1039 cm-1 respectively. For some studies, the sawdust activated carbon is characterized for yield and iodine number [7]. By which, iodine number indicates the porosity of the activated carbon that is defined as the milligrams of iodine adsorbed per gram of carbon and also represents the surface area governed by the pores larger than 10 Ǻ). The Brunauer, Emmett and Teller (BET) technique is also used by other studies to measure surface area including the pore size distribution. Dissolution rate is also predicted here as it is proportional to specific surface area.

4.3. Heavy metal and dye adsorption by sawdust

There are various techniques used to remove heavy metals and dyes in aqueous solutions namely ion exchange, precipitation, coagulation, reverse osmosis, membrane separation, electrolysis, aerobic and anaerobic treatment, bacterial treatment, electrodialysis, magnetic separation, microbial reduction, photochemical reactions, ultrasonic treatment and adsorption [37]. However, inexpensiveness and ease of operation in adsorption makes it to be the most used process among the mentioned techniques. In this section, recent studies regarding the removal of heavy metals and dyes using sawdust as adsorbent were presented.

4.3.1. Adsorption of heavy metals

One of the major contributor of contaminants in most water bodies are the heavy metals. These metals in higher concentration such as cadmium, zinc, lead, nickel, iron, manganese, chromium, copper and cobalt can lead to water pollution that can harm aquatic plants and animals as well as human beings. And in order to reduce the abounding concentration of heavy metals in surface waters, removal of these by adsorption can help the environment.

Gupta et al. (2009) investigated the performance of raw neem sawdust and mango sawdust on removal of hexavalent chromium from aqueous solution by varying pH, concentration of Cr(VI) and contact time. Earlier studies have shown that pH is a major parameter that has a high effect in the adsorption process. Because of this, different pH level was conducted in this study in order to identify the effect of this in the adsorption of chromium.

Another study [31], removing copper and zinc ions from aqueous solutions using sawdust as adsorbent has presented the adsorption capacity of modified and unmodified sawdust. To increase the adsorption capacity of the material, sodium hydroxide and sodium bicarbonate were used to modify the sawdust.

Some investigators have been studying toward the application of conducting polymers such as polyaniline and polypyrrol for the removal of heavy metals. Ansari et al. (2006) chemically synthesize polyaniline which was coated on sawdust and was used as adsorbent for removal of lead ion from aqueous solution. Here, polyaniline was used as a cation exchanger material. Although this study has a different approach, varying parameters such as pH, concentration of lead, sorbent dosage and contact time were also tested.

Begum et al. (2013) on the other hand prepared two different forms of sawdust for the removal of chromium (VI) ion from aqueous solution. One sorbent is an acid-activated sawdust in powder form where sulfuric acid was used as activating agent and the other one is the surface-modified sawdust in bead form where sulfuric and chitosan gel were impregnated in the material. Batch adsorption experiments were carried out to determine the efficiency of two forms of sawdust to remove chromium (VI) ion.

The studies mentioned investigated the capacity of sawdust in adsorption of various heavy metals. Although each research have its own approach in the method such as activating, modifying, and combining sawdust to another substance, the factors that affects adsorption did not vary.

4.3.2. Adsorption of dyes

Like heavy metals, dyes are also contaminants mostly found in water bodies. The color dyes that are used on textile, rubber, plastics, paper etc. are industrial wastes that are capable in harming aquatic life. One of the efficient methods to remove dyes from effluent, is of course, the adsorption process.

Malik P.K. prepared activated carbon from mahogany sawdust as adsorbent for the removal of acid dyes. The study investigated the adsorption capacity of sawdust in removing acid dyes from aqueous solutions. Due to the presence of acid yellow 36 in waste waters, it was selected as adsorbate for the experiment since it was reported toxic and carcinogenic.

