Effect of different phosphorus sources applied with phosphate solubilizing bacteria on bio-geochemical properties and phosphorus release pattern in vertisol

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INTRODUCTION

Phosphorus is an important nutrient that limits the yield of more than 40% of the world’s arable land. World phosphorus (P) reserve is getting depleted faster, and certain reports estimate that there will no soil P reserve left by 2050 (Dey et al., 2017; Bhat et al., 2017). Further, P fertilizers in India are more expensive as it is imported from other countries and due to high cost, only wealthy farmers can utilize for their crops. To liquefy this utility monopoly, farmers need possible low-cost alternatives, or they have to use fertilizers in suitable forms. Phosphorus (P) is one of the major essential nutrient elements and the 10th most abundant element in the earth’s crust, its complex chemistry in the soil makes it a highly deficient nutrient element in most of...
the arable soils across the world. Only 0.1 % of the total P in the soil is available for uptake by plants (FAI, 2016) because of its fixation as various insoluble compounds depending upon the soil reaction. In the Indian context, about 49.3% of districts are reported to have low available P content based on 9.6 million soil test values (India Stat, 2018). Thus, the application of P-fertilizers to maintain soil fertility and sustain crop productivity becomes imperative in today’s input-intensive agriculture. Therefore, research priorities aim to find alternative sources of P-fertilizers with the right source and form to use in various soils. Further, the applied P fertilizers are in a huge problem of getting fixed in soil lattices its availability in soil solution will be very low. The solubilization of fixed P should be done properly for enhancing the fertilizer use efficiency to increase the available P in soil solution. At the same time, solubilization of fixed P requires proton (H⁺), which can be obtained through chemical or microbial intervention. Organic acids are generally responsible for P solubilization various P solubilizers could enhance it without harming the environment. Further, the effectiveness of different fertilizers like conventional, nano-fertilizers, organic manures and composts in agriculture to improve soil moisture retention capacity, neutralizers, and improving plant growth has been assessed in several investigations (Singh et al., 2017). Therefore, it is necessary to identifying the right source and form of P fertilizers to assure its requirement in soil for better crop production and conserve P sources for the future world. Thus, the present investigation was oriented to utilize different P fertilizers along with a P solubilizer on the P release pattern and soil biogeochemical properties in vertisol.

MATERIALS AND METHODS

A bulk surface soil sample (< 20 cm depth) from the farmer’s field, which was identified after surveying available P content at Bhuvanagiri-Mulur village, Chidambaram Taluk in Cuddalore District, Tamil Nadu, India, was collected to study the phosphorus release pattern of different P sources applied with PSB and its effect on physico-chemical and biological properties of soil. The soil was air dried and sieved through 2 mm sieve prior to use in the experiment. One kilogram of 2 mm sieved soil was filled in plastic containers. After that, calculated quantities of fertilizers and organics (w/w basis) were added as per treatments and mixed well. A completely randomized design was used with seven treatments (six treated (PSB alone, SSP + PSB, RP + PSB, DAP + PSB), Nano P + PSB, Phosphocompost (water hyacinth based) + PSB and one absolute control) with three replications and maintained for 3 months (90 days) at room temperature (Table 1). Thus, seven treatment combinations were set aside in 21 containers separately and maintained the soil moisture at about 60 per cent of the field capacity. The moisture level was maintained by adding water daily to compensate the loss due to evaporation. Soil samples were drawn at 30 days interval (30, 60 and 90 DAI) and dried under shade used for analysis of soil organic carbon content (Chromic acid wet digestion method), pH, EC, CEC (Neutral normal ammonium acetate method) and available P (Olsen method - Ascorbic acid method using Spectrophotometr (660 nm)) and microbial population (standard serial dilution plating technique of Pramer and Schenidt (1965)). This incubation experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalainagar, from October-December 2017. Soil classified as clayey texture soil with 54.6 % water holding capacity. Typic haplustrerts having slightly saline pH of 7.4 (1.2, 5, soil:water), electrical conductivity (0.51 dS m⁻¹ at 25°C), low KMnO₄ extractable-N (194 kg ha⁻¹), medium in NaHCO₃-P (17.4 kg ha⁻¹), medium in NH₄OAc-extractable (152 kg ha⁻¹) and 0.61 percent soil organic carbon content.

