

## Phosphorus and Zinc Extractable and Total in Substrate Enriched Coconut Powder and Tomato Cultivation

David Correia dos Anjos<sup>1</sup>, Antônio Alves Maia Neto<sup>1</sup>, Gabrielen de Maria Gomes Dias<sup>2</sup>,  
Fernando Felipe Ferreyra Hernandez<sup>1</sup> & Rebecca Tirado-Corbala<sup>3</sup>

<sup>1</sup> Department of Soil Science, Federal University of Ceará, Fortaleza, Ceará, Brazil

<sup>2</sup> Institute of Rural Development, University of International Integration of Afro-Brazilian Lusophony, Redenção, Ceará, Brazil

<sup>3</sup> Department of Agro-Environmental Sciences, University of Puerto Rico-Mayaguez, Mayagüez, Puerto Rico

Correspondence: David Correia dos Anjos, Department of Soil Science, Federal University of Ceará, Fortaleza, Ceará, Brazil. Tel: 55-85-987-775-247. E-mail: dav\_correia@hotmail.com

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### Abstract

Phosphorus (P) and Zinc (Zn) stand out for their interactions, however, little is known about the interaction of these elements in organic substrates used in the development of plants. The objective of this work was to evaluate the extractable and total P and Zn of the enriched coconut powder substrate and the development of tomato seedlings grown on the same substrate. The work consisted of 10 treatments and 4 replicates with different doses of P and Zn. The substrate used was coconut powder enriched with nutrient solutions in a ratio of 10:1 (v/m). Then, the experiment was conducted using the enriched substrate to observe the effect of P and Zn doses on the development of tomato seedlings in trays. The height, number of leaves and the dry matter of the plants were evaluated. For the water and Mehlich-1 extractors the highest values of P and Zn were for treatments with the highest doses and only the quadratic positive effect of P did not differ statistically in the enriched coconut powder substrate. The results showed that the coconut powder used as substrate is deficient in P and Zn. The highest development of the seedlings was obtained at the doses of 74 mg L<sup>-1</sup> P and 3.25 and 4.75 mg L<sup>-1</sup> Zn of the substrate enrichment solution.

**Keywords:** Mehlich-1, nutrition, organic substrates

### 1. Introduction

Substrate is the medium in which the roots of the plants develop, whose primary function is to provide support to the plants growing in them, and to regulate nutrient and water availability (Fermine & Kampf, 2012; Fonteno, 1996; Zorzeto et al., 2014). There are several scientific studies that characterize physically the different types of substrates (Cardoso et al., 2010; Pagliarini et al., 2012). Among the substrates studied, there is coconut powder that is widely used in different parts of the world (Cardoso et al., 2010).

Plant cultivation using substrate is a technique widely used in most advanced horticulture countries. The use of coconut powder as a substrate is an alternative for the preservation of several trees (for example orchids and bromeliads). In addition, it helps to reduce the volume of waste generated, since after the consumption of water, coconut is often discarded, making it an inconvenience to garbage collectors and shortening the life of public landfills (Kampf & Fermine, 2000). In this way, the most sensible and ecologically correct trend in the destination of solid waste generated is the recycling of these materials, with the consequent preservation of the environment (Kampf & Fermine, 2000).

Plants need for their development to absorb and assimilate adequate amounts of essential nutrients (Souza Júnior et al., 2011). The absence of any of the nutrients in the soil or in the culture medium may limit the production of the plant (Freitas et al., 2011). Deficiency or excess of a mineral element influences the absorption or activity of other nutrients, as a consequence can affect the metabolism of the plant.

The interaction of phosphorus (P) and zinc (Zn) has been studied for a long time in soils, however little is known about the interaction between these two elements in organic substrates, specifically in coconut powder. The description of Zn deficiency induced by P is classic in the literature; high concentrations of P in the soil or nutrient

solution cause decrease in the availability of Zn (Corrêa et al., 2014). This is often attributed to the insolubilization of Zn by the P on the root surface, decreasing its absorption (Malavolta et al., 1997). Phosphorus precipitates Zn as zinc phosphate  $Zn_3(PO_4)_2$ , preventing absorption by plants (Marschner, 1997).