Khattri et al. (2011) tested the adsorption capacity of sagaun sawdust by removing crystal violet dye from simulated waste water. Batch adsorption experiment was conducted and varying parameters such as pH, adsorbate concentration, agitation time and temperature was also explored.

Chakraborty et al. (2006) carried out adsorption for the removal of two reactive dyes namely reactive red and reactive black (commercially known as Cibacron Red RB and Cibacron Black B) from a textile effluent using hardwood sawdust as an adsorbent. Here, phosphoric acid was used for the impregnation of material. The concentration of the two system was predicted by external film mass transfer coefficient and an internal effective diffusivity.

Other than dyes and heavy metals, there are studies conducting an experiment that also removes oil and toxic salts from water bodies using sawdust. Most of the studies that utilized sawdust as an adsorbent tested the adsorption capacity of material by varying parameters. However, there are specific concepts used to analyze clearly the property of sawdust or other materials.

5. *Stable Isotope*

Stable isotopes had been used primarily to track elements like oxygen (O), nitrogen (N), sulfur (S), and carbon (C) during transfers and to understand different interaction during their cycles [17]. Along with the advances of technology, a sophisticated method of tracking isotopes has been developed. Stable isotope analysis, inarguably, have a great contribution in the scientific community and have a huge benefit for environmental and future studies. This section will i) examine theoretical background to the use of $\delta^{18}O_{PO4}$ in studying P; and ii) inspect methodological crisis that usually happens in analysis of $\delta^{18}O_{PO4}$ in different ecosystem.

5.1. Theoretical Background: Isotopic Composition of Phosphate

Stable isotope analysis requires an element to have at least two naturally-occurring stables isotopes, with the changes in the ratio of individual isotopes of an element in a sample with respect to a known ratio as reference [10] Phosphorus (P) lacks stable isotope – it only has one stable isotope (^{31}P). For this reason, stable isotope analyses cannot be performed in P atom in phosphorus containing compounds. So it led many researchers to study the isotopic composition of oxygen (O) in phosphate (PO_4^{3-}), which is the predominant form of P in the natural environment [16]. Thus, attention has really focused on whether the stable isotopic composition of O in PO_4^{3-} can be used to acquire information regarding the cycling of phosphorus. Gruau et al. (2004) found a notable variability of $\delta^{18}O_{PO4}$ within P sources and substantial overlap of $\delta^{18}O_{PO4}$ among different P sources and therefore, seeks validity of this method as a research tool. That being said, a lot of researches use the ratio of isotopic composition of O in PO_4^{3-} ($\delta^{18}O_{PO4}$) to track P in different ecosystem [26][35].

5.2. Methodological Challenges

5.2.1. Phosphate extraction protocols

This type of research tool has been a challenging one especially in the preparation of sample and the analysis itself. Tudge (1960), who first explored many aspect of apatite [6] described the first method to isolate PO_4^{3-} in natural apatite. Tudge (1960) emphasized the removal of chloride and organic matter in apatite sample. This follows the slow precipitation of $BiPO_4$ to avoid bismuth nitrate interference, followed by complete drying of $BiPO_4$ for 3 hours at about 140˚C. This specific method was polished by Kolodny et al. (1983) and has been a standard method through the years. However, using bismuth to isolate PO_4^{3-} is hygroscopic, thus, makes the analysis of $\delta^{18}O_{PO4}$ difficult for future studies [17]. Precipitation of Ag_3PO_4 was introduced by Firsching (1961) and was used as an alternative to $BiPO_4$.

