



## Assessment of Toxic and Essential Heavy metals in Some Commercial Olive Oils brands and their Potential Health Effects in Al Jabal Al Akhdar Region – Libya

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### تقييم المعادن الثقيلة السامة والأساسية في بعض العلامات التجارية لزيوت الزيتون التجارية وتأثيراتها الصحية المحتملة في منطقة الجبل الأخضر – ليبيا

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#### Abstract

This research aims to evaluate the levels of some essential and harmful minerals (Fe, Zn, Cu, Pb, Cd) in a range of commercially available olive oils sold in the Green Mountain region of Libya, specifically Libyan, Tunisian, Italian, and Spanish olive oils. An applied analytical methodology was employed, utilizing atomic absorption spectrometry (AAS) or inductively coupled plasma spectroscopy (ICP-OES), to determine and measure the concentration of these minerals and compare it to internationally permissible limits. The results also indicate a reduction in the risk to consumer health. The findings show that the concentrations of the essential minerals Fe and Zn fall within acceptable dietary ranges, while the levels of Pb and Cd remain below the maximum recommended limits for edible oils, suggesting a low direct health risk from heavy metals, where all maximum Pb levels were determined to be lower than 0.1 mg/kg, a value that is similar to what is typically recommended by many countries when establishing international standards for olive oil.

**Keywords:** Minerals, Olive Oil Concentration, Plasma Spectroscopy, Risk Levels, Consumer Health.

#### الملخص

تهدف هذه الدراسة إلى تقييم مستويات بعض العناصر المعدنية الأساسية والضارة (الحديد Fe، الزنك Zn، النحاس Cu، الرصاص Pb، والكادميوم Cd) في مجموعة من زيوت الزيتون التجارية المتاحة تجاريًا في منطقة الجبل الأخضر بليبيا، وتشمل عينات من زيوت زيتون ليبي، وتونسي، وإيطالية، وإسبانية. وقد اعتمدت الدراسة منهجية تحليلية تطبيقية باستخدام مطيافية الامتصاص الذري (AAS) أو مطيافية البلازما المترنة بالحث (ICP-OES) لتحديد وقياس تركيزات هذه العناصر المعدنية ومقارنتها بالحدود المسموح بها وفق المعايير الدولية. تشير النتائج إلى انخفاض مستوى المخاطر الصحية المرتبطة على استهلاك هذه الزيوت. وأظهرت النتائج أن تركيزات العناصر الأساسية (الحديد والزنك) تقع ضمن النطاقات الغذائية المقبولة، في حين أن مستويات الرصاص والكادميوم بقيت دون الحدود القصوى الموصى بها لزيوت الصالحة للأكل، مما يشير إلى انخفاض مخاطر صحية مباشرة ناتجة عن المعادن الثقيلة. وتم تحديد أن جميع القيم القصوى لتركيز الرصاص كانت أقل من 0.1 مغ/كغ، وهي قيمة تتماشى مع ما توصي به العديد من الدول عند وضع المعايير الدولية لزيت الزيتون.

## Introduction

Olive oil is considered one of the most important essential nutrients, especially in the Mediterranean region. It plays a vital role in the Mediterranean diet and in disease prevention, particularly for heart and metabolic diseases [1]. Olive oil contains a range of essential minerals such as iron (Fe), zinc (Zn), and copper (Cu), as well as some potentially harmful elements like lead (Pb), cadmium (Cd), aspartate (As), and hydrogen (Hg). Whether essential or harmful, excessive concentrations of these elements can lead to oxidative stress, which damages cells. The presence of some toxic elements, such as lead (Pb) and cadmium (Cd), is dangerous even at low concentrations [2]. These elements are linked to neurological and kidney disorders and cancers [3]. The study area is the Green Mountain region of Libya, characterized by its good vegetation cover and suitable climate for olive cultivation. The region possesses the necessary climatic, natural, and environmental conditions, including water, soil, and air, for growing olive trees and producing high-quality oil, especially if the pressing process, type of extraction, and storage methods are controlled. Several studies have shown variations in heavy metal levels in olive oil [4], particularly depending on the origin and source. Data mining of the most commonly used types in this region revealed that four commercial olive oils are the most prevalent among the local population: Libyan olive oil, Tunisian olive oil, Spanish olive oil, and Italian olive oil. It is essential to use techniques such as ICP-OES, ICP-MS, or AAS to measure elements like Pb, Cd, Fe, and Zn, while also assessing health risks using indicators such as THQ and HI [5].

