*Original Research*

# How Fertilization with Farmyard Manure and Nitrogen Affects Available Phosphorus Content and Phosphatase Activity in Soil

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*Received: 2 December 2013*
*Accepted: 2 April 2014*

## Abstract

Our paper presents the results of research on the content of available phosphorus and the activity of alkaline and acid phosphatase in soil under winter triticale fertilized with cattle farmyard manure (FYM) stored in various conditions: aerobic, anaerobic, and aerobic, with burnt lime added. The experiment also involved nitrogen fertilization in the form of ammonium nitrate at various rates (0, 30, 60, 90, and 120 $kgN \cdot ha^{-1}$). The content of available phosphorus from depths of 0-15 cm and 15-30 cm in soil was 58.87 $mgP \cdot kg^{-1}$ and 53.33 $mgP \cdot kg^{-1}$, respectively, which classifies the soil as class III with an average content of that element. We found a significant effect of the experimental factors on the content of available phosphorus. The highest content of that element was found in the soil (0-15 cm) fertilized with FYM stored under aerobic conditions with burnt lime added and non-fertilized with nitrogen (79.57 $mgP \cdot kg^{-1}$). With a greater soil sampling depth (15-30 cm), the content of phosphorus decreased by about 12%. The application of FYM stored under aerobic conditions with burnt lime added resulted in a significant increase in the alkaline phosphatase activity in soil (1.00 mM $pNP \cdot kg^{-1} \cdot hV$ for a depth of 0-15 cm, and 0.830 mM $pNP \cdot kg^{-1} \cdot h^{-1}$ for 15-30 cm). However, we found a significant inhibition of acid activity (1.381 mM $pNP \cdot kg^{-1} \cdot h^{-1}$ for a depth of 0-15 cm, and 1.275 mM $pNP \cdot kg^{-1} \cdot h^{-1}$ for 15-30 cm). We recorded a highly significant correlation between the content of available phosphorus and the activity of alkaline phosphatase in soil ($r=0.731-0.879$; $p<0.05$).

**Keywords:** phosphorus, phosphatases, FYM, nitrogen, soil

## Introduction

Phosphorus, carbon, nitrogen, potassium, and magnesium, are among the basic macroelements indispensable for the proper functioning of all organisms. Their contents in soil are related to many environmental and anthropogenic factors, mainly the type of soil: water content and pH of soil, mineral, organic, and natural fertilizers; cultivation method: crop species, and the amount and quality of organic substance and the presence of calcium, aluminium, and iron ions [1-5]. The main task of sustainable agriculture is the adequate application of fertilizers to ensure the optimal content of micro- and macroelements, including phosphorus in soil for adequate growth and development of the plants [6]. Organic waste can be a valuable and inexpensive fertilizer and a source of plant nutrients. Many studies [7, 8] suggest that organic sources of P are more effective for plant absorption than the inorganic ones. Long-term and repeated applications of manure may lead to the accumulation of soil phosphorus and could contribute to eutrophication [9].

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In the stored FYM there occur numerous changes in the nitrogen bonds in a number of processes, mostly amonification as well as nitrification or denitrification. According to [10], during storage, 20-40% of the nitrogen in deep litter stored in heaps may be lost during the composting process that starts in most deep litter heaps, and most of that N is lost through gaseous emission.

Phosphorus is one of the most essential nutrients in animal manure. Manure contains different forms and varying proportions of organic and inorganic forms of phosphorus. Enzymes are biological catalysts of innumerable reactions and are of great agronomic and ecological value. Alkaline and acid phosphatases are important enzymes that are involved in the phosphorus cycle of soil as they hydrolyze compounds of organic P and transform them into inorganic P forms, which can be assimilated by plant roots [5, 11, 12].

The first step to limit the negative effect of agriculture on the environment was introducing into domestic law in 2003 the Regulation of the Minister of the Environment [J. Law. of 2003 No. 4, Item 44]; under Nitrate Directive [Directive 91/676/EEC] [13] and upon Poland joining the European Union, the environmental protection regulations got stricter, including for waste management. Similarly, Council Directive 96/61/EEC [14] of September 24, 1996 on an integrated prevention and control of pollution makes one obliged to apply the best available techniques (BAT) in terms of animal-keeping systems, fertilizer storage, processing, and the application of natural fertilizers. During FYM storage the processes of transformations of nitrogen bonds via ammonification, nitrification, and denitrification occur. The nature of the transformations depends on physical, chemical, and biochemical factors determining the FYM fertilizer properties [15-17]. Only long-term field experiments can reveal the effects of farming systems on the content of available phosphorus in soil.