Statistical analysis

The data of heavy metal concentrations and soil properties of different amendments were subjected to one-way analysis of variance (ANOVA). All the statistical tests were performed using SPSS software. The significant differences between the means were tested against the critical difference at 5 % probability level.

RESULTS

Effect of P sources and PSB on bio-geochemical properties

An incubation experiment was tried out to unravel certain confounding characteristics of phosphorus release in soil having clay texture with respect to the P sources plus PSB along with NK 100 % RDF added for changing its solubility and availability and changes in bio-geochemical properties, and the results are given in table 2 and 3.

Microbial population (Bacteria, fungi and actinomycetes)

The total microbial loads at 30, 60 and 90 DAI showed a varying trend in all the treatments (Table 3). The microbial population was (Bacteria x 10⁶ CFU g⁻¹, fungi x 10⁸ CFU g⁻¹, actinomycetes x 10⁸ CFU g⁻¹) significantly influenced by the addition of P sources and PSB. Addition of NK100 % RDF + Phosphocompost + PSB (T₇) found to be superior by registering higher microbial population of 51,72,89 ; 17,21,21 and 30,38,37 at 30,60,90 DAI, respectively and it was on par with T₆ and T₅ followed by T₄, T₃, T₂, T₁ (Absolute control), which registered the lower microbial population (28,49,60 ; 7,12,14 and 18,22,24) at all the stages of
incubation, respectively.

**Soil pH**
The pH values recorded at 30, 60 and 90 days after incubation (DAI) ranging from 7.32 to 7.62, 7.31 to 7.62 and 7.28 to 7.61, respectively (Table 2). The results were statistically non-significant. However, higher pH values of 7.62, 7.62 and 7.61 were recorded in absolute control (T₁) and lower pH values of 7.32, 7.29 and 7.28 were recorded in NK 100 % RDF + SSP + PSB (T₅) applied treatment at all the three stages of incubation, respectively.

**Electrical conductivity (EC)**
Treatments implemented in the experiment were not influenced the electrical conductivity of soil (Table 2). The EC values recorded were ranging from 0.46 to 0.47, 0.45 to 0.49 and 0.43 to 0.49 dSm⁻¹ at 30, 60 and 90 days after incubation (DAI), respectively.

**Cation exchange capacity (CEC)**
The application of various P sources plus PSB along with a recommended dose of N and K fertilizers significantly machinates the CEC (Table 2). Values of CEC obtained at 30, 60 and 90 days after incubation (DAI), ranging from 12.4 to 15.6, 12.2 to 15.7 and 12.2 to 16.1 (C mol (p⁻¹) Kg⁻¹), respectively. The highest CEC values of 15.6, 15.7 and 16.1 (C mol (p⁻¹) Kg⁻¹) were recorded in NK 100 % RDF + Phosphocompost + PSB (T₅), which was on par with the treatment T₆ (NK100 % RDF + Nano P+ PSB) followed by T₃, T₅, T₄ and T₂ at 30 DAI. Whereas at 60 and 90 DAI higher CEC values of 15.7 and 16.1 (C mol (p⁻¹) Kg⁻¹) obtained in (T₅) followed by T₆, T₃, T₄ and T₂ (on par with each other), respectively. Further, the lowest CEC values of 12.4, 12.2 and 12.0 (C mol (p⁻¹) Kg⁻¹) were recorded by absolute control (T₁) at all three stages of incubation, respectively.

**Soil organic carbon (SOC)**
The data associated to soil organic content was analyzed during the incubation experiment at 30, 60 and 90 DAI (Table 2). SOC content was significantly (p=0.05) influenced by various P sources and the values ranging from 0.24 to 0.69 at 30 DAI, 0.21 to 0.68 at 60 DAI and 0.18 to 0.71 % at 90 DAI. Application of NK100 % RDF + Phosphocompost + PSB (T₁) registered higher SOC of 0.69, 0.68 and 0.71 % at all the stages of incubation and the treatments followed it in the order of T₆, T₅ and T₄ (on par with each other) and T₂, respectively. Absolute control treatment (T₁) registered the lower values soil organic carbon content of 0.24, 0.21 and 0.18 % at 30, 60 and 90 DAI, respectively.