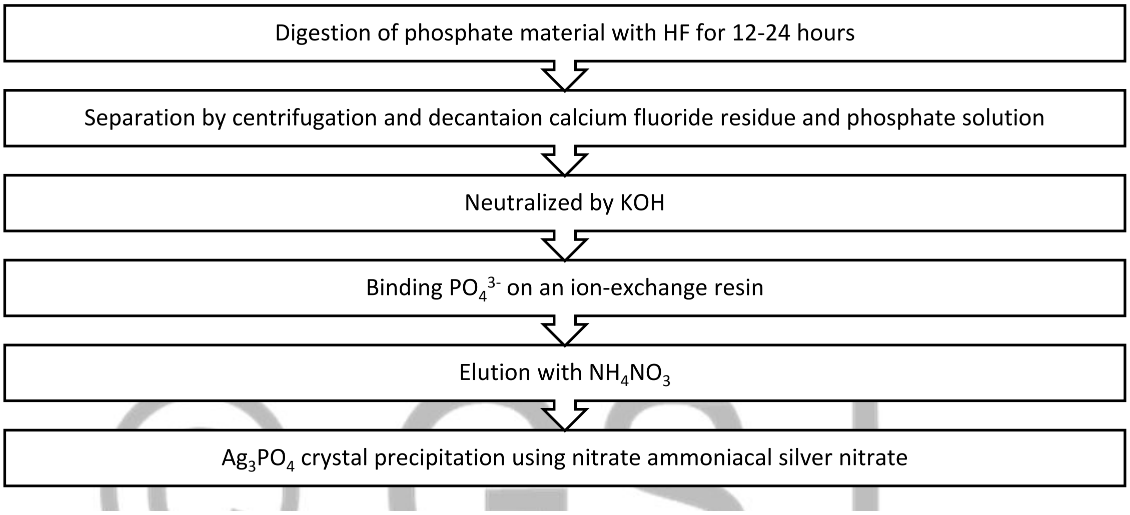

Figure 5.2.1. Ag3PO4 Method – *Summarized chemical protocol to isolate PO_4^{3-} from apatites in the form of Ag_3PO_4* [6]

Ag_3PO_4 method is summarized in Figure 5.2.1. This was directly derived from the original method by Crowson et al. (1991). Elution process was by Firsching (1961). After precipitating Ag_3PO_4 crystal using nitrate ammoniacal silver nitrate, the solution was then separated by centrifugation, and was washed then air-dried at 60˚C. Precise identification of phosphate is possible because of the high gravimetric factor of silver phosphate. The crystal, at this point, is contaminated with some organic compound mainly from an organic solution from the ion-exchange resin, and is a greenish brown color [45].

Furthermore, there are problems that usually arise when producing Ag_3PO_4. The most common one is the presence of metallic interferences such as Fe, Mn, and Al that are present in the sample. To identify its presence, the solution will turn from yellow to red during neutralization with KOH. Low reproducibility yields for duplicated sample is also a common problem encountered during the analysis of $\delta^{18}O_{PO4}$. This is due to the replacing of phosphates with carbonates, silica, or metallic oxides [6].

Table 5.2.1. Comparison of Ag_3PO_4 and $BiPO_4$ method

Ag_3PO_4 method	**$BiPO_4$ method**
Less time-consuming – approximately 3 days	Long operation time – approximately 6 days
Produces Ag_3PO_4 which is not hygroscopic	Produces hygroscopic $BiPO_4$
Easily degassed and dried in vacuum line	Having a difficult time to degassed and dried in vacuum line

One major advantage of using Ag_3PO_4 to isolate PO_4^{3-} is that, Ag_3PO_4 is not hygroscopic [17] therefore, it will be easily degassed and dried in vacuum. However, the purification procedure used to acquire Ag_3PO_4 needs to be adjusted depending on the nature and chemistry of the sample needed to be analyze.

