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Fractionation and availability of phosphorus in acid soils of Hagereselam, Southern Ethiopia under different rates of lime

Alemayehu Kiflu[1*], Sheleme Beyene[1] and Schoenau Jeff[2]

Abstract

Background: Phosphorus (P) availability is commonly assumed to limit productivity in many tropical soils, yet there is relatively little information on the phosphorus chemical forms, distribution, and transformations that P undergoes in Ethiopian soils. We used a sequential fractionation scheme to assess phosphorus fractions of acid soils of Southern Ethiopia. The study area called Hagereselam is characterized by high soil acidity, and the availability of P in the study area is relatively low. As crop production in Ethiopia is dominated by low external input practices, native P remains the main contributor to plant P nutrition in many locations.

Result: Although the total P concentration is 829.7 mg kg^{-1}, the organic P content was relatively low (58 mg kg^{-1}) and constituted on average less than 7% of the total P. The ratio of organic carbon to organic P was generally greater than 240, suggesting the potential for immobilization of P. The Al + Fe-associated P was the dominant inorganic P pool. The majority of the P occurred in recalcitrant form (568.3 mg kg^{-1}). Readily available, exchangeable phosphate, as extracted by anion-exchange resin membranes, was present in very low concentrations (3.3 mg kg^{-1}); moreover, labile P accounted for less than 2% (9.9 mg kg^{-1}) of the total soil P.

Conclusion: Lime was used as a reclamation material for acid soils, and the application of lime significantly affected the different P fractions and was involved in the transformation of P fraction. Organic P forms were significantly lower for higher levels of lime application, suggesting that lime amendment enhanced organic P decomposition. Moreover, application of lime increased Ca-P and decreased Al + Fe–P for acid soils.

Keywords: Inorganic phosphorus, Labile phosphorus, Liming, Occluded phosphorus, Phosphorus fraction, Sequential phosphorus extraction, Resin membrane

Background

Crop productivity on most tropical soils is limited by low availability of essential nutrients. This is especially true for soil P in sub-humid areas. The reasons include low native soil P concentrations and strong soil P retention associated with weathering and other soil properties. The problem is further worsened by nutrient mining due to the low-input agriculture practices. Yet many small scale subsistence farmers in Ethiopia are unable to make regular applications of P fertilizer because of economic constraints.

*Correspondence: alemanchy@gmail.com
[1] College of Agriculture, Hawassa University, Awassa, Ethiopia
Full list of author information is available at the end of the article

Soil P chemistry is complex with numerous forms of P present and possible transformations that P may undergo depending on the physical, chemical, and biological environment. Soil P occurs in organic forms as well as in inorganic compounds in association with aluminum (Al), iron (Fe), and calcium (Ca). The strength of association affects the availability of P to plants [31]. This has generated attention in identifying the pools of soil P, and in quantifying their contribution to plant P nutrition. This is because an understanding of soil P forms and the transformations that affect P availability can be useful when making decisions about how to improve soil P availability for crop production. Phosphorus is among the most limiting nutrients for food production in the sub-humid and humid tropical highlands of East Africa [22]. This deficiency is mainly caused by the inherent low P content characteristics of the parent material and by the strong sorption of PO_4^{3-} to Al and Fe oxides. Limited availability of soil P to plants may be attributed to the inherent low soil phosphorus content and high degree of phosphorus fixation. Significant phosphorus fixation and its precipitation as iron and aluminum phosphates are very common in intensively weathered and sesquioxide-rich acidic soils [3]. There are two ways of addressing such low P availability problems in acidic soils: soil liming for pH neutralization and application to P fertilizer. With liming, added carbonate (CO_3^{2-}) is readily hydrolyzed and produces hydroxide ions (OH^-), increasing soil solution pH. This severely impairs Al oxide activity reduces the ability of the oxide surfaces to retain P and consequently increases P availability [11]. The second way is a more generous P applications to addresses the low P availability rather than addressing the root of the problem but it is not economical.