Phosphorus interactions with Zn can occur in the soil or plant, the latter being considered as the main one (Cakmak & Marschner, 1987). In the first case, the effect may modify the availability of the nutrient or its own absorption. The interactions may occur in the absorption process, in radial or long-distance transport, and in P metabolism (Rober, 2000). The interaction Phosphorus and Zinc (P-Zn) has been well studied, but is a complex phenomenon and still not understood, since there are contradictory results such as: P has no influence on the absorption of Zn; there may be a mutual antagonism between P and Zn, particularly when one of the elements exceeds the critical level (Boawn & Legget, 1963), P can increase Zn uptake (Pauli et al., 1968), and P may decrease Zn uptake (Adriano et al., 1971).

The increase in P levels in the solution can occur by many mechanisms, decrease the concentration of Zn in the leaves, inducing Zn deficiency, with the appearance of deficiency symptoms, even when the concentration of Zn in the leaf appears to be adequate. The excess of P increases the physiological requirement of Zn and stimulates changes in the pectic materials of the cell wall of the roots, suggesting that the low levels of Zn can lead to the concentrations of P to toxic levels (Iorio et al., 2008) and consequently the development of plants.

Under hydroponic conditions, Moreira et al. (2001) and Corrêa et al. (2014) working with lettuce and pitaea, respectively, observed that the level of Zn in the leaves decreased with the increase in the contents of P, being easily increased with the supply of Zn, demonstrating that the balance between these two nutrients is fundamental for the absorption of Zn.

The objective of this work was to evaluate the amount of P and Zn soluble in water, Mehlich-1 and total substrate powder of enriched coconut and in the development of tomato seedlings cv. Santa Clara cultivated on the same substrate.

## 2. Material and Methods

The experiment was conducted in two phases, one in the laboratory and the other in a greenhouse, both located in the Soil Science Department of the Federal University of Ceará (UFC), in Fortaleza, Ceará, Brazil.

### 2.1 Preparation of the Substrate

The substrate used in the experiment was dry coconut powder, purchased from the processing plant of EMBRAPA-AGROTROPICAL (Fortaleza, CE). Before enrichment with P and Zn the material was sieved (2.0 mm sieves) and after washing, to remove excess salts, until reaching an electrical conductivity  $\leq 0.4$  dS  $m^{-1}$ .

### 2.2 Conducting the Experiment

Coconut powder was enriched by incubating it with nutrient solution for a period of 15 days in plastic containers of 10 liters' capacity, using the ratio of 10:1 (volume/massa) between the nutrient solution and substrate. After the incubation period the substrate was separated from the solution and air dried. The treatments applied in the enriched substrate were formed by the combination of five doses of P-(5.7, 39.9, 56.9, 74 and 108 mg  $L^{-1}$ ) in the form of monobasic potassium phosphate ( $KH_2PO_4$ ) and five doses of Zn-(0.25, 1.75, 2.5, 3.25 and 4.75 mg  $L^{-1}$ ) as zinc sulfate ( $ZnSO_4$ ) according to the Pan Puebla II matrix (Table 1) the remaining nutrients are constant. The design was completely randomized blocks with 10 treatments and four replicates, totalizing 40 experimental units.

Table 1. Doses of phosphorus (P) and zinc (Zn) enrichment of the treatment solution according to the experimental matrix Plan Puebla II used in the experiment

Treatment	Levels		Doses (mg L <sup>-1</sup> )	
	P	Zn	P	Zn
T1	-0.3	0.3	39.9	3.25
T2	-0.3	-0.3	39.9	1.75
T3	0.3	0.9	74	4.75
T4	0.3	0.3	74	3.25
T5	0.0	0.0	56.9	2.5
T6	0.3	-0.3	74	1.75
T7	0.9	0.3	108	3.25
T8	-0.3	-0.9	39.9	0.25
T9	-0.9	-0.3	5.7	1.75
T10	Control	Control	0.0	0.0

### 2.3 Determination of the Effect of Phosphorus and Zinc (P-Zn)

After incubation on the air-dried substrate, the water-soluble P and Zn contents, Mehlich-1 extractor and totals were determined. Extracts obtained using water or extractor/substrate ratio of 15:1 (v/m) and 60 minutes of stirring at 200 rpm on a horizontal table were used in the determination of water-soluble P and Zn and in Mehlich-1. The determination of total P and Zn was performed by colorimetric and atomic absorption, respectively, in nitroperchloric (HNO<sub>3</sub>-HClO<sub>4</sub>) extract (Malavolta et al., 1997).