A lot of commonalities in the protocols had been observed across different studies in the analysis of $\delta^{18}O_{PO4}$ particularly in the first procedures before Ag_3PO_4 precipitation. Concentration of PO_4 by co-precipitation with brucite or milk of magnesia following the magnesium-induced co-precipitation (MagIC) method that was introduced by Karl and Tien (1992) was used by McLaughlin et al. (2004) and Goldhammer et al. (2011). McLaughlin et al. (2004) and Weiner et al. (2011) used the cerium phosphate procedure which requires a pH dependent precipitation of pH5.5. Furthermore, Weiner et al. (2011), McLaughlin et al. (2004), Goldhammer et al. (2011), and Tamburini et al. (2010) uses anion-exchange resin to remove cations in H^+ form [10]. Final precipitation of Ag_3PO_4 includes the addition of $AgNO_3$, NH_4NO_3, and NH_4O

5.2.2. Halogenation of bismuth phosphate and silver phosphate

Historically, fluorination (using BrF_3) and bromination (using Br_2) of phosphate precipitate was used to determine isotopic composition of oxygen in phosphate ($\delta^{18}O_{PO4}$). These processes will halogenate the bismuth or silver ion, and will reduce bromine present while releasing O_2 gas which can be converted quantitatively to CO_2. The reaction for these methods are shown in equation 5.1 and 5.2. However, studies have shown disadvantages such as, poor O_2 yields, time-intensive preparation, can produce hazardous chemicals, and requires large sample sizes for both methods.

$$BiPO_4 + \frac{}{3} BrF_3 \longrightarrow BiF_3 + PF_5 + \frac{}{3} Br_2 + 2O_2 \qquad [5.1]$$

$$2Ag_3PO4 + Br_2 \longrightarrow Ag_4P_2O_7 + \frac{1}{2} O_2 + 2AgBr \qquad [5.2]$$

5.2.3. Isotope-ratio mass spectrometer as a detector for $\delta^{18}O_{PO4}$

Isotope-ratio mass spectrometry (IR-MS) is a technique that is used to determine the relative abundance of an isotope in a given sample. Conversion of Ag_3PO_4 to CO for analysis is done through pyrolysis at 1270˚C to 1450˚C usually in the presence of a carbon source to prevent full conversion. The carrier gas is helium, it transfer the gas sample to into a mass spectrometer via a continuous flow mode. Mass signals of 28 ($^{12}C^{16}O$) and 30 ($^{12}C^{18}O$; $^{14}C^{16}O$; $^{13}C^{17}O$) are then integrated and calculate the $^{18}O/^{16}O$ ratio [10].

The lack of internationally certified Ag_3PO_4 standards became a major challenge in the analysis of $\delta^{18}O_{PO4}$, despite of it being used for a long time in determining $\delta^{18}O_{PO4}$ in archeological and paleoclimate studies [10][57]. Using compounds other than Ag_3PO_4 like benzoic acid can introduce matrix to the sample that will have an effect to the analysis [10].

Summary and Conclusion

Phosphate pollution in the ecosystem particularly in different bodies of water might lead to critical degree of contamination and potential ecological risks. In response, several purification techniques were introduced. Various sources of sample in diverse location were analyzed via spectrophotometric methods and instrumental analyzers.

In performing analytical method in a laboratory, solid-phase extraction has been effectively used as a sample preparation technique because of its promising capability for extracting and isolating target analytes away from interferences with high recovery. This technique was more preferred than other separation method because of its advantages that solved some analytical problems before such as usage of large amount of solvents, long operation time, in need of intense labor and errors. In the present time, many scientist is still developing SPE for a higher performance in the future.

Several studies have investigated the potential use of sawdust material in dye and heavy metal adsorption. And it is observed that various factors such as pH, adsorbent dose, temperature, adsorbate concentration and contact time are capable of influencing the adsorption capacity of sawdust. Also, for most studies, Langmuir and Freundlich models played an important role in analyzing adsorbent as they have been useful in examining the adsorption behavior of sawdust. In general, the utilization of this low-cost material would be quite useful in developing technology for treating water and waste water. Furthermore, application of this development in solid-phase extraction can be possible to increase the industrial importance of sawdust as an adsorbent.

Stable isotope analysis can be a useful tool for identifying history, sources and a great help for tracking a certain compound in its corresponding cycles. However, this method is still in its developing stage and there are a plenty of room for improvement. The potential of this analysis as a tracer can be further developed especially with this kind of technology nowadays.