Although most soils of the highlands are characterized by soil acidity and high P fixation due to intensive weathering and leaching attributed to high rainfall [7], there is limited knowledge about soil P distribution, transformations, and fertility in Ethiopia, but the general impression about the soils is that availability of P in the dominant soils of in the country is relatively low [2, 7]. Although much of the Ethiopian soils have a high potential for crop production, no detailed work has been done on the P status, forms, and dynamics [19]. Most past studies have focused only on the plant-available inorganic P fraction [15, 28, 34]. Because inorganic phosphorus (Pi) and organic phosphorus (Po) differ in their rates of phosphorus release, it is necessary to separate them into different biologically meaningful pools. This can be accomplished by sequentially fractionating soil P into different inorganic and organic pools. This study is therefore intended to quantify the various labile and stable pools of inorganic and organic P in acid soils of southern Ethiopia and the influence of reclamation of acid soils via application of lime on soil P fractions and availability.

Methods

Description of the study sites

The study was conducted in Southern Ethiopia. The study area is called Hagereselam located in southern region between geographical coordinates of 6° 24′09″–6° 36′29″ N latitude and 38°26′21″–38°42′25″ E longitude with an average altitude of 2765 m.a.s.l. (Fig 1). The annual rainfall of the area varies from 900 to 1400 mm with mean monthly temperature ranging from 6° to 22° C (Fig. 2). The major soil type of the area is typic paleustults Alemayehu et al. [1] former from basaltic parent material. Crops grown in Hagereselam region include barley