This study aims to identify and measure the levels of some essential and harmful minerals in a range of commercial olive oils of different origins and sources traded in the Green Mountain region of Libya. These include Libyan, Tunisian, Italian, and Spanish olive oils. The study also aims to determine the extent of health risks arising from excessive concentrations of these minerals. The importance of this study stems from its comprehensiveness and objectivity, avoiding bias in both results and data. Furthermore, it provides an analysis linking the findings to potential health risks for consumers, thus making it a valuable literature review on the subject. Despite the importance of this topic, there is a lack of recent data and studies accurately assessing the levels of minerals, particularly in olive oil from the Green Mountain region, and the extent of these elements' impact on human health through cytotoxicity or what is known as cellular antioxidant activity, despite increased consumption and diversification of oil sources. This lack of knowledge represents a scientific gap that warrants further investigation [6].

## Method and Materials

The main study methodology is the applied analytical methodology, in addition to several auxiliary methodologies, including the descriptive methodology for describing the data and results, the quantitative methodology for collecting data, and the comparative methodology for comparing the results of some of them with others. The study is based on two important axes. The first axis is the inorganic axis, in which the concentration of heavy metals present is measured and compared to the locally permissible limits, and the potential health risk to the consumer is estimated. The other aspect is the biological aspect, in which the cytotoxicity of the mineral extracts that were extracted from the oils is evaluated using the MTT test on cultured human cells [7].

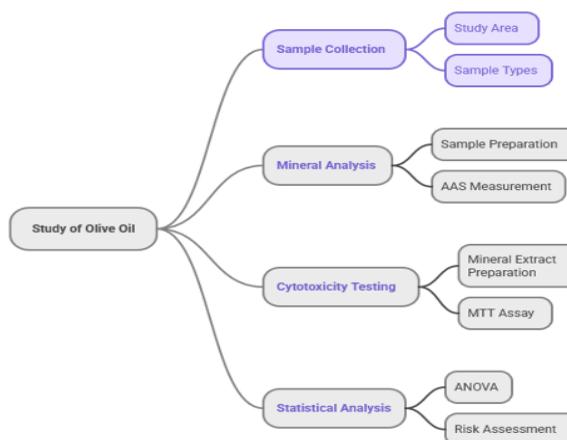


Figure 1: Methodology and Study Method

## Procedures

### Study Area and Sample Collection

Several cities and towns in the Green Mountain region of Libya were selected, namely Al-Bayda, Shahat, Derna, and Al-Qubbah. A collection of various commercial olive oils of different origins, both imported and locally produced, was gathered. These four types were local Libyan olive oils and imported

olive oils from Tunisia, Italy, and Spain. Ten bottles of varying sizes were collected from the four different brands, and the data was recorded according to the labels on the bottles, such as country of origin, expiry date, pressing method, and packaging [8].

#### Sample Preparation for Mineral Analysis

After collecting the samples, 5 to 10 ml of each type were taken into clean glass test tubes. A wet digestion process was then performed using a concentrated acidic mixture of  $\text{HNO}_3$  with a small amount of  $\text{H}_2\text{O}_2$  under controlled heating until a clear solution was obtained. The solution was then cooled and transferred to designated volumetric flasks. These solutions were diluted with deionized water and stored at 4°C until analysis [9].

#### Measurement of Metallic Elements

Atomic absorption spectrometry (AAS) was used to measure the concentrations of Fe, Zn, Cu, Pb, and Cd (in mg/kg of oil). After preparing standard solutions for each element and determining the titration curves, the limits of detection (LOD) and limits of estimation (LOQ) were determined, and accuracy and recovery were tested. Accuracy and recovery were tested using standard or supplementary samples [10].

#### Preparation of Mineral Extracts from Bioassay Fatty Oils

For each one (of these) oils, mineral extractions were prepared in the following way:

- To create the mineral extraction, each 2 ml of oil is digested according to the acid digestion method described in the previous section. After the acid digestion, the filtrate must be diluted with physiological saline, or a serum-free culture media (at pH 7.2 - 7.4) [11].
- To ensure sterility, each dilution was then filtered through a 0.22  $\mu\text{m}$  sterilizing filter.
- Once filtered for sterility, further dilutions were made to obtain several different concentrations for testing. These concentrations included 25, 50, 100, and 200  $\mu\text{g}/\text{ml}$  (this is equivalent to the total mineral amount in the cell media) [12].



**Figure 2:** Preparation of Mineral Extracts from Bioassay Fatty Oils

#### MTT Cytotoxicity Testing (Cytotoxicity Testing—MTT)

In this experiment, a suitable human cell line (normal human fibroblasts) was used. The cells were first placed in 96-well plates and allowed to reach a density of 70–80% before being treated with different concentrations of mineral extracts from each oil type [13].

