

# MERCURY QUANTIFICATION IN GRASS TREATED WITH BIOSOLIDS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY WITH HYDRIDE GENERATOR

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## ABSTRACT

Solid byproducts of organic nature generated by the processes of treatment of municipal wastewater are commonly used as a nutrient source for soil treatment. These biosolids contain nutrients inorganic contaminants such as mercury, which is used to study by his knowledge widely documented about the toxicity in living systems and their effects on human. In this sense and since plants are the basis of the food chain the focus is to the grass, due to its easy growth and use in animal husbandry. Mercury in grass was quantified by atomic absorption spectrophotometry technique using hydride generator cool mist. Some validation parameters were checked with desirable acceptance criteria such as linearity ( $r^2 = 0,999$ ), accuracy ( $R = 93,14-96,12\%$ ), precision ( $CV = 1,11-1,69\%$ ), limits of detection and quantification ( $0,001-0,003$  ppb). It became clear that the mercury concentrations are below the maximum values permitted by international standards, presenting the biosolids as a reliable source of nutrients for agriculture. This way establishes a methodology that can be a good tool for the analytical determination of mercury in grass.

**KEYWORDS:** atomic absorption, quantification, validation, mercury, toxicity, hydride generator.

## CUANTIFICACIÓN DE MERCURIO EN PASTO TRATADO CON BIOSÓLIDOS POR ESPECTROFOTOMETRÍA DE ABSORCIÓN ATÓMICA CON GENERADOR DE HIDRUROS

## RESUMEN

Los subproductos sólidos de carácter orgánico generados por los procesos de tratamiento de aguas residuales municipales son utilizados comúnmente como fuente de nutrientes para tratamiento de suelos. Estos biosólidos contienen nutrientes contaminantes inorgánicos como el mercurio, el cual es empleado para el estudio, por el conocimiento ampliamente documentado de su toxicidad en sistemas vivos y sus efectos en seres humanos. En este mismo sentido,

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y puesto que las plantas constituyen la base de la cadena trófica, se centra la atención sobre el pasto, debido a su fácil crecimiento y uso como pastura de ganadería. El mercurio en pasto fue cuantificado mediante espectrofotometría de absorción atómica utilizando la técnica de generador de hidruros con vapor frío. Se verificaron algunos parámetros de validación con criterios de aceptación deseables tales como linealidad ( $r^2 = 0,999$ ), exactitud ( $R = 93,14-96,12\%$ ), precisión ( $CV = 1,11-1,69\%$ ), límites de detección y cuantificación ( $0,001-0,003$  ppb). Se evidenció que las concentraciones de mercurio se encuentran por debajo de los valores máximos permitidos por la normatividad Internacional, presentando el biosólido como fuente confiable de nutrientes para el agro. De esta forma se establece una metodología que puede ser una buena herramienta para la determinación analítica de mercurio en pastos.

**PALABRAS CLAVES:** absorción atómica; cuantificación; validación; mercurio; toxicidad; generador de hidruros.

## QUANTIFICAÇÃO DE MERCÚRIO NA GRAMA TRATADO COM BIOSSÓLIDO POR ESPECTROFOTOMETRIA DE ABSORÇÃO ATÔMICA COM GERADOR DE HIDRETO.

### RESUMO

Subprodutos sólidos de natureza orgânica gerado pela cidade de processos de tratamento de águas residuais municipais são geralmente usados como uma fonte de nutrientes para o tratamento do solo. Estes contêm nutrientes do resíduo de contaminantes inorgânicos tais como o mercúrio, o qual é usado para estudar o seu conhecimento amplamente documentado sobre a toxicidade em sistemas vivos e os seus efeitos na saúde humana. Neste sentido, e uma vez que as plantas constituem a base da cadeia alimentar o foco é a grama, devido ao seu fácil crescimento e usar na criação de animais. Mercury na grama foi quantificada por espectrofotometria de absorção atômica técnica usando gerador de hidreto de névoa fría. Alguns parâmetros de validação foram verificados com os critérios de aceitação desejáveis, tais como linearidade ( $r^2 = 0,999$ ), exatidão ( $R = 93,14-96,12\%$ ), precisão ( $CV = 1,11-1,69\%$ ), limites de detecção e quantificação ( $0,001-0,003$  ppb). Tornou-se claro que as concentrações de mercúrio estão abaixo dos valores máximos permitidos pelos padrões internacionais, apresentando os bioossólido como uma fonte confiável de nutrientes para a agricultura. Desta forma, estabelece uma metodologia que pode ser uma boa ferramenta para a determinação analítica de mercúrio na grama.