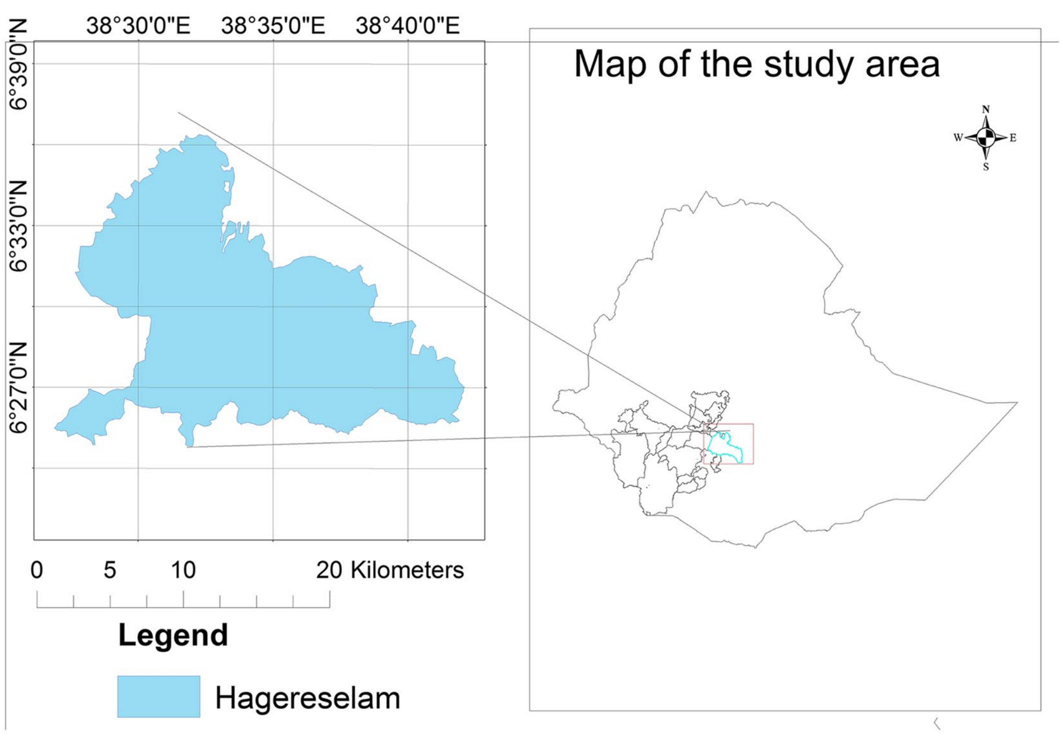

Fig. 1 Location map of the study area

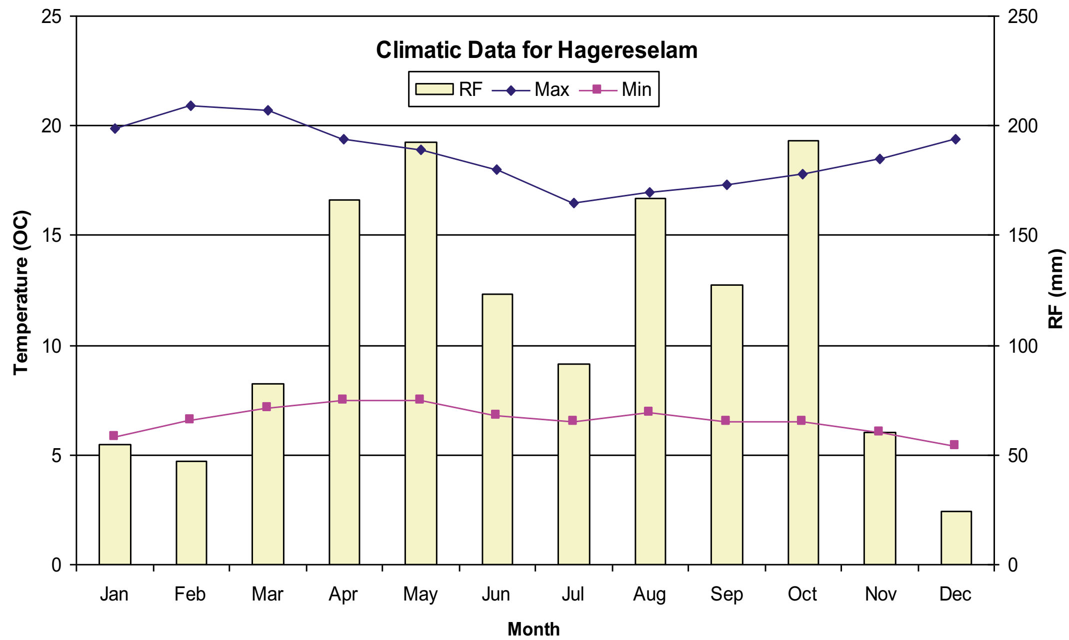

Fig. 2 Climatic data for the study area

(*Hordeum vulgare*), enset (*Ensete ventricosum*), potato (*Solanum tuberosum*), and wheat (*Triticum aestivum*).

Soil sampling and analysis

Composite surface soil samples of the 0–15 cm depth were collected using random sampling technique from representative (15) crop fields using auger. In the remediation pot study, 4 kg of soil was crushed to pass through a 2 mm sieve and were placed in plastic pots. The treatments were arranged in a completely randomized design (CRD) with five replications. The lime requirement (LR) was determined according to a method described by Shoemaker et al. [27]. This method is frequently used in Ethiopia and southern Ethiopia in particular because it is suitable for soils, requiring lime correction of more than 1 ton ha^{-1} and soils with pH less than 5.8 Pansu and Gautheyrou [18]. Accordingly, the lime requirement was found to be 6.5 ton ha^{-1}. Four different rates of lime (0, 3.25, 6.5, and 9.75 ton ha^{-1}) were used and the soil was incubated for 3 months at field capacity moisture.