**Effect on Olsen P acquisition**
Effect of P and PSB sources on Olsen P content in soil observed at 30, 60 and 90 DAI (Table 3). Significantly higher Olsen P content in soil was recorded in the treatment (T₃) NK100 % RDF + SSP + PSB (35.8 and 40.1 mg kg⁻¹) which was on par with (T₅) NK100 % RDF + Nano P + PSB (33.9 and 38.6 mg kg⁻¹) at 30 and 60 DAI. These treatments were followed by T₇, T₃, T₄ and T₂ (significantly different). Whereas at 90 DAI the application of NK100 % RDF + Nano P + PSB (T₆) registered higher P content in soil (42.3 mg kg⁻¹) which was on par with treatment (T₅). i.e. NK 100 % RDF + Phosphocompost + PSB (40.4 mg kg⁻¹). This was followed by T₃, T₅, T₄ (on par with each other) and T₂, whilst the lowest values of Olsen P content (15.2,13.9 and 11.8 mg kg⁻¹) were observed in the absolute control treatment (T₁) at 30, 60 and 90 DAI, respectively.

**DISCUSSION**

**Effect of different phosphorus sources combined with PSB on bio-geochemical properties and release pattern of P in vertisol**

**Soil biogeochemical properties**
The present study observed that bio-geochemical properties viz., pH, EC, CEC, SOC, microbial population (bacteria, fungi, actinomycetes) and Olsen P had a varying trend on its values due to the addition of various P sources plus PSB along with NK 100 % RDF.
fertilizers at 30, 60 and 90 DAI.

**Soil microbial population**

About the microbial population of incubated soil, the various P sources and PSB applied treatments significantly influenced the microbial biomass at 30, 60, and 90 DAI (Table 3). The higher microbial biomass (soil bacteria, fungi, and actinomycetes) observed in NK 100 % RDF + Phosphocompost + PSB (T₃) followed by NK 100 % RDF + Nano P + PSB (T₆) applied treatments at all the stages of incubation. This might be ascribed due to the addition of organic and inorganic fertilizers, which may affect the population, composition, and function of soil microorganisms. Inorganic fertilizers had relatively less effect on soil microbial biomass and activities than organics and bio-fertilizers. Further, this higher population of soil bacteria, fungi, and actinomycetes attributed higher organic carbon content duly by addition of phosphocompost plus PSB. Present study results are in line with those of Marschner et al., 2003; Amitava et al., 2008; Chести et al. (2015), they opined that soil microbial community structure was affected by addition of organic amendments, composted water hyacinth material could serve as quality manure for improving soil health and manural application attributes direct addition OC content to soil which stimulate growth and activity of microorganisms, respectively.

Further, Zhao et al. (2010) reported a close relationship between microorganism growth and activity to organic matter content in the soil. This provides carbon and energy sources for the growth of microorganisms. Lower microbial populations were recorded by NK 100 % RDF + PSB (T₂) and absolute control (T₁) treatments. This might be caused by induced pH (variable charge) that may not favorable for microbial growth. This is in confirmation with the findings of Kang et al. (2005) who proved that the use of either chemical or organic fertilizers of insufficient levels resulted in poor soil microbial index.

