Determination of total and available phosphorus in soil samples collected from Tarhuna, Libya using spectrophotometry.

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الملخص

أجريت هذه الدراسة لتحديد مستويات الفوسفور الكلي والمُتاح في عينات من تربة منطقة ترهونة باستخدام تقنية أبحريت هذه اللونية. وفي هذه الدراسة تم تجميع 12 عينة من الطبقة السطحية للتربة، ومن ست مواقع رئيسية تُمثل المطيافية اللونية. وفي هذه الدراسة تم تجميع 12 عينة من الطبقة السطحية للتربة، ومن ست مواقع رئيسية تُمثل (HNO3) المنطقة المدروسة. وبعد المُعالجة الأولية للعينات تم هضمها باستخدام خليط من حمضي النيتريك (HNO3) والبيروكلوريك (HCO4) بنسبة 2:3 وفقاً للخطوات المُتبعة في الطريقة المدرجة بالمرجعين (27، 28). وفي نفس الوقت تم استخدام محلول من بيكربونات الصوديوم (HNO3) وفي نفس الوقت تم استخلاص الفوسفور المُتاح من العينات المدروسة باستخدام محلول من بيكربونات الصوديوم (NaHCO3) وفي نفس الوقت تم استخلاص الفوسفور المُتاح من العينات المدروسة باستخدام محلول من بيكربونات الصوديوم (NaHCO3) تركيزه وماستخلاص الفوسفور المُتاح من العينات المدروسة باستخدام محلول من بيكربونات الصوديوم (NaHCO3) تركيزه محلول من بيكربونات الصوديوم (NaHCO3) تركيزه والنيو حسب الطريقة المُتبعة في المرجع [29]. وبعد ترشيح المحاليل المُتحصل عليها في الحاليتين تم إضافة محلول من مولاري حسب الطريقة المُتبعة في المرجع [29]. وبعد ترشيح الماليل المُتحصل عليها في الحاليتين تم إضافة محلول من مولاري حسب الطريقة المناحة المرجع [29]. وبعد ترشيح الماليل المُتحصل عليها في الحاليتين تم إضافة محلول من مولاري حسب الطريقة المحاليل للحصول على لون أزرق ناتج من تكون مُعقد الفوسفور مع الموليبدات، وقد محلول من موليدات الأمونيوم إلى هذه المحاليل للحصول على لون أزرق ناتج من تكون مُعقد الفوسفور مع الموليبدات، والذي تم قياس الشدة اللونية له عند الطول الموجي 800 خانومتر باستخدام مقياس الشدة الفوسفور مالم كم عوالذي يوقد محصل عليها ان تراكيز الفوسفور الكلي في عينات التربة تراوحت ما بين 100 ح 400 ملجم/كجم أظهرت النتائع المني على وبمعدل بلغ 212 ملجم/كجم، في حين تراوح تركيز الفوسفور المتاح لهذه العينات ما بين 24 ما ح 50 ملجم/كجم وبمعدل بلغ 212 ملجم/كجم، في حين تراوح تركيز الفوسفور المتاح لهذه من أن العينات المروسة تحتوي على وبمتوسط قُدُر بحوالي 42 ملجم/كجم، وخلُصت الدراسة إلى أنه وعلى الرغم من أن العينات المروسة يوي على وراكيز مافوسفور المتاح لها ما من الموسفور الكم من أن العينات المرمكي عا

تحوي كميات مُعتبرة من الفوسفور المُتاح. وقد أرجع سبب ذلك إلى عدة عوامل منها ما قد يكون طبيعية، أو بسبب النشاطات الزراعية المحدودة، أو إلى قلة معدلات سقوط الأمطار في السنوات الأخبرة.

الكلمات المفتاحية: الفوسفور الكلي، الفوسفور المُتاح، مُعقد الفوسفور والموليبدات، تربة، هضم، مقياس الشدة الطيفية اللوني.

Abstract

This study aimed to evaluate the levels of total and available phosphorus in soil samples from Tarhune, Libya using spectrophotometry technique. Twelve representative top layer soil samples were collected from six different sites and pre-treated for analysis. For total phosphorus determination, the samples were digested with a mixture of concentrated HNO₃ and HClO₄ in a 2:3 ratio, as described in [27, 28]. Meanwhile, available phosphorus was extracted from the samples using 0.5 M NaHCO₃, following the method outlined in [29]. Both mixtures were filtered, and ammonium molybdate was added to the filtrate solutions to form a blue phosphorus molybdenum complex. The intensity of the blue color at 830 nm was measured using a spectrophotometer. The results showed that the total phosphorus concentration ranged from 100 to 405 mg/kg, with an average of 212 mg/kg. The available phosphorus concentration fluctuated between 24 and 56 mg/kg, with an average of 42 mg/kg. The study concluded that although the soil samples had a lower total phosphorus content compared to the global mean value, the amounts of available phosphorus were significant. This may be attributed to various factors including natural occurrence, limiting cultivation activities, and rainfall rates.