**PALAVRAS-CHAVE:** absorção atômica, quantificação, validação, mercúrio, toxicidade, gerador de hidretos.

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### 1. INTRODUCTION

The use of solid organic byproducts generated by municipal wastewater treatment as a source of organic matter and nutrients for crops is a practice that is commonly used for the purpose of improving the physicochemical properties of the soil and reducing the need for inorganic fertilizers (Park, *et al.*, 2011; Bernal, *et al.*, 2009; Baldwin and Shelton, 1999). It has been reported that organic amendments with low levels of heavy metals can be used as sinks for the reduction of the bioavailability of the metals in the soil to the plants due to processes

such as absorption, complexing, oxidation-reduction reactions, and volatilization (Violante, *et al.*, 2010; Park, *et al.*, 2011; Roca-Perez, *et al.*, 2010; Yong-Kui, *et al.*, 2007).

Among the heavy metals encountered is mercury, an inorganic contaminant that nonetheless plays an indispensable role in vital processes according to the characteristics and concentrations found in the environment due to the fact that it tends to accumulate along the food chain, which is one of the reasons why it is usually employed in catchment studies given the widely documented knowledge on its toxicity in living systems and its effects on human

beings. In the same sense, and since plants make up the base of the trophic chain, some implications have been suggested about toxic concentrations of mercury and the transport of plants to higher levels of the chain (Peralta-Videa, *et al.*, 2009). The capacity that vegetables have for capturing mercury has made it possible to establish factors that estimate transference as well as phytoremediation (Arroyave, *et al.*, 2010; De Temmerman, *et al.*, 2009).

Plants are frequently used on mined soils to carry out the phytoextraction of mercury, nonetheless, the studies on agroecosystems are few. The concentrations of mercury in rice and cultivated vegetables on agricultural land near industrial zones in China, in the south of Jiansu, revealed a transference of 0.006 and 0.002 mg/kg respectively, values that were under the maximum allowed concentration for foods (Cao, *et al.*, 2010). In corn crops the accumulation of mercury is seen mostly in the roots, indicating that the risk of bioaccumulation in human beings is low (Peralta-Videa, *et al.*, 2009). The cell wall of the root plays an important role in the uptake and retention of mercury in such a way that the metal does not enter into other organs in the plant. (Cavallini, *et al.*, 1999). This phenomenon was confirmed in the plants *Lavandulastoechas* (Sierra, *et al.*, 2012) and *Silene vulgaris* (Perez-Sanz, *et al.*, 2012), where the concentration of mercury found was greater in the root than in the above-ground part of the plant, confirming the importance of the root in the processes of immobilizing mercury.

These results constitute a key element in studies of mercury tolerance. Nevertheless, elevated concentrations of mercury can generate transference from the root to the other organs; therefore, it has also demonstrated the direct relationship between mercury concentration in the soil and the catchment level, as is the case with the *pasto llanero* (*Brachiariadictyoneura*) grass, whose mechanism for tolerance was related to the accumulation and exclusion of mercury (Arroyave, *et al.*, 2010). Finally, regarding leaf absorption in various plant species exposed to atmospheres contaminated with mer-

cury, effects have been established if they have two points of entry: root and leaf (Cavallini, *et al.*, 1999; Ci, *et al.*, 2011).

The *Kiktuvo* grass (*Pennisetum clandestinum*) is a tropical perennial species native to the central region of Africa, but it is now common in many more areas. The optimal temperature for growth of the grass is between 6 and 24 °C, but it also responds to a temperature range between 2 and 8 °C. The attention given to this grass on the behalf of the researchers is due to its being easy to grow, its root system, and its content of highly digestible proteins (Muscolo, 2003). Furthermore, it is used for cattle pastures in the municipality of Entrerríos in the Antioquia department of Colombia, and the lands there are treated with biosolids as a source of soil nutrients.