Total nitrogen of the soil was determined by wet acid Kjeldahl digestion method [32]. Soil organic carbon was determined by dry combustion method using a LECO CR-12 Carbon Determinator (LECO Instruments Ltd, Mississauga, ON L5T 2H7). The exchangeable base cations (K^+, Ca^+, Mg^{2+}, and Na^+) were extracted with 1 M—ammonium acetate at pH 7.0. Cation exchange capacity (CEC) of the soil was estimated by measuring the sum of exchangeable cations from the ammonium acetate extracted sample. The Bouyoucos hydrometer method was used for the determination of soil particle size [4]. The soil pH was measured using a glass combination electrode pH meter with the electrode inserted in the filtered supernatant solution of a 1:2.5 soil to water suspension. DTPA extraction was used to extract available Cu, Fe, Mn, and Zn from the samples [14]. As recommended by Sahlemedhin and Taye [21] for most Ethiopian soils, soil available phosphorus was determined by the Olsen extraction method. A sequential extraction procedure was applied to distinguish between different soil P pools (Fig. 3). First, the most labile inorganic phosphorus was extracted using an anion-exchange resin membrane (Resin-P). Sodium bicarbonate 0.5 M (pH 8.5) was added to removed labile Pi (NaHCO3-Pi) and Po ($NaHCO_3$-Po) sorbed to the soil surfaces [5]. Sodium hydroxide 0.1 M was added to extract P associated with amorphous and some crystalline Al and Fe oxides [30] and Po associated with humic compounds (NaOH-Po). Before the acid digests, 1 M hydrochloric acid (HCl–P) was added to extract mainly apatitic phosphorus. It is unavailable in the short term. The residue containing the most chemically stable Po and Pi forms was digested using concentrated $H_2SO_4 + H_2O_2$ (Resid-P) [32]. Phosphorus in the extracts or digests was determined using the ascorbic acid molybdenum blue method of Murphy and Riley [17].

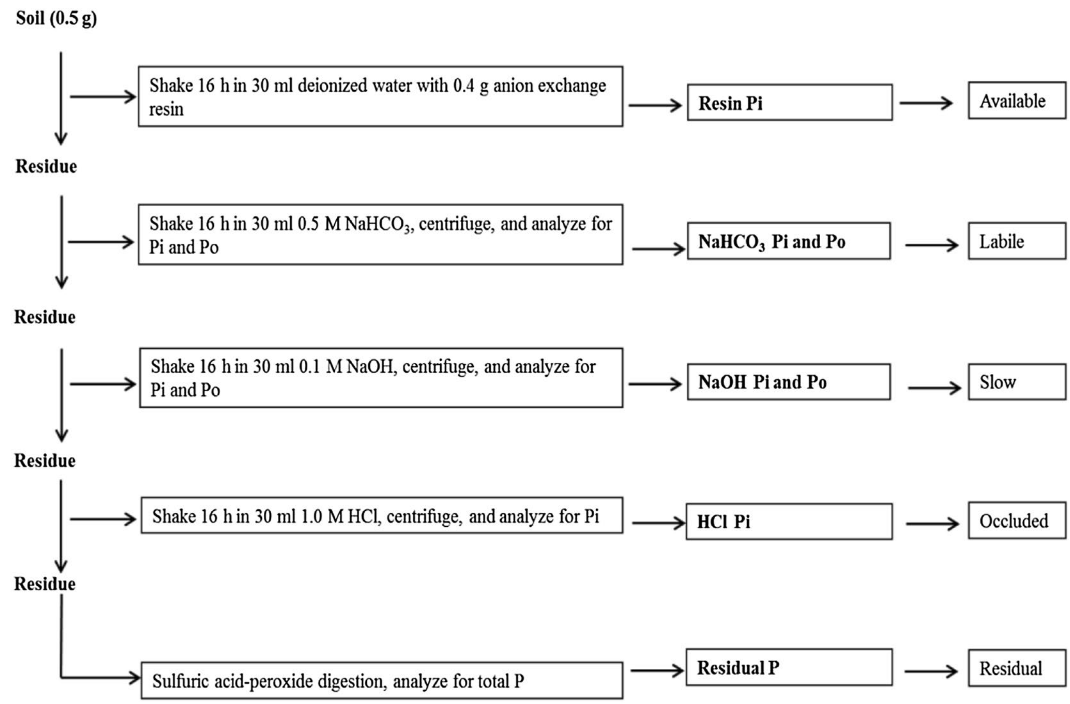

Fig. 3 Flow chart of sequential phosphorus (P) extraction procedure (Pi is inorganic P, Po is organic P) (Adapted from [20])

Statistical analysis

Analysis of variance and mean separation were carried out for the selected soil physicochemical properties and P fractions using SAS software [24] to differentiate the extent of relations and associations between parameters and treatments. The significance was then evaluated using the least significant difference (LSD) at 5% level significance.