Acknowledgment

The authors wish to thank Department of Physical Sciences of Polytechnic University of the Philippines for this opportunity given to them to make this review paper. The authors are also grateful for the help of the people whom they've asked for advises for this particular topic.

References

[1] A. Ouakouak, L. Youcef, D. Boulanouar and S. Achour. "Adsorptive Removal of Phosphate from Groundwater Using Granular Activated Carbon". International Journal of Engineering Research in Africa, volume 32, page 53-61, December 2017. Available: https://doi.org/10.4028/www.scientific.net/JERA.32.53

[2] A. Shukla, Y. Zhang, P. Dubey, J. Margrave, and S. Shukla, "The role of sawdust in the removal of unwanted materials from water," Journal of Hazardous Materials, Elsevier, vol. 95, issues 1–2, pp. 137-152, November 2002.

[3] A.P. Cid-Andres, "A review on useful concepts for stable isotope of oxygen in phosphate extraction, purification and analysis of freshwater samples and other potential sources," Japan, Microchemical Journal, Elsevier, 2015

[4] A.P. Tudge, "A method of analysis of oxygen isotopes in orthophosphate – its use in the measurement of paleotemperatures," Geochimica et Cosmochimica Acta, Pergamon Press Ltd., 1960.

[5] A. Attia, R. Aboelenin, S. Kheder, G. Mohmed, S. El-Shafey, "The Effect of Activation Method on the Adsorption Performance of Sawdust Activated Carbon," British Journal of Applied Science & Technology, vol. 7, issue 3, pp. 302-315, February 2015.

[6] C. Lécuyer, "Oxygen isotope analysis of phosphate," Handbook of Stable Isotope Analytical Techniques, Vol.1, Chapter 22, Elsevier, 2004.

[7] C. Srinivasakannan and M.Z. Abu Bakar, "Production of activated carbon from rubber wood sawdust," Biomass and Energy, Elsevier, vol. 27, issue 1, pp. 89-96, July 2004.

[8] C.F. Poole, "New Trends in Solid-Phase Extraction" Trends in Analytical Chemistry, Vol. 22, No.6, 2003.

[9] C.F. Poole, "Solid-Phase Extraction with Discs" Encyclopedia of Separation Science, pp. 4141-4148, 2000.

[10] C.L. Davies, B.W.J. Surridg, D.C. Gooddy, "Phosphate oxygen isotopes within aquatic ecosystems: Global data synthesis and future research priorities," United Kingdom, Science of the Total Environment, Elsevier, 2014.

[11] Markel, Y. Kolodny, B. Luz, A. Nishri, "Phosporus cycling and phosphorus sources in Lake Kinneret: Tracing by oxygen isotopes in phosphate," Laser Pages Publishing Ltd, 1992.

[12] D.A. Skoog, D.M. West, F.J. Holler and S.R. Crouch. 2013. "Solid-Phase Extraction" Fundamentals of Analytical Chemistry, Page 838-839, 2013.

[13] D.A. Wells, "Bioanalytical Applications: Solid-Phase Extraction", 2013.

[14] D.A. Wells, "Solid-Phase Extraction with Cartridges", 2000.

[15] D.P. Jaisi, R.E. Blake, R.K.Kukkadapu, "Fractionation of oxygen isotopes in phosphate during its interaction with iron oxides," Geochimica et Cosmochimica Acta, Elsevier, 2009.

[16] E.S. Melby, D.J. Soldat, P. Barak, "Synthesis and detection of Oxygen-18 labeled phosphate," PLoS ONE. 2011.

[17] F. Tamburini, V. Pfahler, C. von Sperber, E. Frossard, S.M. Bernasconi, "Oxygen isotopes for unravelling phosphorus transformations in the soil-plant system: A review," Switzerland, Soil Science Society of America Journal, 2014.