In greenhouse to observe the effect of the doses of P and Zn on the development of plants, the incubated substrate was placed in plastic trays of 63 cells and after sowing three seeds per cell of tomato cv. Saint Clara. Then, 7 days after germination, the thinning was done leaving one plant per cell. In treatments with enriched coconut powder the plants received no additional fertilization. Irrigation was performed with water used in the public supply of the Fortaleza region.

After 24 days of cultivation, the tomato plants were evaluated for: plant height, number of leaves and determination of the dry matter of the plants.

### 2.4 Statistical Analysis

The values obtained for the evaluated characteristics were submitted to analysis of variance. The averages were compared by the Tukey's test ( $p > 0.01$ ) and the doses of P and Zn used in the substrate incubation were submitted to regression analysis, at 1% probability of error.

## 3. Results and Discussion

The water-soluble P ranged from 25 to 93 mg kg<sup>-1</sup> (Table 2) representing 13.4 to 64% of the P extracted in Mehlich-1 and from 1.7 to 2.4% of the total P.

Table 2. Water-soluble phosphorus (P) and zinc (Zn) contents, Mehlich-1 and total substrate coconut powder enriched

Treatment	Soluble in water		Soluble in Mehlich-1		Nutrient Totals	
	P	Zn	P	Zn	P	Zn
	----- mg kg <sup>-1</sup> -----					
T1	34	11.57	194	17.88	1624	32.3
T2	42	10.38	186	17.78	1800	29.17
T3	45	15.95	313	35.55	2674	54.73
T4	50	11.71	370	21.01	2813	37.82
T5	58	12.78	308	21.84	2270	34.74
T6	44	10.81	357	16.61	2759	28.34
T7	93	12.74	531	21.53	3864	35.86
T8	28	8.02	198	14.83	1503	22.65
T9	31	8.89	59	17.71	1497	29.46
Control	25	0.53	39	12.71	1487	22.94
DMS	4.81	0.510	176	3.80	395	2.53
C.V. (%)	3.91	1.71	4.44	6.66	6.12	2.664

The treatment with the highest dose of P (108 mg L<sup>-1</sup>) combined with the dose of Zn 3.25 mg L<sup>-1</sup> (T7) presented the highest content of water-soluble P (93 mg kg<sup>-1</sup>) differing statistically from other treatments. In this treatment, the water soluble P was 272% higher than the control treatment, which did not receive fertilization, and soluble P came from the substrate coconut powder itself. Similar results were found by Lima et al. (2011) that verified the content of P is significantly lower in the coconut-based substrate compared to other substrates used in the production of tomato seedlings.

In the treatment enriched with 56.9 mg L<sup>-1</sup> P solution and 2.5 mg L<sup>-1</sup> Zn-P water-soluble P increased 132% when compared to the control. The treatments with 74 mg L<sup>-1</sup> of P + 3.25 and 4.75 mg L<sup>-1</sup> of Zn the increases of soluble P in relation to the control treatment were 100 and 80%, respectively (Table 2). In the other treatments, these values were lower than 80%. Anand et al. (2002) working with coconut powder enrichment found values of water-soluble P at 60 days after incubation of 200 mg kg<sup>-1</sup> and at 120 days of incubation the amount of water-soluble P was 250 mg kg<sup>-1</sup>. The highest amount of soluble P found by these researchers in water relative to the present study (93 mg kg<sup>-1</sup>), are explicable because they incubated the coconut powder directly with phosphate rock and manure and no immersion in nutrient solution.

The water-soluble P content of the enriched substrate presented a linear and quadratic regression coefficient positive for the effect of P and Zn respectively and negatively for the interaction of P-Zn (Table 3).