Key words: total; available; phosphorus; blue molybdate; soil; digestion; spectrophotometer.

1- Introduction

Phosphorus is the eleventh most common element in the Earth's crust and is mostly found in phosphate rocks [1,2]. It is one of the seventeen essential nutrients required for plant growth [3] and is the second most important macronutrient for crop growth, after nitrogen [4]. Phosphorus plays a significant role in various plant processes, including photosynthesis, respiration, energy generation, nucleic acid biosynthesis, and as a crucial component of plant structures like phospholipids [4,5]. In natural water, phosphorus is a minor component because most inorganic phosphorus compounds have low solubility. Dissolved concentrations in natural waters typically fluctuate between 0.01 - 0.1 ppm and rarely exceed 0.2 ppm. The environmental behavior of phosphorus is mainly influenced by the low solubility of its inorganic compounds, its strong adsorption to soil particles, and the fact that it is an essential nutrient for most life forms, including animals, plants, and microbes [6].

The original source of phosphorus in natural soils is the weathering of primary minerals such as apatite, strengite, and variscite in igneous, sedimentary, and metamorphic rocks [7,8]. These minerals are highly stable, and the release of phosphorus to the soil solution from them is generally low [9, 10]. On the other hand, secondary phosphorus minerals, including calcium (Ca), iron (Fe), and aluminum (Al) phosphates, have different dissolution rates depending on the size of the mineral particles and soil pH [9, 11-13]. As soil pH increases, the solubility of Fe and Al phosphates increases, while the solubility of calcium phosphate starts

to increase until reaching neutral pH values. Calcium phosphate becomes insoluble when the pH exceeds 8.0 [14, 15].

In acidic soils, phosphorus is commonly bound to iron and aluminum oxides and hydroxides through chemical precipitation or physical adsorption [16]. However, in neutral-to-alkaline soils, the retention of phosphorus is mainly influenced by precipitation reactions [17], although phosphorus adsorption can also occur on the surface of calcium carbonate [18] and clay minerals [19]. Both precipitation and adsorption reactions play an essential role in the retention mechanism of phosphate. When the phosphate concentration is low, the primary process is the adsorption of phosphate ions onto the surfaces of more crystalline clay compounds, sesquioxides, or carbonates [8, 20-22]. Conversely, when the phosphate sin acidic soils, and Ca and Mg phosphates in alkaline soils [8, 20, 22, 23].

Despite its high abundance in soils, a number of soils throughout the world are suffering from phosphorus deficiency. It has been reported that the available portion of phosphorus for plants is only 1-3% of the total phosphorus in the soil due to the aforementioned precipitation and adsorption reactions [24]. Therefore, the objective of this study is to evaluate the total and available phosphorus levels in soil samples in Tarhuna (which has been classified as neutral-to-alkaline soils [25, 26]) in order to determine the extent of phosphorus sufficiency for plants in these soils. As far as our knowledge goes, no related study has been conducted in this area.

2- Experimental

2.1- Sites, Sampling, and Pre-treatment

The region of Tarhuna is situated in the northwest of Libya, approximately 85 km southeast of the capital city, Tripoli (see Figure 2.1). Tarhuna consists of five main districts: Addawoun, Al-Khadra, the city center, Sidi as Sayd, and Fammmolgha, along with several smaller sub-districts. A total of twelve soil samples were collected from various agricultural areas in Tarhuna. Two samples were taken from each main district, except for the city center area, which had four soil samples collected. The map (Figure 2.1) and table 2.1 display the sites of samples were collected from the top surface layer using a plastic spoon and stored in plastic bags. In the laboratory, the soil samples were air-dried for three days at room temperature. Subsequently, they were gently crushed with a wooden roller and passed through a 2 mm sieve.



Figure 2.1 In the top right corner, the Libyan map displays the location of Tarhuna, which is represented by a dark color. The larger map provides an

overview of the investigated area, Tarhuna, and includes the sites where the samples were collected, along with their corresponding numbers.