**Soil reaction (pH)**

According to the ANOVA test, the application of P sources plus PSB did not show any statistical significance on soil reaction (pH). However, the soil pH was altered (increased or decreased) by the treatments when compared to the initial soil pH level (7.45) at all stages of incubation. The pH values in different treatments tried in the experiment at different stages (30, 60, and 90 DAI) ranged from 7.32 to 7.62, 7.31 to 7.62 and 7.28 to 7.61, respectively. Decreased pH values were noticed in the treatments NK 100 % RDF + SSP + PSB (T₃), NK 100 % RDF + Nano P + PSB (T₆) and NK 100 % RDF + Phosphocompost + PSB (T₇), whereas all other treatments showed a trend of increased pH values from 30 to 90 days of incubation (Fig.1A). The lower soil pH values were observed in the treatments NK 100 % RDF + SSP + PSB (T₃), NK 100 % RDF + Nano P + PSB (T₆) and NK 100 % RDF + Phosphocompost + PSB (T₇) at all the stages of incubation experiment. This may be attributed due to the changes in electrochemical properties of soil through the added P sources and PSB. P solubilizers owing to the release of low molecular weight organic acids like citric, oxalic, malic etc., during the course of microbial degradation have a positive influence on the soil reaction reported by Mandal (2014); Mandal et al., (2015). Also, a decrease or increase in pH over the period of both aerobic and anaerobic incubation in this study may be ascribed to the process of H⁺ consumption or shift in the H⁺/OH⁻ during soil reduction or oxidation influenced by microbes. However, changes in soil pH were slightly altered due to the low dissociation constant of low molecular weight organic acids contributing low proton activity in soil solution. The activity of P solubilizing bacteria might be very meagre in the treatments, which in turn increased soil pH throughout the incubation period. Further, an increased level in pH was observed in the absolute control treatment (T₁) might be attributed due to little or no availability of microbial activities to produce acidity. These findings confirm with those of Katkar et al. (2011) and Kannan et al. (2013), whose results evidenced that integrated fertilizers application has a positive significant correlation with physico-chemical and biological parameters compared to unfertilized treatments in Vertisol.

**Electrical conductivity (dSm⁻¹)**

Considering the electrical conductivity (EC), according to the ANOVA test, the application of P sources plus PSB did not show any statistical significance like soil pH. The electrical conductivity of incubated soil decreased with the incubation period in all treatments. The EC values in different treatments at different stages (30, 60 and 90 DAI) ranged from 0.46 to 0.47, 0.45 to 0.49 and 0.43 to 0.49 dSm⁻¹ at 30, 60 and 90 days after incubation (DAI), respectively. However, slightly higher EC values were observed in absolute control (T₁) at all the experiment stages. This may be attributed to the changes in electrochemical properties of soil without the addition of PSB. Microbes generally added organic acids during their degradation to soils for solubilizing nutrients (Fazli et al., 2016), and solubilization has a positive direct relation with EC, which may be absent without PSB i.e. cationic solubilizing processes (reduction of Ca⁰ concentration in soil) solution by microbes may be less or nil. By increasing, divalent calcium radicals or ions (salts) content in soil solution would cause the EC as increased. Further, the addition of inorganic fertilizers and biofertilizers might have swung the soil’s electrical conductivity. In addition to that, the biogeochemical processes in soil and functions of added nutrient sources on soil reaction coupled with other
soil physicochemical characteristics increase the EC of the soil solution. The study results are in line with Dushyant et al. (2015), who reported that application of 100 % RDF through inorganic form had experienced higher pH and EC values when compared to integrated nutrient management treatments.

Cation exchange capacity (C mol (p+)' kg⁻¹)

Cation exchange capacity (CEC) is the direct measure of the quantum of negative charges in soil. Colloidal fractions in soil due to higher specific surface area and charge (dominantly under normal pH) contribute largely to the soil's cation exchange capacity. Application of P sources plus PSB significantly influenced the cation exchange capacity of experimental incubation soil. The CEC was altered (increased or decreased) by the treatments when compared to the initial soil CEC level (13.04 C mol(p+)* kg⁻¹) at all stages of incubation. The CEC values in different treatments at different stages (30, 60 and 90 DAI) ranged from 12.4 to 15.6, 12.2 to 15.7 and 12.2 to 16.1 C mol (p+) Kg⁻¹, respectively. Increased CEC values were noticed in all the treatments except absolute control (T₁). The Highest CEC values were observed in NK 100 % RDF + Phosphocompost + PSB (T₆) and NK 100 % RDF + Nano P + PSB (T₆) applied treatment at all the stages of incubation experiment may be due to charge contribution upon dissociation of low molecular weight organic acids. In general, higher CEC observed in Vertisol is due to the increased number of net negative charges on soil colloids with the increase in finer fraction (Hazalton and Murphy, 2007; Wei et al., 2010). Also, PSB addition might have stimulated the soil biological activity, which consequently increases the organic matter content and negative charges on the soil surface, thereby increasing exchangeable cations. Present results conform with those of Alamgir et al., 2011. Whereas the lowest CEC was observed in the absolute control treatment (T₁). This may be attributed to no molecular and or bio-fertilizers supplied to it, increasing the pH, where sources of negative charges in the adsorbent site would be reduced by soil aggregation, thus decreasing the CEC.