Results and discussion

General properties of soils of the study area

The general physical and chemical properties of the soils prior to the application of the treatments are presented in Table 1. Soils are loam with low pH (4.51). At this, pH solubility and availability of most nutrients could be strongly affected Hazelton and Murphy [9]. The available soil P content is also low indicating the fixation of P to other unavailable sites. The Fe content of the soil is 39.8 mg kg^{-1} with low (8.4 mg kg^{-1}) calcium content [13].

Table 1 General characteristics of the soil for the study areas

Soil property	Amount
Soil pH	4.51
Sand	47%
Silt	31%
Clay	22%
Cu	0.41 ppm
Fe	39.8 ppm
Mn	24.19 ppm
Zn	0.59 ppm
Ca	8.4 ppm
CEC	33.90 C molc kg^{-1}
TN	0.35%
OC	3.44%
Av. P	7.1 ppm

Soil phosphorus fractions

Inorganic and organic phosphorus concentrations in sequential extracts are shown in Table 2. Phosphate extracted by anion-exchange membranes was found to be very low 3.3 mg kg^{-1} (0.4%). $NaHCO_3$ extracted inorganic and organic phosphate fractions were also found to be low 3.6 and 3.0 mg kg^{-1}, respectively. In general, the biologically available forms (Phosphate extracted by anion-exchange membranes, $NaHCO_3$-Pi, and $NaHCO_3$-Po) were found to be very low (less than 2%). Therefore, plant growth could be affected due to the presence of low biologically available P forms and this indicates management of P is a must for a better production. The NaOH extractable P (Pi and Po) also called Fe–Al associated P was

Table 2 Sequentially extracted soil phosphorus fractions (mg P kg^{-1}) for the soils of the study site

P-form	Amount	% of total
Resin-P	3.3	0.40
$NaHCO_3$ Pi	3.6	0.43
$NaHCO_3$ Po	3.0	0.36
NaOH-Pi	182.3	21.97
NaOH-Po	41.5	5.00
HCl Pi	14.2	1.71
HCl Po	13.5	1.63
Total Pi	203.4	24.51
Total Po	58.0	6.99
Residual	568.3	68.49
Total P	829.7	100.00

Values are means of three replicate analyses

found to be very high (21.97 and 5% for inorganic and organic NaOH-P). The relative abundance of Al + Fe–P could be as a result of the presence of variable Al and Fe contents in soils at various stages of relative development and their reaction with soil P. The range of Al + Fe–P found in these soils is comparable to other Ethiopian soils reported by Solomon and Lehmann [29] and Piccolo and Huluka [19]. This pool is considered to be important to plant P nutrition and important in P transformation [12]. On the other hand, the HCl–P (Pi and Po) was detected in small concentrations (<4%) (Table 2). This could be due to low soil Ca content (Table 1). The concentrated H_2SO_4 digest P (residual P) is the P which is not readily removed by 0.5 M $NaHCO_3$, 0.1 M NaOH or 1 M HCl extracting solution and is considered to be a recalcitrant P form of very low solubility and availability, with the residual P as the most resistant fraction Tiessen and Moir [33]. It was found that residual P was very high indicating that most of the soil P was found in residual form. For the study soils, the distribution of P forms was similar with the previous studies of strongly weathered soils of Ethiopia [19, 28, 34].