Table 3. Multiple regression coefficients of water soluble P, Mehlich-1 and total coconut powder content as a function of the P and Zn rates

Variable	Soluble Water	Soluble Mehlich-1	Total
P	0.197546 × 10 <sup>-02**</sup>	0.0160089**	0.0876385**
Zn	0.0247701**	0.131950**	0.670040**
P <sup>2</sup>	0.145620 × 10 <sup>-04**</sup>	0.633687 × 10 <sup>-04**</sup>	0.692578 × 10 <sup>-03**</sup>
Zn <sup>2</sup>	0.994116 × 10 <sup>-02**</sup>	0,0798644**	0.594980**
P-Zn	-0.139820 × 10 <sup>-02**</sup>	-0.942120 × 10 <sup>-02**</sup>	-0.0655047**
Constant	-0.0394261	-0.333604	-1.30569
R <sup>2</sup>	0.676421	0.846094	0.851993

Note. \*\*, \*, 0: Significant at 1, 5 and 10% probability by the T test, respectively; ns: not significant.

The water soluble Zn contents were influenced by the combined doses of P and Zn applied to the substrate. The highest Zn dose (4.75 mg kg<sup>-1</sup>) (T3) also had the highest water soluble Zn content (15.9 mg kg<sup>-1</sup>) differing statistically from the other treatments and showed an increase of 209% in relation to the control treatment (Table 4).

Table 4. Multiple regression coefficients of the contents of water soluble Zn, Mehlich-1 and total in the enriched coconut powder as a function of the doses of P and Zn

Variable	Soluble Water	Soluble Mehlich-1	Total
P	0.150277**	0.242997**	0.459704**
Zn	-1.7733 <sup>ns</sup>	-8.05740**	-8.56924*
P <sup>2</sup>	0.24988 × 10 <sup>-03**</sup>	0.0012338 <sup>ns</sup>	0.00301452 <sup>0</sup>
Zn <sup>2</sup>	0.960938**	2.50064**	4.27254**
P-Zn	-0.0686756**	-0.138346**	-0.300553*
Constant	9.80026	24.5577	31.8304
R <sup>2</sup>	0.600365	0.724586	0.652137

Note. \*\*, \*, 0: Significant at 1, 5 and 10% probability by the T test, respectively; ns: not significant.

Anand et al. (2002) working with enriched coconut powder after 120 days of incubation found levels of water-soluble Zn around 1 mg kg<sup>-1</sup>. The water soluble Zn content of the enriched substrate was positively influenced by the dose of P and Zn applied, presenting highly significant regression coefficients for the simple P and Zn effect of P and Zn, respectively, while in the interaction P and Zn influence was negative (Table 4).

The P and Zn contents extracted by the double acid (Mehlich-1) are presented in Table 2. In the treatment with the highest dose of P ( $108 \text{ mg L}^{-1}$ ) the P extracted was 126% higher than the control treatment ( $39 \text{ mg kg}^{-1}$ ), differing statistically from the other treatments.

At the lowest dose of P ( $5.7 \text{ mg L}^{-1}$ ) combined with the dose of Zn ( $1.75 \text{ mg L}^{-1}$ ) the P content extracted by Mehlich-1 was  $59 \text{ mg kg}^{-1}$ , 461% lower than that extracted with the highest dose ( $531 \text{ mg kg}^{-1}$ ).

At the dose of  $74 \text{ mg L}$  of P combined with the doses of 2.5 and  $4.75 \text{ mg L}^{-1}$  of Zn or P extracted by Mehlich-1 was five to 7 times greater than the control. The Mehlich-1 soluble P content of the enriched substrate was positively influenced by the simple and quadratic effect of P and Zn respectively and negatively by the interaction of P-Zn (Table 3). Zinc extracted with Mehlich-1 from the treatment (T3) with the highest Zn dose ( $4.75 \text{ mg L}^{-1}$ ) was 180% higher ( $35.55 \text{ mg kg}^{-1}$ ) when compared to treatment (T10) received fertilization ( $12.7 \text{ mg kg}^{-1}$ ), differing statistically from the other treatments (Table 2).

The Zn-soluble Zn content of the enriched substrate was positively influenced by the simple P and quadratic effect of Zn respectively and negatively by the simple Zn effect and by the P-Zn interaction and not being influenced by the quadratic effect of P (Table 4).

The total P and Zn contents varied from 1.487 to  $3.864 \text{ mg kg}^{-1}$  and from 22.9 to  $35.9 \text{ mg kg}^{-1}$ , respectively, in the coconut powder enriched by incubation (Table 2). Presented lower values the control treatment and the higher values the treatment that received the highest dose of nutrients.