It should be noted that no regulations exist yet as far as the maximum allowed levels of toxic elements in pasturage. As a reference, the maximum permitted mercury load in soils (mg/kg) established by different international regulations indicates the concentration that represents a threat to the environment based on regulations in the Netherlands (McBride, 1995), as seen in **Table 1**.

TABLE 1. CONCENTRATION OF MERCURY IN SOILS ACCORDING TO INTERNATIONAL REGULATIONS					
Maximum allowable mercury load in soils					Risk concentration
International regulations (mg/kg)					Netherlands (mg/kg)
Element	U.S	Germany	Netherlands	Canada	
Mercury	-	8	8	1.6	40

Currently, in accordance with Colombian regulations, the ministries of Environment, Agriculture and Rural Development and the Ministry of Social Protection are in the regulatory phase of Decree 1287, “by which are established criteria for the use of biosolids generated in municipal wastewater treatment plants,” classifying biosolids into categories A, B, and C as seen in **Table 2**.

**TABLE 2. CONCENTRATION OF MERCURY IN BIOSOLIDS ACCORDING TO COLOMBIAN REGULATIONS**

Maximum allowable mercury load in biosolids (mg/kg)			
Element	Biosolid category		
	A	B	C
Mercury	17	57	Does not meet any parameter defined for either A or B

The validation of the methodology for the quantitative determination of mercury in pasture grass through atomic absorption with hydride generation attempts to not only document the quality of the pasture grasses, but also validate the analytic method as an essential part of the quality assurance program for any service lab, thus satisfying applicable parameters and criteria from a statistical, operative, and economic standpoint.

## 2. MATERIALS AND METHODS

### Reagents

Sulfuric acid ( $H_2SO_{4(ac)}$ ), Nitric acid ( $HNO_{3(ac)}$ ), Hydrochloric acid ( $HCl_{(ac)}$ ), potassium permanganate ( $KMnO_{4(s)}$ ), potassium persulfate ( $K_2S_2O_{8(s)}$ ), sodium chloride ( $NaCl_{(s)}$ ), hydroxylamine sulfate ( $(NH_3OH)_2SO_{4(s)}$ ), sodium borohydride ( $NaBH_{4(s)}$ ) and sodium hydroxide ( $NaOH_{(s)}$ ), were of analytical grade from Aldrich Chemical Co. Ltd., Pro-analysis.

### Solution preparation

#### Potassium permanganate solution $KMnO_{4(ac)}$

The potassium permanganate was prepared in deionized water at 25°C. The content of potassium permanganate in the aqueous solution corresponds to 5% g/mL.

#### Potassium persulfate solution $K_2S_2O_{8(ac)}$

The potassium persulfate was prepared in deionized water at 25°C. The content of potassium persulfate in the solution corresponds to 5% g/mL.

#### Sodium chloride and hydroxylamine sulfate solutions $[NaCl; (NH_3OH)_2SO_{4(ac)}$

The sodium chloride and the hydroxylamine sulfate were prepared in deionized water at 25°C. The contents of the sodium chloride and hydroxylamine sulfate in solution both correspond to 12% g/mL.

#### Sodium borohydride and sodium hydroxide solutions $[NaBH_4; NaOH]_{(ac)}$

The sodium borohydride and sodium hydroxide were prepared in deionized water at 25°C. The contents of sodium borohydride and sodium hydroxide in solution both correspond to 0.6% g/mL.

### Mercury Hg standards

The standard solution of 100 mg/L of Hg was prepared using a standard solution of 1000 mg/L of Hg in nitric acid at 1%. The standard solution of 10 mg/L of Hg was prepared using a solution of 100 mg/L of Hg in nitric acid at 1%. The standard solution of 100 µg/L of Hg that was used to prepare the standards for the calibration curve was obtained by adding 1 mL of the standard solution of 10 mg/L of Hg, and that was then added to 100 mL with nitric acid at 1%.

### Equipment and spectroscopic conditions

ASensAA® atomic absorption spectrophotometer from GBC Scientific Equipment (Dandenong, Australia) with a peristaltic air pump for mercury measurement with the hydride generation technique, and a mercury lamp at a wave length of 253.7 nm with a deuterium lamp for background radiation correction were used. It was set at a bandwidth of 0.5 nm with a lamp current of 4.0 without flame. Due to the use of the atomic absorption technique with cold vapor, the carrier gas was argon.