Effect of liming on phosphorus fractions

As defined by Schoenau et al. [25], the resin Pi, $NaHCO_3$–P (Pi and Po) are considered as easily desorbable or labile phosphorus pools. These labile fractions increased more than threefold for the application of lime at a rate of 9.75 ton ha^{-1}. Therefore, liming of these acidic Ethiopian soils is anticipated to have a large positive impact on increasing P availability for crop production. Several reports have shown that liming results in increase in labile P [8, 10]. Liming intensity also affects the distribution of phosphorus among the fractions in the soil. Analysis of the Pi fraction (resin, $NaHCO_3$-Pi, NaOH-Pi, and HCl-Pi) revealed that the major part of mineral phosphorus (66.6–89.7% of total mineral phosphorus) was composed of Al + Fe phosphates (extracted by NaOH-Pi) (Table 2). These could be due to the presence of high Al and Fe oxides and hydrous oxides with large specific surface areas, which provide large number of adsorption sites [10, 26]. As the amount of lime added increased, the concentration of Al + Fe–P decreased significantly from 182.3 to 113.7 mg kg^{-1} (Table 3) as the P associated with Al and Fe hydrous oxides is transformed and released by desorption reactions to increase plant-available phosphorus pools in soil such as water soluble P [10] and $NaHCO_3$-Pi and PO fractions.

Hagereselam soil with different rates of lime application

Liming at a rate of 9.75 ton ha^{-1} significantly increased the amount of Ca phosphates as revealed in HCl extractable Pi. It increased from 14.2 to 53.1 mg kg (Table 3). Similar results were reported by Hartono et al. [8] and Jokubauskaite et al. [10]. It is likely related to an increase in adsorption and precipitation of P with the addition of excess Ca derived from lime. Our data are consistent with those obtained in recent studies conducted in acid soils by [6, 23]. Liming at lower rates creates optimal conditions for plant growth as it has low Ca–P compared with higher rates of lime [16].

Soil organic P occurs in a variety of chemical forms and can be released through mineralization processes

Table 3 P concentration (mg P kg^{-1}) in P fractions extracted from Hagereselam soil with different rates of lime application

LR (ton ha^{-1})	Resin	$NaHCO_3$		NaOH		HCl		Residual digest	Total	AP	OP	pH
		Pi	Po	Pi	Po	Pi	Po					
0	3.3d	3.6b	3.0b	182.3a	41.5	14.2b	13.5	568.3	829.7	9.8c	58.0	4.51
3.25	4.4c	4.7b	4.1b	168.4a	38.7	16.9b	14.1	574.0	825.3	13.2c	56.8	5.71
6.5	9.8b	5.8b	8.9a	122.9b	35.2	45.1a	11.2	576.1	814.8	24.4b	55.3	6.54
9.75	10.9a	13.0a	11.4a	113.7b	35.5	53.1a	7.5	577.3	822.4	35.3a	54.4	7.08

Means with the same latter are not significant at $p = 0.05$

mediated by soil organisms and plant roots. These processes are highly influenced by the soil pH. Organic P transformation has a great influence on the overall bioavailability of P in soil [26]. Analysis of the organic P fractional composition showed that the major portion of the total extractable organic P was extracted by NaOH solutions (Table 3). These phosphorus compounds are more readily mineralized because they are constituents of the newly incoming organic residues as well originating from cells of microorganisms [10]. Although not statistically significant, the organic P contained in both NaOH and HCl fractions decreased with addition of lime. A possible explanation for the decrease of organic P might be related to the intensified microbial activity at higher pH values, enhancing the mineralization processes in soil [10]. In general, as the amount of lime increases, the available P form increases from 9.8 to 35.3 (Table 3) and become adequate to plant indicating the benefit of liming.

Conclusions

The total P concentrations in the acid soils of southern Ethiopian are generally comparable to those found elsewhere. Among the fractions extracted and for which separation into organic and inorganic P forms was conducted, there appears to be a low partitioning of soil P into the organic pool. The inorganic Al + Fe and Ca associated pools are the most affected fractions by the addition of lime. The preponderance of a large pool of "residual" P in these soils brings forward the issue of how this P may be brought back into a more actively cycling available pool. The low available P supply creates crop production limitations in these soils. This is despite the reasonably good total P contents. This constitutes a real challenge to crop production systems based upon low-input management. From the results of this study it appears that the addition of lime to the acidic soils is an effective strategy to mobilize and render more phosphorus available for plant uptake and management of soil pH which further have impact on the availability of nutrients.

Authors' contributions

AK: researcher and author. SB and SJ: editor and advisor. All authors read and approved the final manuscript.