Table 2.1: The number and sites of the soil samples, as well as their cultivation activities, were not consistent across all sites (none of the sites were irrigated).					
Sample No.	Site	Cultivation activities			
1	Addawoun	includes olive and almond trees, along with a			
		designated grazing area.			
2	Addawoun	includes olive and almond trees, along with a			
		designated grazing area.			
3	Al-Khadra	only grazing area.			
4	Al-Khadra	only grazing area.			
5	North city center	few trees.			
6	North city center	few trees.			
7	South city center	few trees.			
8	South city center	few trees.			
9	Sidi as Sayd	few trees and a grazing area.			
10	Sidi as Sayd	few trees and a grazing area.			
11	Fammmolgha	olive trees and grazing area.			
12	Fammmolgha	olive trees and grazing area.			

2.2- Reagents and Glassware

The glassware and low-density polyethylene containers were cleaned using phosphate-free detergent. They were then rinsed with double-distilled water. The reagents were prepared using water that had been double distilled. All chemicals used in this study were of analytical reagent grade. The used reagents were potassium dihydrogen phosphate, ammonium molybdate, hydrazine sulfate, sodium hydrogen carbonate, concentrated acids (nitric, perchloric, and sulfuric), and two pH buffers: 7.0 and 10.0.

A 100 mL standard measuring flask was used to prepare 100 ppm of phosphate. This involved weighing 14.1 mg of potassium dihydrogen phosphate (KH₂PO₄)



and transferring it into the flask. The potassium dihydrogen phosphate was then dissolved in water and diluted to the mark with distilled water.

For ammonium molybdate solution, 1.708 g of ammonium molybdate $[(NH_4)_6 Mo_7O_{24}.4H_2O]$ was dissolved in 250 mL of double-distilled water.

A solution of hydrazine sulfate was prepared by weighing 0.125 g of $(N_2H_5)(HSO_4)$ and dissolving it in a small amount of water. The resulting solution was then transferred into a clean 100 mL standard measuring flask.

Lastly, a 0.5 M sodium bicarbonate solution was made by dissolving 42 g of NaHCO₃ in 1.0 L of double-distilled water.

2.3- pH Measurements

The pH meter was calibrated with 7.0 and 10.0 standard buffers according to the manufacturer's instructions. Then, 10.0 grams of dried soil samples were weighed and placed into a 100 mL beaker, and 25 mL of double-distilled water was added. The soil-water mixture was shaken thoroughly for 60 minutes. The glass electrode was immersed into the sample solution, and pH measurements were taken for each sample.

2.4- Digestion of soil samples for total phosphorus measurements

According to [27, 28], two grams of air-dried soil were weighed out and placed in a 250 ml Erlenmeyer flask. The flask was then heated on a hot plate. Next, 20 mL of concentrated nitric acid (HNO₃) was added to the flask and mixed well. The soil-HNO₃ mixture was allowed to cool slightly. After that, 30 mL of concentrated perchloric acid (HClO₄) was added to the sample at boiling temperature (\sim 200 °C)

for 20 min. During this time, dense white fumes volatilized and the insoluble solid material was left at the bottom of the flask. If necessary, a little extra HClO₄ acid was used to wash any black particles that had stuck to the sides of the flask. After allowing the residual mixture to cool, it was filtered through Whatman No. 40 and transferred into a 100 mL volumetric flask. The flask was then filled up to the mark with double-distilled water. Aliquots of this solution were taken for determining the total phosphorus using spectroscopic measurements.

2.5- Soil solution extraction for available phosphorus concentration

Five grams of soil samples were precisely weighed and placed in a 250 mL beaker. They were then shaken with 100 mL of 0.5 M NaHCO₃ solution for 30 minutes, ensuring a constant shaking rate. The mixture was subsequently filtered through Whatman No. 40 or another appropriate filter paper [29]. The resulting filtrate was used to determine the available phosphorus in the soil samples via spectrophotometer.

2.6- Spectrophotometric measurements for phosphorus determination

To prepare a series of phosphorus standards, 1, 2, 3, 4, and 5 mL of 100 ppm stock solution were taken into 100 mL volumetric flasks to obtain 1, 2, 3, 4, and 5 ppm, respectively. To each of these flasks, 4 mL of hydrazine sulfate, 10 mL of ammonium molybdate, and 10 mL of 0.2 M sulfuric acid solutions were added and diluted to the mark with double-distilled water. The standard measuring flasks were kept in a water bath for 30 min. The temperature of the water bath was set to 60 °C. A blue color was developed due to the formation of ammonium phosphomolybdate complex [28, 30]. The formed complex was allowed to settle,

and its absorbance was measured at a wavelength of 830 nm. An experimental blank solution was used for carrying out the correction of the baseline. For determining phosphorus in the soil solutions, similar procedures were followed with each sample solution and their absorbance were measured.