The aim of this paper was to evaluate the effects of nitrogen fertilization on changes in the content of available phosphorus against the activity of select phosphomonoesterases in the soil under winter triticale.

## Material and Methods

### Field Experiment

Our research was based on a multiple, static field experiment set up by the Sub-Department of Plant Nutrition and Fertilization of the Institute of Soil Science and Plant Cultivation in Puławy, located at the Agricultural Experiment Station at Grabów nad Wisłą, in Mazowieckie Province, Zwoleń County, Przyłęk Commune (Central Poland). The location of the experimental station is determined by latitude (51º21'8"N) and longitude (21º40'8"E). The experiment was performed in four-year crop rotation sites: spring barley, winter wheat, silage corn, and white triticale on typical soil in Poland, classified as light loamy and sand texture according to USDA soil classification [18], represent the type of Luvisols [19]. Cattle farmyard manure (FYM) was applied at a rate corresponding to 170 kg N·ha$^{-1}$ every 2 years in autumn after winter cereals harvest. Strict experiments were performed in May 2009 in a many-year fixed experimental field. The experiment was carried out as a two-factor, split-plot design. The following experimental factors were applied:

First-order factor: – FYM: A – without FYM, B – FYM stored under anaerobic conditions (from cold fermentation, the heap was lagering throughout summer in the amount of 30 tons), C – FYM stored under aerobic conditions, the so-called composted FYM (from hot fermentation), D – FYM stored under aerobic conditions with quick lime added in the form of calcium oxide. FYM was applied in autumn 2008 after the harvest of corn in the amount of 20 t·ha$^{-1}$. The chemical composition of cattle FYM applied in the experiment (nutrient% fresh weight) was as follows: dry matter 21%, nitrogen (N) 0.5%, phosphorus ($P_2O_5$) 0.29%, potassium ($K_2O$) 0.68%, calcium (CaO) 0.45%, magnesium (MgO) 0.16%, and sodium ($Na_2O$) 0.11%.

Second-order factor – nitrogen fertilization in the form of ammonium nitrate at five levels: N0 – 0 kgN·ha$^{-1}$, N1 – 30 kgN·ha$^{-1}$, N2 – 60 kgN·ha$^{-1}$, N3 – 90 kgN·ha$^{-1}$, and N4 – 120 kgN·ha$^{-1}$. Fertilization with phosphorus and potassium in all the experimental treatments was the same and equal to, respectively, $P_2O_5$ – 80 kg·ha$^{-1}$ (granulated triple superphosphate -46% $P_2O_5$) and $K_2O$ – 120 kg·ha$^{-1}$ (60% potassium salt). The experiment was performed in randomized block design, in four replications.

The soil was sampled in May 2010 with the Egner stick from two depths: 0-15 cm (surface layer) and 15-30 cm (sub-surface layer) under winter triticale.

### Soil Analysis

The following were assayed in the soil material:

1. pH in 1 M KCl measured potentiometrically (ISO 10390).
2. The content of available phosphorus ($P_{E-R}$) according to the Egner-Riehm method – DL (PN-R-04023, 1996) [20], which involves a spectophotometric measurement of the intensity of color of phosphorus-molybdenum blue formed by orthophosphoric ions with molybdenum ions in the acid environment in the presence of $SnCl_2$.
3. The activity of alkaline phosphatase (AlP) [EC 3.1.3.1] and acid phosphatase (AcP) [EC 3.1.3.2] according to Tabatabai and Bremner [21] is based on the colorimetric assaying of released substrate: *p-nitrophenol* after the incubation of soil with MUB (modified universal buffer) at pH 5.5 for acid phosphatase and pH 8.5 for alkaline phosphatase samples for 1h at 37ºC.