[18] F.H. Firsching, "Precipitation of silver phosphate from homogeneous solution,"1961.

[19] G. Gruau, M. Legeas, C. Riou, E. Gallacier, F. Martineau, O. Hénin, "The oxygen isotope composition of dissolved anthropogenic phosphates: A new tool for eutrophication research?," France, Water Research, Elsevier, 2004.

[20] H.L.Q. Stuart-Williams and H.P. Schwarcz. "Oxygen isotopic determination of climatic variation using phosphate from beaver bone, tooth enamel, and dentine" Geochimica et Cosmochimica Acta, volume 16, no. 12, page 2539-2550, 1997. Available: https://doi.org/10.1016/S0016-7037(97)00112-9

[21] I. Liska, "On-line versus off-line solid-phase extraction in the determination of organic contaminants in water: Advantages and Limitations" Journal of Chromatography A, 655, pp. 163-176, August 1993.

[22] I.T. Miettinen, T. Vartiainen and P.J. Martikainen. "Phosphorus and Bacterial Growth in Drinking Water". Applied and Environmental Microbiology, volume 63, page 3242-3245, August 1997. Available: https://aem.asm.org/content/63/8/3242

[23] J.L. Bonnefous and R. Boulieu, "Comparison of Solid-Phase Extraction and Liquid-Liquid Extraction Methods for Liquid Chromatographic Determination of Diltiazem and its Metabolites in Plasma" Journal of Liquid Chromatography, 1990.

[24] J.L. Domagalski and H. Johnson. “Phosphorus and Groundwater: Establishing Links Between Agricultural Use and Transport to Streams”. United States Geological Survey Fact Sheet 2012-3004, January 2012.

[25] K. McLaughlin, S. Silva, C. Kendall, H.S. Williams, A. Paytan, “A precise method for the analysis of delta O-18 of dissolved inorganic phosphate in seawater,” American Society of Limnology and Oceanography, Inc., 2004.

[26] K.E. Elsbury, A. Paytan, N.E. Ostrom, C. Kendall, M.B. Young, K. McLaughlin, M.E. Rollog, S. Watson, “Using oxygen isotopes of phosphate to trace phosphorus sources and cycling in Lake Erie,” American Chemical Society, 2009.

[27] K.G. Stollenwerk. “Simulation of phosphate transport in sewage-contaminated groundwater, Cape Cod, Massachusetts”. Applied Geochemistry, volume 11, page 317-324, 1997. Available: https://doi.org/10.1016/0883-2927(95)00041-0

[28] K.M. Begum and N.M. Alhaji, “Adsorption Efficiency of Sawdust in Activated And Surface-Modified Forms - A Comparative Study,” Chemical Science Transactions, pp. 1364-1369, August 2013.

[29] L. Randrimanantsoa, C. Morel, L. Rabeharisoa, J.M. Douzet, J. Jansa, E. Frossard, “Can isotopic exchange kinetic method be used in soils with a very low water extractable phosphate content and a high sorbing capacity for phosphate ions?,” Geoderma, Elsevier, 2013.

[30] L. Zhang, L. Wan, N. Chang, J. Liu, C. Duan, Q. Zhou, X. Li and X. ssssssWang. 2011. “Removal of phosphate from water by activated carbon fiber loaded with lanthanum oxide”. Journals of Hazardous Materials 190, Page 848-855, April 2011. Available: https://www.sciencedirect.com/science/article/pii/S0304389411004596

[31] M. Šćiban, M. Klašnja, and B. Skrbic “Modified softwood sawdust as adsorbent of heavy metal ions from water,” Journal of Hazardous Materials, Elsevier, vol. 136, issue 2, pp. 266-27, August 2006.