2.7- Instrumentation

For absorbance measurements, a JENWAY 6300 visible spectrophotometer was utilized. This spectrophotometer has a wavelength range of 340–900 nm. The absorbance measurements were taken by inserting the sample tube containing the solution into the spectrophotometer. In order to measure pH, a HANA 8014 potentiometer and HNAN glass electrode were employed.

3- Results and Discussion

Table 3.1 presents the pH values, total phosphorus concentrations, and available phosphorus concentrations in the soil samples from Tarhuna. The pH values range from 8.27 to 8.90, with an average of 8.63. These values indicate that the soils being studied are slightly to moderately alkaline, and can be classified as neutral-calcareous soils. This type of soil is known to have a low availability of phosphorus due to its precipitation as calcium phosphate [25].

It can also be observed from Table 3.1 that the total phosphorus concentrations varied between 100 - 405 mg/kg, with an average of approximately 212 mg/kg. Sample 12 had the highest total phosphorus concentration, while sample 3 had the lowest concentration. The overall concentration of soil samples in Tarhuna is lower than the average total phosphorus concentration in soils worldwide, which ranges from 500 - 800 mg/kg [31]. This is reasonable because of the main source

of phosphorus in these soils is only the weathering of primary minerals in igneous, sedimentary, and metamorphic rocks; and no fertilizers were added to studied sites.

Table 3.1: pH values, total phosphorus, and available phosphorus in soil samples from Tarhune.					
Sample No.	pН	Total P	Available P	Available-to-total ratio	
		(mg/kg)	(mg/kg)		
1	8.63	245	50	20.4%	
2	8.65	220	52	23.6%	
3	8.27	100	24	24.0%	
4	8.81	165	32	19.4%	
5	8.51	165	53	32.1%	
6	8.90	180	41	22.7%	
7	8.50	245	48	19.6%	
8	8.68	115	26	22.6%	
9	8.52	190	52	27.3%	
10	8.84	270	34	12.6%	
11	8.46	240	56	23.3%	
12	8.76	405	32	08.0%	
An average		212	42	21.3%	





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On the other hand, the phosphorus available in the studied samples ranges from 24 to 56 mg/kg, with an average of 42 mg/kg. Despite the low total concentration of phosphorus in the samples and the relatively high pH values (which limit phosphorus availability in the soil), the studied samples still contain significant amounts of available phosphorus. This can be attributed to several factors. Firstly, the forms of phosphorus in soils change over time as the soil evolves. In young calcareous soil, phosphorus is mainly in the form of apatite phosphorus. However, as plants grow and organic matter accumulates in the soil, a significant amount of apatite phosphorus is converted into organic phosphorus. Secondly, apatite weathers slowly, releasing phosphate ions in the form of HPO_4^{2-} into the soil solution. These ions are absorbed by roots and microorganisms, which convert the phosphorus into organic compounds. As a result, a significant amount of phosphorus is present in the soil as organic phosphorus. It is common for 20 to 30 percent of phosphorus in plow layers of mineral soils to be organic phosphorus. Microorganisms mineralize organic phosphorus, releasing it back into the soil solution as soluble species of HPO_4^{2-} [32]. Thirdly, the presence of naturally occurring competing ions in the soil can encourage phosphorus release. Organic anions from stable manure and silicates have been reported to be effective in reducing phosphorus fixation [33, 34]. Humic acids, in particular, have been found to play an important role in preventing or decreasing phosphorus fixation by chelating the ions responsible for the reaction [34-36]. Additionally, the scarcity of rainfall in recent years may result in reduced leaching of soluble phosphorus into deeper layers of the soil, thus maintaining higher levels of available phosphorus in the top layers of soil. Lastly, the low cultivation activities in the sampling points, where all samples were collected from locations containing olive

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trees and seasonal grasses, contribute to the maintenance of available phosphorus levels.

Moreover, it can be seen from Figure 3.1 that there is no clear relationship between the total and available phosphorus in the samples. In other words, while the samples contain a high amount of total phosphorus, it does not necessarily mean that there is also a high amount of available phosphorus, and vice versa. This lack of correlation may be attributed to the different conditions of cultivation of each soil sample, as well as the weathering processes for each sample.

4- Conclusion

This study has deduced that the total phosphorus in the parent rocks of the investigated soils is relatively low compared to the global average. The results showed an average phosphorus concentration is around 212 mg/kg. However, an interesting finding of this study is presence of significant amount of available phosphorus in Tarhune. The average available phosphorus has been estimated to be around 42 mg/kg. This relatively high quantity of available phosphorus may be attributed to natural causes, such as transformation from one form to another, decomposition of residual plants and animals, low rainfall rates that minimize leaching into deep layers, or low cultivation activities. Finally, the results of the pH measurements have indicated that the soil is slightly to moderately alkaline.

5- References

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