### Statistical Analysis

The results were exposed to the analysis of variance and the significance of differences between means was verified with the Tukey test at the significance level of $<0.05$. The calculations involved the use of ANOVA based on Microsoft Excel. With the results there was also performed the analysis of simple correlation ($p<0.05$), which deter-

Table 1. pH in KCl of the soil.

| FYM | 0-15 cm | | | | | 15-30 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrogen [kgN·ha$^{-1}$] | | | | | | | | | |
| | 0 | 30 | 60 | 90 | 120 | 0 | 30 | 60 | 90 | 120 |
| A* | 6.0 | 5.8 | 5.8 | 5.7 | 5.6 | 5.8 | 5.8 | 5.6 | 5.7 | 5.7 |
| B | 5.9 | 5.7 | 5.6 | 5.3 | 5.2 | 5.6 | 5.5 | 5.3 | 5.2 | 5.0 |
| C | 6.7 | 5.9 | 5.8 | 5.8 | 5.9 | 5.7 | 5.8 | 5.7 | 5.7 | 5.6 |
| D | 7.2 | 6.8 | 6.6 | 6.4 | 6.2 | 6.5 | 6.3 | 6.3 | 6.3 | 6.1 |

*A – without FYM, B – FYM stored under anaerobic conditions, C – FYM stored under aerobic conditions, the so-called composted FYM, D – FYM stored under aerobic conditions with quick lime added.

Table 2. Content of available phosphorus [mgP·kg$^{-1}$] in the soil under triticale.

| FYM I factor [t·ha$^{-1}$] | 0-15 cm | | | | | | 15-30 cm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrogen [kgN·ha$^{-1}$] II factor | | | | | | | | | | | |
| | 0 | 30 | 60 | 90 | 120 | Mean | 0 | 30 | 60 | 90 | 120 | Mean |
| A* | 56.68 | 52.97 | 49.05 | 45.34 | 42.51 | 49.31 | 54.93 | 50.57 | 45.99 | 40.33 | 34.88 | 45.34 |
| B | 64.74 | 61.04 | 56.24 | 53.62 | 47.96 | 56.72 | 59.07 | 56.46 | 51.88 | 45.12 | 34.22 | 49.35 |
| C | 71.72 | 65.40 | 58.64 | 54.71 | 51.01 | 60.29 | 68.01 | 61.69 | 53.84 | 50.57 | 47.96 | 56.41 |
| D | 79.57 | 70.85 | 68.23 | 65.40 | 61.69 | 69.15 | 69.97 | 66.05 | 61.91 | 58.20 | 54.93 | 62.21 |
| Mean | 68.18 | 62.56 | 58.04 | 54.77 | 50.79 | 58.87 | 63.00 | 58.69 | 53.41 | 48.56 | 43.00 | 53.33 |
| $LSD_{0.05}$ | | | | | | | | | | | | |
| I factor | 4.280 | | | | | | 1.618 | | | | | |
| II factor | 1.998 | | | | | | 0.794 | | | | | |
| Interaction | | | | | | | | | | | | |
| I/II | 5.248 | | | | | | 2.023 | | | | | |
| II/I | 3.996 | | | | | | 1.588 | | | | | |
| SD | 9.669 | | | | | | 10.03 | | | | | |
| *CV* [%] | 16.42 | | | | | | 18.80 | | | | | |

*For symbols, see Table 1, SD – standard deviation, *CV* – coefficient of variation

mines the level of dependence between respective features. The analysis of the correlation was made using ‘Statistica 6’ software. The coefficient of variation of analyzed parameters was calculated:

$$CV = (SD/X)\times 100\%$$

...where: *CV*– coefficient of variation (%), *SD* – standard deviation, *X*– arithmetic mean.

Values, in which values of 0-15%, 16-35%, and > 36% indicate low, moderate, or high variability, respectively.

## Results and Discussion

$pH_{KCl}$ of soil sampled at in 0-15 cm and 15-30 cm soil depths was in the range of 5.0-7.2 (Table 1), depending on the fertilizer applied. With those values in mind, one can classify the soil as acid, slightly acid, or neutral. Added FYM stored under aerobic conditions with lime added an increase of pH value.