### Soil preparation

0.5g of soil was measured and treated with 5 mL of  $H_2SO_{4(ac)}$  and 2.5 mL of  $HNO_{3(ac)}$  with continuous agitation, afterwards 15 mL of  $KMnO_{4(ac)}$  were added

until the purple color remained for 15 minutes. Later, 8 mL of  $K_2S_2O_8$  were added followed by heating for 2 hours at 95°C. The heat was removed, and when the solution reached room temperature  $NaCl-(NH_3OH)_2SO_4$  was added for the purpose of eliminating the excess  $KMnO_4$ . It was allowed to rest for one hour, and finally the mercury was quantified.

### *Sample preparation*

Between 0.5 and 1.0 g of the sample (dried grass) was measured into a 250 ml Erlenmeyer flask and was treated with 5 mL of  $H_2SO_4$  and 2.5 mL of  $HNO_3$  with continuous agitation. Afterwards, 15 mL of  $KMnO_4$  were added until the color purple reached a permanence of 15 minutes. Then 8 mL of  $K_2S_2O_8$  were added followed by 2 hours of heating at 95°C. The heat was removed, and when the sample reached room temperature  $NaCl-(NH_3OH)_2SO_4$  was added for the purpose of eliminating the excess  $KMnO_4$ . It was left to rest for one hour, and finally the mercury was quantified.

### *Statistical analysis*

The results were compared statistically using analysis of variance (ANOVA), followed by the multiple comparison test for the differences in means using STATGRAPHICS Centurion XVI.II.

### *Sampling site*

The study was performed on the farm La Pradera, located in the Pío XII vereda in the municipality of Entrerriós in the department of Antioquia. The land was examined with the purpose of selecting the experimental plot (with biosolids) and the control plot (without biosolids), keeping in mind that both plots were to have the same physiographic components in terms of slope, soil, and water sources (Peláez, *et al.*, 2011).

### *Plot selection*

The soil of the plots was found to be developed on reworked volcanic ash, to have an Ap horizon (with a thickness that varies between 10 and 30 cm

depending on the degree of erosion), and to be seeded with Kikuyo grass. The experimental plot had an area of 1.4 hectares and had been treated 8 years ago with biosolids (every forty-five days as the only source of fertilization). The control plot had an area of 0.5 hectares and had been treated with urea and diammonium phosphate (DAP) as fertilizers with a frequency of a month and a half (100 kg of urea plus 50 kg of DAP). It is important to take into account that after fertilization the plots are used as pasture for varying animal stock for a period of no longer than 6 days according to the management that the farm administrator sets out (Peláez, *et al.*, 2011).

### *Plot division*

The plots were divided into four lots, keeping the slope morphology in mind: one in the upper part with a slope of approximately 5%, two in the middle part of the hill with a slope between 15 and 25%, and a lot at the thalweg with a slope between 5 and 8%, at the end of which there is a free flow of water across the surface.

### *Biosolid application*

In the experimental plot, the diluted biosolids from the San Fernando wastewater treatment plant (Medellin, Antioquia) were applied manually using hoses. In order to determine the amount of biosolids applied, blankets, each measuring 0.52 m<sup>2</sup>, were placed on the ground (Peláez, *et al.*, 2011). These blankets were placed in plastic bags after the treatment and measured later to determine the mass and volume of the biosolids applied.

### *Sampling design*

Four soil samples were taken in each of the lots. The samples were made up of 10 subsamples per lot with a depth of 20 cm. 15 subsamples at random of Kikuyo grass were taken from each of the lots in

the plots for leaf analysis. The soil and leaf sampling were done one week after each application.

### *Spectroscopic method*

The validated and implemented analytical parameters for the quantification of mercury were linearity, accuracy, precision, detection limit, and quantification limit.

### *Linearity and linear range*

In order to evaluate the linearity, five concentrations were prepared (1, 5, 10, 20, 30 ppb). Each concentration was prepared through a standard mercury solution at 1000ppm. The prepared solutions were subjected to the digestion process and injected in triplicate.