Soil organic carbon (%)

SOC is an important soil chemical property that decides the fertility of the soil. It gives an indirect indication of nutrient status in soil. Application of P sources plus PSB significantly influenced the soil organic carbon content. The SOC values in different treatments at different stages (30, 60 and 90 DAI) ranged from 0.24 to 0.69, 0.21 to 0.68 and 0.18 to 0.71 %, respectively. Increased SOC values were noticed in all the treatments except (T₂) and (T₁) treatments (Fig.2). Higher SOC (%) values were observed in NK 100 % RDF + Phosphocompost + PSB (T₁) applied treatment at all the stages of incubation experiment compared to other P sources plus PSB tried. (Table 2 and Fig. 2). This addition of organic carbon might be due to the accumulation of plant residual lignin in water hyacinth enriched compost, directly flowing to the structural and metabolic soil carbon pool, and increased biologically active soil organic carbon such as microbial biomass carbon mineralizable carbon in the present experiment. Further, the non-labile or residual SOC fraction constitutes the percentage of SOC under integrated application of molecular and biofertilizers potentially increased SOC. The present study results conform with those of Dushyant et al. (2015); Aditya Kumar et al. (2018). Furthermore, it is confirmed with a linear relationship between CEC and SOC at 30, 60, 90 DAI. (Y=6.244x + 10.58, R² = 0.702, Y=6.408x + 10.57, R² = 0.753, Y=6.413x + 10.50, R² = 0.707), respectively. Whereas the absolute control treatment (T₁) received nothing and thus did not influence the carbon pool. Organic manure was the most effective in increasing SOC when compared with or without molecular fertilizers or other soil amendments. The N and P fertilization through organic manures stimulates microbial activity. It enhances C turnover (Thakur et al., 2011), which explains the negative values of C stabilization in the nutrient sources applied plots which were hindered or nil in the absolute control treatment.

P release pattern (Available P- Olsen's) (mg kg⁻¹):

Soil available P increased with increasing P application due to the increase in water-soluble P in the soil. The quantity of the P available in soil solution decides by the physical, biological, and chemical attributes of soil. In the present study, various phosphorus sources combined with P solubilizing bacteria applied were entirely and significantly differed, especially in soil chemical environments (Fig.1B). Olsen P content ranged from 15.2 to 35.8 mg kg⁻¹ at 30 DAI, 13.9 to 40.1 mg kg⁻¹ at 60 DAI and 11.8 to 42.3 mg kg⁻¹ at 90 DAI. The maximum available P observed at 90 DAI and the minimum values were at 30 DAI. Application of NK 100 % RDF + SSP + PSB (T₃) clearly showed higher availability at 30 and 60 DAI and it was comparable with NK 100 % RDF +Nano P + PSB (T₆) treatment. Whereas at 90 DAI the application of NK 100 % RDF + Nano P + PSB (T₆) registered higher Olsen P content which was on par with treatment (T₃) i.e. NK 100 % RDF + phosphocompost + PSB (Table 3 and Fig. 1B). Soil available P clearly showed that PSB inoculation could have solubilized more P from the soil than un-inoculated with PSB or treatment without P fertilizers. PSB might have helped in increasing the availability of insoluble P in the soil pool. Further, the addition of P solubilizers stimulated the growth and activities of microorganisms, which increased N and P release. The effect was further enhanced by the addition of inorganic or organic fertilizers that are aptly available at different durations. These
that phosphorus availability in soil not only relied upon post + PSB applied one. These results evidently proved er P availability and it was on par with Phosphocom-But at 90 DAI Nano P applied treatments showed high-
by Nano P + PSB applied treatments at 30 and 60 DAI. 