The content of available phosphorus ranged from 42.51 to 79.57 mgP·kg$^{-1}$ (on average 58.87 mgP·kg$^{-1}$ for the soil sampled from 0-15 cm). In the soil sampled 15-30 cm deep, the content was slightly lower (34.22-69.97 mgP·kg$^{-1}$; on average 53.33 mgP·kg$^{-1}$) (Table 2). According to PN-R-04023 [20], it classifies the soil to class III of the average content of that element. Based on the analysis of variance we found a significant influence of the factors applied on the content of available phosphorus in soil. The highest content of $P_{E-R}$ (69.15 mgP·kg$^{-1}$ for 0-15 cm and 62.21 mgP·kg$^{-1}$ for 15-30 cm) was reported in the soil sampled from the object fertilized with FYM stored in aerobic con-

Table 3. Activity of alkaline phosphatase [mM pNP $kg^{-1}·h^{-1}$] in the soil under triticale.

| FYM I factor [$t·ha^{-1}$] | 0-15 cm | | | | | | 15-30 cm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrogen [$kgN·ha^{-1}$] II factor | | | | | | | | | | | |
| | 0 | 30 | 60 | 90 | 120 | | 0 | 30 | 60 | 90 | 120 | Mean |
| A* | 0.875 | 0.940 | 0.735 | 0.705 | 0.650 | 0.781 | 0.785 | 0.695 | 0.615 | 0.580 | 0.420 | 0.619 |
| B | 0.905 | 0.820 | 0.775 | 0.820 | 0.620 | 0.788 | 0.815 | 0.880 | 0.720 | 0.655 | 0.600 | 0.734 |
| C | 1.030 | 0.935 | 0.825 | 0.740 | 0.700 | 0.846 | 0.880 | 0.815 | 0.785 | 0.675 | 0.635 | 0.758 |
| D | 1.285 | 1.120 | 0.990 | 0.825 | 0.780 | 1.000 | 0.610 | 1.015 | 1.035 | 0.775 | 0.715 | 0.830 |
| Mean | 1.024 | 0.954 | 0.831 | 0.773 | 0.688 | 0.854 | 0.772 | 0.851 | 0.789 | 0.671 | 0.593 | 0.735 |
| $LSD_{0.05}$ | | | | | | | | | | | | |
| I factor | 0.020 | | | | | | n.s. | | | | | |
| II factor | 0.031 | | | | | | 0.235 | | | | | |
| Interaction | | | | | | | | | | | | |
| I/II | 0.055 | | | | | | n.s. | | | | | |
| II/I | 0.062 | | | | | | n.s. | | | | | |
| SD | 0.162 | | | | | | 0.143 | | | | | |
| *CV* [%] | 18.96 | | | | | | 19.72 | | | | | |

n.s. – non-significant, *For symbols, see Table 1. SD – standard deviation, *CV* – coefficient of variation

ditions with quick lime added. The soil can be classified to class II with a high content of $P_{E-R}$. Aerobic degradation of organic residues enriched with bacteria capable of solubilization of inorganic phosphate is responsible for the increase of phosphorus availability to plants [22]. A lower content of available phosphorus was noted in the soil from the objects fertilized with FYM stored under anaerobic conditions (56.72 mgP·$kg^{-1}$ for 0-15 cm and 49.35 mgP·$kg^{-1}$ for 15-30 cm).

During composting a mineralization of organic compounds occurs and nutrients get released to soil [23]. The anaerobic digestion of swine manure did not affect the mechanisms controlling P solubility and, therefore, no significant differences were observed in phosphorus content after raw and treated manure application [15]. For this reason, the content of available phosphorus in soil from the objects fertilized with composted FYM was higher (60.29 mgP·$kg^{-1}$ for 0-15 cm and 56.41 mgP·$kg^{-1}$) in the objects with FYM stored under anaerobic conditions. Similar results were earlier reported by Sommer [10]. The concentration of P increased during composting as a consequence of the low doses of P and a reduction in the amount of deep litter. Additionally, a lack of liming by decreasing soil pH decreases the intensity of mobilizing the reserves of soil phosphates, which involves the transition of hardly available forms of phosphorus into the ones available to plants. FYM, irrespective of management type, is a precious source of organic matter and macroelements in soil.