Author details

[1] College of Agriculture, Hawassa University, Awassa, Ethiopia. [2] College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada.

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Competing interests

The authors declared that they have no competing interests.

Availability of data and materials

We declare that all the data are available in the manuscript.

Declarations

I am enclosing herewith a manuscript entitled "[Availability and Chemical fractionation of phosphorus in Acid soils of Hagereselam, Southern Ethiopia under different rates of lime] for possible evaluation.

With the submission of this manuscript, I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute's [Hawassa University] representative is fully aware of this submission.

The type of Submitted manuscript is original article. For the Editor-in-Chief, I would like to disclose the following information about the project in that the research project was conducted under the supervision of Beyene Sheleme (Major Advisor) and Jeff Schoenau (Co-advisor) and the project was run as my Ph.D. project.

This research project was conducted from September 2013–December 2015. My Research Project was fully sponsored by IDRC-CRID Hawassa University.

I would also like to share the following information with Editor-in-Chief in that I do not have the following similar manuscripts already published from this project:

Here in this paper, the importance of addition of lime for soil phosphorus fractions is quantified. Accordingly although the soil has high total phosphorus addition of reclamation materials like lime will increase available phosphorus which is very important for plant production. This implies the need for site specific soil studies especially for problem soils where there is limited fertility status. In addition the findings of this result will be used as a base for further researches on the soils to alleviate similar problems and initiate ideas for other types of soils with different problem.

A paragraph explaining why the manuscript is appropriate with the scope of the journal.

The journal is a highly reputable journal publishing articles related soils and plant. These research is about the relationship between plant-available soil phosphorus and soils. By this paper, it is tried to explain about the relationship between available plant phosphorus and the different fractions with different rates of lime. Therefore, it is highly related with the scope of the journal.

Ethics approval and consent to participate

The research does not involving human subjects, human material, or human data.

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References

1. Kiflu Alemayehu, Sheleme Beyene, Schoenau Jeff. Characterization of problem soils in and around the south central Ethiopian Rift Valley. J Soil Sci Environ Manage. 2016;7:191–203.
2. Asmare M. Phosphorus status, adsorption characteristics, kinetics and availability to wheat crop as influenced by applications of various amendments on acid soils of Farta District, Northwestern Highlands of Ethiopia. PhD Dissertation, Haramaya University, Ethiopia. 2014.
3. Birru Y. Phosphorus status and sorption characteristics of soils in Northwestern Highlands of Ethiopia M.Sc. thesis, School of Graduate Studies, Alemaya University. 2000. p 106.
4. Bouyoucos GJ. Hydrometer method improved for making particle size analysis of soils. Agron J. 1962;54:464–5.
5. Bowman RA, Cole CV. Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Sci. 1978;125:49–54.