### *Precision*

Two analysts independently prepared 6 replicas of a standard solution per day and subjected them to digestion the same as the test sample. The variation coefficient was determined as the ratio between the mean of the replicas of the solution (MRS) and the standard deviation of the replicas (DSR) for each analyst.

### *Accuracy*

In order to find the accuracy as the recovery rate, the method of standard addition was used, for which known quantities of mercury were added to one or more real samples in three levels of concentration (low, medium, and high) within the working linearity range. The results were expressed as a percentage of recovery (% *R*), calculated as shown in **Equation 1**.

$$\% R = \frac{C_{SA} - C_S}{C_A} * 100 \quad (1)$$

Where  $C_S$  is the concentration of mercury in the sample,  $C_{SA}$  the concentration of mercury in the sample plus the concentration of the mercury added, and  $C_A$  is the known concentration of mercury added to the sample.

### *Detection and quantification limits*

In order to evaluate the detection limit, 10 blanks were prepared as a minimum and were subjected to the usual preparation process for the samples and the reading of the signal through the selected process. The detection limit was determined for the digested matter (*Dld*) through the signal average (*v*) and the standard deviation of the blanks (*s*), as seen in **Equation 2**.

$$LDd = y + 3,3 * s \quad (2)$$

The method detection limit for the grass (*MDL*), was determined through the relation of the detection limit of the digested material, the volume of digested matter (*Vd*), and the mass of the sample (*m*) as seen in **Equation 3**.

$$LD = LDd \left( \frac{mg}{L} \right) * \frac{Vd (mL)}{m (g)} \quad (3)$$

The method quantification limit (*MQL*) was calculated through the method detection limit as represented in **Equation 4**.

$$LC = 3 * LD \quad (4)$$

## 3. RESULTS

### *Linearity and linear range*

The statistical results of the analysis of the mercury and area standards are shown in **Table 3**. The obtained values presented a statistically significant correlation (*p*-value < 0.05), with a 95% confidence level. The correlation coefficient for the model was 0.999, indicating good proportionality between the concentration and the area. The errors of slope and intercept present a good fit for the linear model.

**TABLE 3. SPECTROSCOPIC PARAMETERS FOR THE MERCURY QUANTIFICATION**

Linear Range	Regression equation	r <sup>2</sup>	MDL	MQL
1 - 30	Y = (0.0152 ± 0.0004) *C - 0.0004 ± 0.0001	0.999	0.001	0.003

**Y**: area, **C**: mercury concentration and **r<sup>2</sup>**: coefficient of determination. The linear range, detection limits (**MDL**) and quantification limits (**MQL**) are expressed in ppb (µg/L).

### Linear dynamic range

The linear dynamic range was determined through the ratio between the area and the concentration of the mercury evaluated. The upper limit (slope + 10%) and lower limit (slope - 10%) were 0.0165 and 0.0135 respectively. In this way, it was established that the concentrations of mercury: 1.0, 5.0, 10, 20, and 30 ppb, are found within the defined linear range.

### Detection and quantification limits

In **Table 3**, you can see the estimated values for both. The detection and quantification limits were 0.001 and 0.003 ppb respectively, indicating that the analytical method shows good sensitivity.

### Precision

The coefficients of variation (**CV**) for analysts 1 and 2 are summarized in **Table 4**. It can be observed that the coefficients of variation are less than or equal to 2%. For coefficient of variation values less than 10%, which is a value of a measurement of dispersion that is acceptable within the set of experimental measurements formulated from the model adopted by IUPAC (International Union of Pure and Applied Chemistry) it can be inferred that the analytical approach allows for the establishment of conditions for repeatability and reproducibility by applying the methodology to the same sample under the instrumental conditions with a different operator and in a different period of time, given that there was no significant difference between the process followed by the analysts on the quantification days.