Nitrogen application at a rate of 120 kgN·$ha^{-1}$ decreased $P_{E-R}$ in the soil sampled from both depths by about 29% as compared with the soils non-fertilized with nitrogen (Table 2), which is connected with the yield-forming effect of nitrogen and the resultant increase in phosphorus uptake, accompanied by an increase in soil acidity caused by nitrogen fertilizers. As a result of increasing nitrogen rates, the soil changed its richness class from average to low [24].

All the transformations of biogenic elements in soil are stimulated by enzymes conditioning their transformation into the forms available to plants. The activity of alkaline and acid phosphatase depended significantly on the factors applied, except for the activity of alkaline phosphatase in the soil sampled from 15-30 cm deep (Table 4).

The activity of alkaline phosphatase in the soil was lower than the activity of acid phosphatase. Similar results were earlier reported by Samuel et al. [25] (soil of mellow loam texture). A low alkaline activity of phosphomonoesterase can be accounted for by the sensitivity of phosphatases to changes in the soil [26].

Fertilization of soil, besides affecting soil physicochemistry, also affects soil biology. The application of composted FYM with quick lime resulted in a significant increase of alkaline phosphatase activity (1.000 mM pNP·$kg^{-1}·h^{-1}$ in soil from a depth of 0-15 cm and 0.830 mM pNP·$kg^{-1}·h^{-1}$ in the soil from a depth of 15-30 cm). Added lime increased soil pH and thus the conditions for the activity of that enzyme were optimal. The activity of acid phosphatase, on the other hand, got clearly lower (1.381 mM pNP·$kg^{-1}·h^{-1}$ and 1.275 mM pNP·$kg^{-1}·h^{-1}$) as compared to the activity of that enzyme in soil from the objects with composted FYM only (1.701 mM pNP·$kg^{-1}·h^{-1}$ and 1.483 mM pNP·$kg^{-1}·h^{-1}$).

Table 4. Activity of acid phosphatase [mM pNP·kg$^{-1}$·h$^{-1}$] in the soil under triticale.

| FYM I factor [t·ha$^{-1}$] | 0-15 cm | | | | | | 15-30 cm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nitrogen [kgN·ha$^{-1}$] II factor | | | | | | | | | | | |
| | 0 | 30 | 60 | 90 | 120 | Mean | 0 | 30 | 60 | 90 | 120 | Mean |
| A* | 1.100 | 1.180 | 1.275 | 1.325 | 1.465 | 1.269 | 1.000 | 1.125 | 1.265 | 1.330 | 1.410 | 1.225 |
| B | 1.165 | 1.195 | 1.245 | 1.355 | 1.395 | 1.271 | 1.060 | 1.180 | 1.360 | 1.545 | 1.545 | 1.322 |
| C | 1.555 | 1.615 | 1.700 | 1.785 | 1.850 | 1.701 | 1.355 | 1.405 | 1.485 | 1.625 | 1.625 | 1.483 |
| D | 1.220 | 1.300 | 1.380 | 1.455 | 1.550 | 1.381 | 1.195 | 1.085 | 1.225 | 1.570 | 1.570 | 1.275 |
| Mean | 1.260 | 1.323 | 1.400 | 1.480 | 1.565 | 1.406 | 1.153 | 1.199 | 1.334 | 1.410 | 1.538 | 1.327 |
| $LSD_{0.05}$ | | | | | | | | | | | | |
| I factor | 0.035 | | | | | | 0.033 | | | | | |
| II factor | 0.014 | | | | | | 0.029 | | | | | |
| Interaction | | | | | | | | | | | | |
| I/II | 0.042 | | | | | | 0.057 | | | | | |
| II/I | 0.028 | | | | | | 0.058 | | | | | |
| SD | 0.213 | | | | | | 0.182 | | | | | |
| *CV* [%] | 15.14 | | | | | | 13.72 | | | | | |

*For symbols, see Table 1, SD – standard deviation, *CV* – coefficient of variation

The activity of both phosphomonoesterases in soil was higher after composted FYM application, as compared to the object where FYM was stored under anaerobic conditions, the application of which could have been connected with greater losses of organic material during composting as a consequence of the process of fast biochemical and microbiological mineralization [23]. Increased activity of alkaline and acid phosphatase in soils with FYM is indicative of the effects of increased organic carbon content in these soils on size or metabolic activity of the soil microbial population [27].