6. Crews TE, Brookes PC. Changes in soil phosphorus forms through time in perennial versus annual agro ecosystems. Agr Ecosyst Environ. 2014;184:168–81.
7. Desta HA. Reclamation of phosphorus fixation by organic matter in acidic soils. Glob J Agric Sci. 2015;3:271–8.
8. Hartono A, Vlek PLG, Moawad A, Rachim A. Changing in phosphorus fraction on an acid soil induced by phosphorus, organic matter and lime. J Il. Tan Lingk. 2000;3(2):1-7. http://repository.ipb.ac.id/handle/123456789/29754.
9. Hazelton P, Murphy B. Interprating soil test results. What do all the numbers mean?. Australia: CSIRO Publishing; 2007.
10. Jokubauskaite I, Karcauskiene D, Antanaitis S, Mazvila J, Slepetiene A, Končius D, Piaulokaite-Motuziene L. The distribution of phosphorus forms and fractions in *Retisol* under different soil liming management. Zemdirb Agric. 2015;102:251–6.
11. Kisinyo PO, Othieno CO, Gudu SO, Okalebo JR, Opala PA, Ng'Etich WK, Nyambati RO, Ouma EO, Agalo JJ, Kebeney SJ, Too EJ, Kisinyo JA, Opile WR. Immediate and residual effects of lime and phosphorus fertilizer on soil acidity and maize production in western Kenya. Exp Agric. 2014;50:128–43.
12. Kumoyo K, Yerokun OA, Damaseke MI. Changes in inorganic phosphorus following incubation of some Zambian soils. S Afr J Plant Soil. 2005;22:149–53.
13. Landon JR. Booker tropical soil manual. A handbook for soil survey and agricultural land evaluation in the tropics and sub tropics. New York: John Wiley and Sons; 2014. p. 474.
14. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J. 1978;42:421–8.
15. Mesfin A. Nature and management of acid soils in Ethiopia. Dire Dawa: Alemaya University of Agriculture; 2007. p 99.
16. Murphy PNC, Sims JT. Effects of lime and phosphorus application on phosphorus runoff risk. Water Air Soil Pollut. 2012;223(8):5459–71. doi:10.1007/s11270-012-1293-3.
17. Murphy J, Riley JP. A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31–6.
18. Marc P, Gautheyrou J. Handbook of soil analysis mineralogical, organic and inorganic methods. Heidelberg: Springer-Verlag Berlin; 2006. p. 995.
19. Piccolo A, Huluka G. Phosphorus status of some Ethiopian Soils. Trop. Agric. 1986;63(2):137–42.
20. Rehemuti M. Effect of forage legumes in short-term rotation on phosphorus fertility of four Saskatchewan soils. MSc Thesis. University of Saskatchewan. Saskatoon. Canada. 2015. P 164.
21. Sahlemedhin S, Taye B. Procedures for soil and plant analysis. National Soil Research Center, EARO, Technical Paper NO. 74. Addis Ababa, Ethiopia. 2000. p. 110.
22. Sanchez PA, Shepherd KD, Soule MJ, Place FM, Buresh RJ, Izac AM, Mokwunye AU, Kwesiga FR, Ndiritu CN, Woomer PL. Soil fertility replenishment in Africa: an investment in natural resource capital. 1997.
23. Sarker A, Kashem MA, Osman KT. Phosphorus availability, uptake and dry matter yield of Indian spinach (*Basella alba* L.) to lime and phosphorus fertilization in an acidic soil. Open J Soil Sci. 2014;16:2014.
24. SAS. SAS Institute Inc., Cary. 1997.
25. Schoenau JJ, Stewart JWB, Bettany JR. Forms and cycling of phosphorus in prairie and boreal forest soils. Biogeochemistry. 1989;8:223–37.
26. Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F. Phosphorus dynamics: from soil to plant. Plant Physiol. 2011;156(997):1005.
27. Shoemaker HE, McLean EO, Pratt PF. Buffer methods for determining lime requirement of soils with appreciable amounts of extractable aluminium. Proc Soil Sci Soc Am. 1961;25:274–7.
28. Solomon D, Lehmann J, Mamo T, Fritzsche F, Zech W. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. Geoderma. 2002;105:21–48.
29. Solomon D, Lehmann J. Loss of phosphorus from soil in semi-arid northern Tanzania as a result of cropping: evidence from sequential extraction and 31P-NMR spectroscopy. Eur J Soil Sci. 2000;51:699–708.
30. Syers JK, Jackson ML, Berkheiser VE, Clayton RN, Rex RW. Eolian sediment influence on pedogenesis during the Quaternary. Soil Sci. 1969;107:421–7.
31. Tan KH. Principles of soil chemistry. 3rd ed. New York: Marcel Dekker Inc; 1998.
32. Thomas RL, Sheard RW, Moyer JP. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digest. Agron J. 1967;59:240–3.
33. Tiessen H, Moir JO. Characterization of available P by sequential extraction. In: Carter MR, editor. Soil sampling and methods of analysis. Boca Raton: Canadian Society of Soil Science (Lewis Publishers); 1993. p. 75–86.
34. Tigist Y. 2007. Soil phosphorus forms as influenced by land use systems and topography in the ultisols of kindo koye watershed, Southern Ethiopia. MSc thesis. Hawassa University, Awassa, Ethiopia. p 92.