**TABLE 4.** PRECISION OF THE SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF MERCURY

	Analyst 1	Analyst 2
<b>Day 1</b>	9.933	10.092
	10.011	10.106
	10.279	10.112
	10.008	10.178
	10.459	10.187
	10.180	10.193
<b>MRS ± DSR</b>	10.1 ± 0.2	10.14 ± 0.05
<b>CV</b>	2.0	0.5
<b>Day 2</b>	10.016	10.105
	10.009	9.895
	10.040	10.056
	10.001	10.006
	9.886	9.893
	9.866	9.952
<b>MRS ± DSR</b>	10.0 ± 0.1	10.0 ± 0.1
<b>CV</b>	1.0	1.0

**MRS**: mean of the replicas of the solution **DSR**: standard deviation of the replicas

### Accuracy

The recovery percentage of mercury in the three levels of concentration were as follows: for the low level 93.14%-95.24%, for the middle level 94.35%-96.12%, and for the high level 92.59%-95.18%, indicating that the method does not have analyte loss (< 93.14%) or interference in the matrix (> 96.12%). Therefore, the spectroscopic method is considered accurate.

### Biosolid characterization

The amount of biosolids and the content of nitrogen (N), phosphorus (P) and potassium (K) delivered in each application is shown in **Table 5**.

**TABLE 5.** AMOUNT OF BIOSOLIDS ON A DRY BASIS AND N, P<sub>2</sub>O<sub>5</sub> AND K<sub>2</sub>O APPLIED IN THE EXPERIMENTAL PLOT

Application (months)	Biosolid	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
	(kg/ha)			
0	3012	54.2	28.0	9.0
2	3546	63.8	33.0	10.6
5	1895	34.1	17.6	5.7
7	2721	49.0	25.3	8.2

(\*) The amounts of N, P and K were calculated according to the analysis of the collected biosolids on the day of application

### Mercury determination

The quantification of mercury in the soils is seen in **Table 8**. Upon evaluating the possible differences through a comparison test between the two treatments, an estimated average of the mercury content as a function of the treatment is obtained which indicates that the control plot has a basal mercury concentration of  $0.32 \pm 0.02$  mg/kg, corresponding to the 38% reported for both cases. On the other hand, the experimental plot has a mercury concentration of  $0.52 \pm 0.05$  mg/kg, representing 62% of the total mercury observed and demonstrating a statistically significant difference ( $p$ -value =  $4.20 \times 10^{-9}$ ), with a 95% confidence level.

**TABLE 6.** MERCURY CONTENT (MG/KG) IN SOILS± (STANDARD DEVIATION)

Lot	Experimental PLOT	Control plot
4	$0.570 \pm 0.004$	$0.280 \pm 0.004$
3	$0.437 \pm 0.002$	$0.327 \pm 0.003$
2	$0.570 \pm 0.010$	$0.313 \pm 0.004$
1	$0.487 \pm 0.006$	$0.334 \pm 0.006$

The mercury content in the grass is shown in **Table 7**. It did not show differences between the grass in the control plot and that of the experimental plot in regards to growth and color. The mercury content in the control plot was not detected in contrast to the experimental plot, which showed an av-

erage mercury concentration of  $0.065 \pm 0.001$  mg/kg. Although there was mercury transference, the risk of bioaccumulation in humans is low (Peralta-Videa, *et al.*, 2009).

**TABLE 7.** MERCURY CONTENT (MG/KG) IN GRASSES± (STANDARD DEVIATION)

Lot	Experimental plot	Control plot
4	$0.0570 \pm 0.0010$	<0.01
3	$0.0637 \pm 0.0005$	<0.01
2	$0.0650 \pm 0.0020$	<0.01
1	$0.0740 \pm 0.0020$	<0.01

## 4. CONCLUSIONS

In this study the quantification of mercury in grasses treated with biosolids by atomic absorption spectroscopy with hydride generation was achieved. The method is linearly acceptable due to its coefficient of correlation of 0.999, precise with a CV less than or equal to the 2% accepted within the model adopted by IUPAC, and meeting the repeatability parameter. Furthermore, the method is considered accurate due to the recovery rate between 93.14% and 96.12%. The results of this study suggest that the method is safe and the methodology can be applied in mercury analysis by spectroscopy of atomic absorption with hydride generation on grass-type systems.

The soils treated with biosolids do not represent a threat to the environment given that they are found to be under the international standard and Colombian regulations. Furthermore, the quality of the grasses analyzed shows that biosolids are an alternative for farmers with respect to chemical fertilizers and raises the possibility of using a material considered waste.

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