[32] M. Suzumura, S. Ueda, and E. Sumi “Control of phosphate concentration through adsorption-desorption processes in groundwater and seawater mixing at sandy beaches in Tokyo bay, Japan”. Journal of Oceanography, volume 56, Page 667-673, July 2000. Available: https://link.springer.com/article/10.1023/A:1011125700301

[33] M.A. Karimi, A. Hatefi-Mehrjardi, A. Mohadesi, S.Z. Mohammadi, M. Taghdiri, J. Yarahmadi, H. Mahmoodian and S.N. Khorasani. November 2014. “Solid phase extraction-spectrophotometric determination of phosphate using modified magnetite nanoparticles as extractor”. Iranian Journal of Analytical Chemistry, volume 1, page 7-11. Available: http://ijac.journals.pnu.ac.ir/article_524_1.html

[34] M.A. Yahya, H.M. Mansor, W. Zolkarnaini, N.S. Rusli, A. Aminuddin, K. Mohamad, F.A. Sabhan, A.B. Atik, L.N. Ozair, “A brief review on activated carbon derived from agriculture by-product,” American Institute of Physics, June 2018.

[35] M.B. Young, K. McLaughlin, C. Kendall, W. Stringfellow, M. Rollog, K. Elsbury, E. Donald, A. Paytan“Characterizing the oxygen isotopic composition of phosphate sources to aquatic ecosystems,” American Chemical Society, 2009.

[36] M.L. Larrivee and C. F. Poole, “Solvation Parameter Model tor the Prediction of Breakthrough Volumes in Solid-Phase Extraction with Particle-Loaded Membranes” Analytical Chemistry, Vol. 66, No.1, January 1994.

[37] M.N. Sahmoune and A.R. Yeddou, “Potential of sawdust materials for the removal of dyes and heavy metals: examination of isotherms and kinetics,” Balaban Desalination Publication, vol. 57, pp. 24019-24034, January 2016.

[38] M.P. Juhascik and A.J. Jenkins, “Comparison of liquid/liquid and solid-phase extraction for alkaline drugs” Journal of Chromatographic Science, Vol. 47, August 2009.

[39] N. Srinivasa Rao and P. Rajendra Prasad. “Phosphate pollution in the groundwater of lower Vamsadhara river basin, India”. Environmental Ecology 31, May 1997.

[40] N. Yousefi, A. Fatehizedeh, K. Ghadiri, N. Mirzaei, S.D. Ashrafi and A.H. Mahvi. 2015. “Application of nanofilter in removal of phosphate, fluoride and nitrite from groundwater”. Desalination Water Treatment., page 1-7. Available: http://dx.doi.org/10.1080/19443994.2015.1044914

[41] P. Atkins and J. De Paula, “Physical chemistry” 9th edition, New York, pp. 889-894, 2010.

[42] P.K. Malik, “Use of activated carbons prepared from sawdust and rice-husk for adsorption of acid dyes: a case study of Acid Yellow 36,” Centre for Surface Science, Elsevier, vol. 56, issue 3, pp. 239-249, March 2003.

[43] Q. Shen, W. Dong, Y. Wang, L. Gong, Z. Dai and H. Y. Cheung, “Pipette tip solid-phase extraction and ultra-performance liquid chromatography/mass spectrometry based rapid analysis of picrosides from Picrorhiza scrophulariiflora” Journal of Pharmaceutical and Biomedical Analysis 80, pp. 136-140, 2013.

[44] R. Ansari and F. Raofie, “Removal of Lead Ion from Aqueous Solutions Using sawdust Coated by Polyaniline,” E-journal of chemistry, vol. 3, issue 1, pp. 49-59, January 2006.

[45] R.A. Crowson, W.J. Showers, E.K. Wright, T.C. Hoering, “Preparation of phosphate samples for oxygen isotope analysis,” American Chemical Society, 1991.

[46] R.E. Blake, J.C. Sae, A. Lepland, “Phosphate oxygen isotopic evidence for a temperate and biologically active Archean ocean,” Norway, Macmillan Publishers Limited, 2010.