The results have shown that increasing nitrogen rates significantly changed the activity of alkaline and acid phosphatase. We noted a significant decrease in the activity of alkaline phosphatase and an increase in acid phosphatase following the application of nitrogen at the rate of 120 kgN·ha$^{-1}$. The high rate of nitrogen fertilizer decreased the soil pH, which changed the activity of phosphomonoesterases [28].

The phosphatase activity is increased in the soil collected from 0-15 cm, while from 15-25 cm it is slightly lower (Table 4). This variation is dependent on the response of existing microbial populations as a consequence of carrying out agricultural works [25, 28, 29].

Enzymatic soil pH index (AlP:AcP) [30] as calculated based on the values of activity of alkaline and acid phosphatase ranged from 0.39 to 0.72 in studied soil (Fig. 1). Optimal for plant growth and development can be considered such soil pH value under the conditions in which the adequate ratio of the AlP:AcP activity, namely 0.50, occurs [30]. The ratio AlP:AcP lower than 0.50 points to acid soil reaction and liming is recommended. Such a law value was

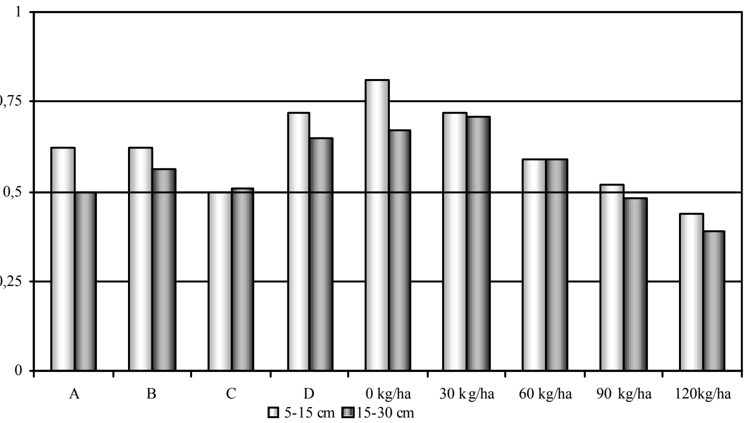


Fig. 1. Ratio of alkaline-to-acid phosphatase AlP:AcP in soil.

detected in the objects fertilized with FYM at the rate of 120 kgN·ha$^{-1}$ (0.39-0.44). The index can be used as an alternative method to determine the soil pH as well as the changes that occur in it [5, 24, 30, 31].

Moderate variation was observed for the available phosphorus content and the alkaline phosphatase activity, and it was both at a depth of 0-15 and a depth of 15-30 cm. For the acid phosphatase variations was low (*CV* =15.14%, depth 0-15 cm and *CV*=13.72% depth 15-30 cm).

We recorded significant statistical correlation between the content of available phosphorus and the activity of alkaline phosphatase in soil (r=0.731-0.879; $p<0.05$), which suggests that the enzyme was the adequate parameter defining the soils analyzed, unlike the activity of acid phosphatase. Similar results were earlier reported by Sinegani and Rashidi [32], and Piotrowska-Długosz and Wilczewski [33]. According to Kizilkaya et al. [34], a significant and positive relationship between phosphatase activity and phosphorus availability is obtained in soils that have not been fertilized and in those with a low abundance of nutrients, where a phosphorus deficiency occurs.

## Conclusions

The highest activity of alkaline phosphatase was noted in soil from the objects where composted FYM with lime was added.

Under the soil conditions investigated, the application of composted FYM with burnt lime added increased the content of available phosphorus, which classified the soil to class II with a high content of that element.

The inhibition of the alkaline phosphatase activity and the stimulation of acid phosphatase due to fertilization with increasing nitrogen rates shows the applicability of the research into the activity of those enzymes as an indicator sensitive to soil pH changes, and thus the enzymatic soil pH index can be used as an alternative to determine pH.

Further studies are required to confirm the positive long-term effect of FYM stored under aerobic conditions with lime added on the content of available phosphorus and the activity of phosphatases in soil.

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