The total P in the coconut powder increased with the dose of P applied in the enrichment solution, presenting on average contents of  $1.487 \text{ mg kg}^{-1}$ ;  $1.497 \text{ mg kg}^{-1}$ ;  $1.624 \text{ mg kg}^{-1}$ ;  $2.270 \text{ mg kg}^{-1}$ ;  $2.759 \text{ mg kg}^{-1}$  and  $3.864 \text{ mg kg}^{-1}$  for the doses of Zn 0.0; 5.7; 39.9; 74 and  $108 \text{ mg L}^{-1}$ , respectively. However, when considered as constant the doses of  $39.9 \text{ mg L}^{-1}$  of P combined with increasing doses of Zn 0.25; 1.75 and  $3.25 \text{ mg L}^{-1}$  the total P amounts in the substrate were on average  $2.270 \text{ mg kg}^{-1}$  and these treatments did not differ from each other. At the dose of  $74 \text{ mg L}^{-1}$  of P (T6, T4 and T3) and increasing doses of Zn 1.75; 3.25 and  $4.75 \text{ mg L}^{-1}$  on average the total P content was  $2.748 \text{ mg kg}^{-1}$ . The total P content of the enriched substrate was positively influenced by the simple and quadratic effect of P and Zn respectively and negatively by the interaction of P-Zn (Table 3). Similarly, to total P the treatments with increasing doses of P 5.7; 39.9 and  $74 \text{ mg L}^{-1}$  that received the same dose of  $1.75 \text{ mg L}^{-1}$  did not show significant differences among them in the quantification of total Zn contents (Table 2).

Differently from the treatments that received constant doses of Zn  $3.25 \text{ mg L}^{-1}$  and increasing doses of P 39; 74 and  $108 \text{ mg L}^{-1}$  the total Zn contents in the substrate increased statistically significant with increasing doses of P. This behavior suggests an interaction between P and Zn, where the increase in Zn contents in the substrate explained by its precipitation in the form of zinc phosphate (Marschner, 1997; Muner et al., 2011). This result also explains the coefficient of linear and quadratic regression positive and statistically significant for the effect of P (Table 4).

The height, number of leaves and dry mass of tomato plants grown in the enriched coconut powder can be seen in Table 5. The plants grown on the enriched substrate had a mean height of 17.45 cm (Table 5). In the treatment that received the lowest dose of Zn presented the lowest height. The treatments that received doses of  $39.9 \text{ mg L}^{-1}$  and  $5.7 \text{ mg L}^{-1}$  of P combined with the doses of  $0.25 \text{ mg L}^{-1}$  and  $1.75 \text{ mg L}^{-1}$  of Zn presented the lowest heights of 11.7 and 15.5 cm, respectively, and the treatment with the dose of P  $74 \text{ mg L}^{-1}$  and Zn of  $1.75 \text{ mg L}^{-1}$  presented the highest height of all treatments. The control treatment was the one with the lowest value for height, showing that the amount of nutrient was not enough for the plant growth. Muner et al. (2011) observed with the application of excessive phosphate fertilization can lead to Zn deficiency in the substrate, because P renders Zn unavailable at the root surface or precipitates it, impairing the assimilation of the nutrient by the plants.

Table 5. Height, number of leaves and dry matter of tomato seedlings cv. Santa Clara at 24 days of culture in the substrate coconut powder with different doses of P and Zn

Treatment	Variables evaluated		
	Height	Sheet	Dry mass
T1	23.5	2.90	2.21
T2	21.2	2.52	1.82
T3	23.3	2.34	2.25
T4	22.8	2.08	2.29
T5	23.5	2.36	2.18
T6	24.4	2.58	2.08
T7	21.9	2.33	2.08
T8	11.7	2.04	1.11
T9	15.5	2.01	1.39
Control	9.55	1.96	0.68
DMS	3.50	0.48	0.28
C.V.	7.35	8.55	6.52

The plants grown on the enriched substrate had a mean height of 17.45 cm (Table 5). In the treatment that received the lowest dose of Zn presented the lowest height. The treatments that received doses of 39.9 mg L<sup>-1</sup> and 5.7 mg L<sup>-1</sup> of P combined with the doses of 0.25 mg L<sup>-1</sup> and 1.75 mg L<sup>-1</sup> of Zn presented the lowest heights of 11.7 and 15.5 cm, respectively, and the treatment with the dose of P 74 mg L<sup>-1</sup> and Zn of 1.75 mg L<sup>-1</sup> presented the highest height of all treatments. The control treatment (T10) was the one with the lowest value for height, showing that the amount of nutrient was not enough for the plant growth. Muner et al. (2011) observed with the application of excessive phosphate fertilization can lead to Zn deficiency in the substrate, because phosphorus renders zinc unavailable at the root surface or precipitates it, impairing the assimilation of the nutrient by the plants.