[47] R.E. Majors, “New Designs and Formats in Solid-Phase Extraction Sample Preparation” LC•GC Europe, December 2001.

[48] R.F. Holt, D.R. Timmons and J.J. Latterell. "Accumulation of Phosphates in Water". Journal of Agricultural and Food Chemistry, volume 18, page 781-784, 1970.

[49] S. Benyoucef, M. Amrani, “Removal of Phosphate from Aqueous Solution with Modified Sawdust,” Procedia Engineering, Elsevier, vol. 23, pp. 58-69, December 2012.

[50] S. Chakraborty, J.K. Basu, S. De, and S. DasGupta, "Adsorption of Reactive Dyes from a Textile Effluent using Sawdust as the Adsorbent," Industrial and Engineering Chemistry Research, pp. 4732–4741, May 2006.

[51] S. Gupta and B.V. Babu, "Modeling, simulation, and experimental validation for continuous Cr (VI) removal from aqueous solutions using sawdust as an adsorbent," Bioresource technology, Elsevier, vol. 100, issue 23, pp. 5633-5640, December 2009.

[52] S. Ötles and C. Kartal, "Solid-Phase Extraction (SPE): Principles and Applications in Food Samples" Acta Sci. Pol. Technol. Aliment., 15(1), 5–15, 2016.

[53] S.D. Khattri and M.K. Singh, "Use of Sagaun Sawdust as an Adsorbent for the Removal of Crystal Violet Dye from Simulated Wastewater," Wiley online library, July 2011.

[54] Supelco Bulletin, "Guide to Solid Phase Extraction" Sigma-Aldrich Co., 1998.

[55] T. Goldhammer, B. Brunner, S.M. Bernasconi, T.G. Ferdelman, M. Zabel, "Phosphate oxygen isotopes: Insights into sedimentary phosphorus cycling from the Benguela upwelling sytem," Germany, Science Direct, Elsevier, 2011.

[56] T. Yamashita, and R. Yamamoto-Ikemoto "Nitrogen and phosphorus removal from wastewater treatment plant effluent via bacterial sulfate reduction in an anoxic bioreactor packed with wood and iron". International Journal of Environmental Research and Public Health, 11, page 9835-9853, Available: doi:10.3390/ijerph110909835

[57] T.W. Venemann, H.C. Fricke, R.E. Blake, J.R. O'Neil, A. Colman, "Oxygen isotope analysis of phosphate: A comparison of techniques for analysis of Ag_3PO_4," Chemical Geology, Elsevier, 2001.

[58] V. Campos. "Arsenic in groundwater affected by phosphate fertilizers at Sao Paulo, Brazil". Environmental Geology volume 42 page 83-87, 2002.

[59] V. Daux, C. Lecuyer, M.A. Heran, R. Amiot, L, Simon, F. Fourel, F. Martineau, N. Lynnerup, H. Reychler, G. Escarguel"Oxygen isotope fractionation between human phosphate and water revisited," France, page 1138-1147, Journal of Human Evolution, Elsevier, 2008.

[60] V. Daux, C. Lecuyer, M.A. Heran, R. Amiot, L. Simon, F. Fourel, F. Martineau, N. Lynnerup, H. Reychler, and G. Escarguel. June 2008. "Oxygen isotope fractionation between human phosphate and water revisited". Journal of Human Evolution volume 55 page 1138-1147. Available: https://www.sciencedirect.com/science/article/pii/S004724840800119Xssssss

[61] V. Walker and G.A. Mills, "Solid-Phase Extraction in Clinical Biochemistry", Ann Clin Biochem, Vol. 39, pp. 464-477, 2002.

[62] Y. Liu, Y. Zhu, X. Qiao, B. Zheng, S. Chang and Q. Fu. December 2018. "Investigation of nitrogen and phosphorus contents in water in the tributaries of Danjiangkou Reservoir". R.Soc. opensci.5:170624.