m P availability was higher with SSP + PSB followed the efficiency of applied P fertilizers. For example, Ol-
results are in corroboration with those of Pandey et al. (2010); Panhwar et al., 2011; Panhwar et al., 2013;Yan et al., 2017). They opined that microbes harbouring in soil with or without crop rhizosphere provide better nut-
rient availability or solubilizing insoluble forms of nutri-
ents and play a key role in organic matter decomposi-
tion, nutrient cycling, and other chemical transfor-
mations in soil. Further, a significant difference in the availability of P at different incubation periods showed the efficiency of applied P fertilizers. For example, Olsen P availability was higher with SSP + PSB followed by Nano P + PSB applied treatments at 30 and 60 DAI. But at 90 DAI Nano P applied treatments showed higher P availability and it was on par with Phosphocompost + PSB applied one. These results evidently proved that phosphorus availability in soil not only relied upon source but also with the form of fertilizers applied i.e. Molecular P fertilizer like SSP, DAP, etc or Nano P fer-
tizers or composts enriched with molecular fertilizers or Nano fertilizers. But it will be a big question in making P availability in soil solution for a longer duration with less P fixation under aerobic or anaerobic soil en-
vIRONMENT. However, the present study results conform the findings of Sharmila Rahale (2011), Kannan et al., 2012), Tarafdar et al. (2012c), and Selva Preetha and Balakrishnan (2017).

Conclusion
In this study, among the different P sources, the availa-
ble P increased significantly due to the application of different P fertilizers compared with the control treat-

Table 2. Effect of phosphorus sources and PSB on soil pH, EC (dS m⁻¹), CEC (C mol (p⁺) Kg⁻¹) and soil organic carbon content (%).

| Parameters | Treatments / Parameters | CD (P=0.05) |
|------------|-------------------------|-------------|
|            | Absolute Control        | NS          |
|            | T₂ - NK 100 % RDF + PSB | NS          |
|            | T₃ - NK 100 % RDF + SSP | NS          |
|            | T₄ - NK 100 % RDF + RP  | NS          |
|            | T₅ - NK 100 % RDF +     |             |
|            | T₆ - NK 100 % RDF +     |             |
|            | T₇ - NK 100 % RDF +     |             |

Table 3. Effect of P sources and PSB on soil microbial population and Olsen P (mg/kg).

| Parameters | Bacteria (CFU/g soil×10⁵) | Fungi (CFU/g soil×10⁵) | Actinomycetes (CFU/g soil×10⁴) | Olsen P (mg kg⁻¹) |
|------------|---------------------------|-----------------------|-------------------------------|-----------------|
|            | 30 DAI  | 60 DAI  | 90 DAI | 30 DAI  | 60 DAI  | 90 DAI | 30 DAI  | 60 DAI  | 90 DAI |
| T₁         | 28      | 49      | 60     | 15.2    | 13.9    | 11.8   | 7       | 12      | 14      |
| T₂         | 31      | 54      | 65     | 24.3    | 25.1    | 26.0   | 9       | 14      | 16      |
| T₃         | 37      | 59      | 80     | 35.8    | 40.1    | 37.1   | 11      | 21      | 24      |
| T₄         | 44      | 65      | 75     | 27.9    | 29.4    | 31.0   | 16      | 19      | 18      |
| T₅         | 40      | 61      | 72     | 28.1    | 32.1    | 33.9   | 12      | 17      | 19      |
| T₆         | 48      | 69      | 86     | 33.9    | 38.6    | 42.3   | 16      | 19      | 23      |
| T₇         | 51      | 72      | 89     | 31.1    | 35.2    | 40.4   | 17      | 21      | 21      |
| SEd±       | 1.79    | 1.85    | 2.01   | 1.21    | 1.36    | 1.43   | 0.94    | 0.89    | 0.91    |
| CD (P=0.05)| 3.77    | 3.89    | 4.23   | 2.59    | 2.92    | 3.07   | 1.98    | 1.87    | 1.92    |
Available P significantly (p=0.05) increased with the application of SSP or Nano P along with PSB. The increased P may have been improved due to the more solubilizing effect of PSB in the soil. This finding has useful implications for P fertilizer management to reduce applied P fixation and increased availability of P in soil solution. However, the results proved that application of phosphocompost (water hyacinth based) with PSB significantly (p=0.05) influenced the soil microbial population, pH, EC, CEC, and SOC than other P sources applied; also, it recorded higher P availability next to SSP and Nano P application. Water hyacinth-based phosphocompost has an added advantage as it is a no-cost organic manure except collection and P enriching material cost. Being not only a cheapest composting source for P but also helps in environmental pollution management and it paves a new way to input cost cutting on P fertilizers.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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