Analyzing the effect of fertilization with P and Zn in relation to plant height, it was verified that there was a significant difference between the control that did not receive P and Zn and those that received the lower doses of P and Zn. There were also significant differences between the treatments with the higher doses of P and Zn and the lower doses of these nutrients. Similar results were found by Batista et al. (2011) that observed the increase of the availability of P in the substrate favored positive result on the vegetative growth of the plants. The polynomial regression analysis showed a positive and highly significant linear coefficient ( $p \leq 0.01$ ) for P and Zn, while the quadratic coefficient was not significant for P and significant at 5% probability for Zn. This variable had a low coefficient of variation (CV = 7.35%) and a high coefficient of determination ( $R^2 = 0.86$ ) (Table 6).

Table 6. Coefficient of multiple regression and determination of height, leaf number and dry matter yield of aerial part of tomato seedlings cv. Santa Clara at 24 days of substrate cultivation coconut powder with different doses of P and Zn

Variable	Height	N° of leaves	Mass
P	0.0673182**	0.00618447**	0.00549146**
Zn	3.76557**	0.275858**	0.306936**
P <sup>2</sup>	-0.314133E-4 <sup>ns</sup>	0.79979E-5*	-0.544234E-5**
Zn <sup>2</sup>	-0.10737*	0.0147051*	-0.0114257**
P-Zn	-0.00710115*	-0.00170356**	-0.314955E-30
Constant	0.298931	0.735564	0.178695
R <sup>2</sup>	0.859252	0.490221	0.929936

Note: \*\*, \*, 0: Significant at 1, 5 and 10% probability by the T test, respectively; ns: not significant.

The number of leaves was also influenced by increasing doses of P and Zn (Table 5). The lower doses of P and Zn presented values that did not differ statistically and the treatment with the dose of P and Zn 39.9 and 2.5 mg L<sup>-1</sup> presented higher results than the other treatments for the plants grown in enriched substrate. When the polynomial regression model was applied to this variable, the linear coefficient for P and Zn was positive and highly significant. The quadratic coefficients were significant at 5% probability. However, the coefficient of determination was the lowest ( $R^2 = 0.50$ ) suggesting that the number of leaves is the least reliable variable of the three determined (Table

6). This behavior was also observed Corrêa et al. (2014), who concluded that the application of P and Zn, and the zinc phosphorus interaction affect the availability of both nutrients in the substrate, root system and shoot, thus influencing initial plant growth.

The dry matter yield of the plants, considering all the treatments varied from 0.68 to 2.29 g plant<sup>-1</sup> with the doses of P and Zn (Table 5). The doses of 74 mg L<sup>-1</sup> of P and 3.25 mg L<sup>-1</sup> of Zn presented the highest dry mass production (2.29 g plant<sup>-1</sup>) of all treatments. For this variable the DMS was 0.284 and the CV of 6.52%. According to Muner (2011), well-supplied plants in P and Zn present significant dry mass averages.

In the polynomial regression of the dry matter of the tomato seedlings presented linear coefficients positive and quadratic negative highly significant for the doses of P and Zn. The coefficient of determination of dry matter was the highest ( $R^2 = 0.93$ ) indicating it as the most reliable of the variables studied (Tables 6).

#### 4. Conclusions

High doses of P reduce the Zn content of the solution and increase its total content in the substrate dry coconut powder. Increase in the doses of P and Zn also increase the P extracted in water, Mehlich-1 and total in the substrate dry coconut powder. The coconut powder used as a substrate is deficient in P and Zn. The highest development of tomato seedlings cv. Santa Clara on the substrate coconut powder is obtained with the doses of 74 mg L<sup>-1</sup> P and 3.25 and 4.75 mg L<sup>-1</sup> Zn of the substrate enrichment solution.

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