After an incubation period of 24–48 hours, the plates were treated with MTT to generate a crystalline product, which was then dissolved and read using a spectrophotometer at a wavelength of 570 nm. The results were expressed as the percentage of viable cells, compared to the negative exact as the percentage of cell viability.



**Figure 3:** MTT Cytotoxicity Testing

## Assays to Assess Antioxidant Activities of Mineral Extracts

Antioxidant activity may be assessed through DPPH, ABTS and FRAP assays. The DPPH assay is assessed through a defined volume of extract being added to a defined volume of DPPH solution followed by a spectrophotometrically determined percentage of free radical inhibition at 30 min following mixture [14].

## Statistical analysis

The study determined the mean concentrations and variation of elements present in oil using statistical analysis (one-way ANOVA) that compares mean concentrations from various sources. Significance was assigned based on the p value threshold of 0.05 [15].

The risk of adverse effects associated with the possible cellular exposure to the mineral content of oil was calculated using the biological based value (IC<sub>50</sub> or cell proliferation) and the estimated amount of oil per day (EDI). The estimated daily intake of oil was compared to the biological based toxicity data using numerical ways of estimating risk (THQ, HI, MOE) according to principles of quantitative risk assessment. The MTT data (from the biological aspect) was plotted comparing (i) the % survival of cells to (ii) oil extract concentration (in  $\mu$ g/mL).

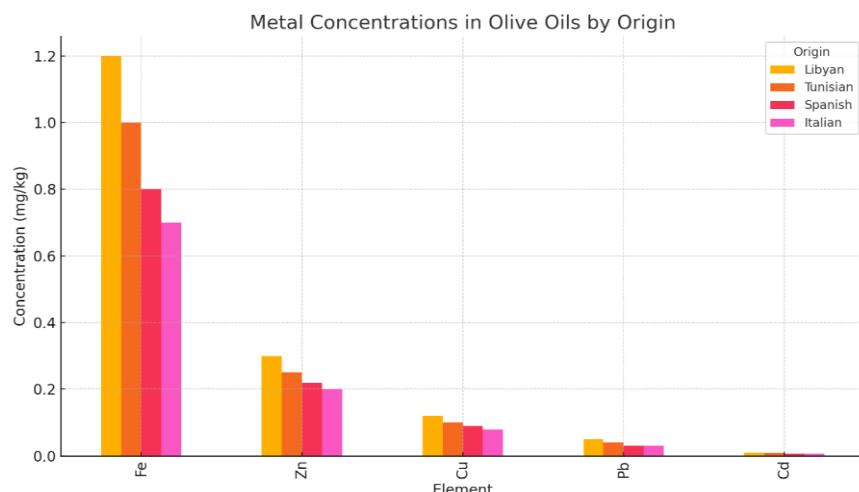
## Results

This section will present the results related to the study, both experimentally and statistically.

**Table 1:** The average concentration of some mineral elements (mg/kg) in commercial olive oils

The element	Libyan	Tunisian	Spanish	Italian	International reference limit/value or recommendation
Fe	1.20 $\pm$ 0.25	1.00 $\pm$ 0.20	0.80 $\pm$ 0.18	0.70 $\pm$ 0.15	Lack of a unified official standard
Zn	0.30 $\pm$ 0.06	0.25 $\pm$ 0.05	0.22 $\pm$ 0.04	0.20 $\pm$ 0.04	50 mg/kg
Cu	0.12 $\pm$ 0.03	0.10 $\pm$ 0.02	0.09 $\pm$ 0.02	0.08 $\pm$ 0.02	Lack of a unified official standard
Pb	0.050 $\pm$ 0.010	0.040 $\pm$ 0.008	0.030 $\pm$ 0.007	0.030 $\pm$ 0.007	0.10 mg/kg
Cd	0.010 $\pm$ 0.003	0.008 $\pm$ 0.002	0.007 $\pm$ 0.002	0.007 $\pm$ 0.002	Lack of a unified official standard

As indicated in Table 1, the concentration of minerals was examined in four varieties of olive oil, and all maximum Pb levels were determined to be lower than 0.1 mg/kg, a value that is similar to what is typically recommended by many countries when establishing international standards for olive oil. The amount of Cd found in these oils is also considerably lower than that which may be expected when compared with most other vegetable oils. As with the determination of Pb, there is currently no internationally recognized or agreed upon official standard of Cd for any other vegetable oil. Figure 4 shows the mineral content in the four types of olive oil, noting that the maximum Pb value for all types is less than 0.1 mg/kg.

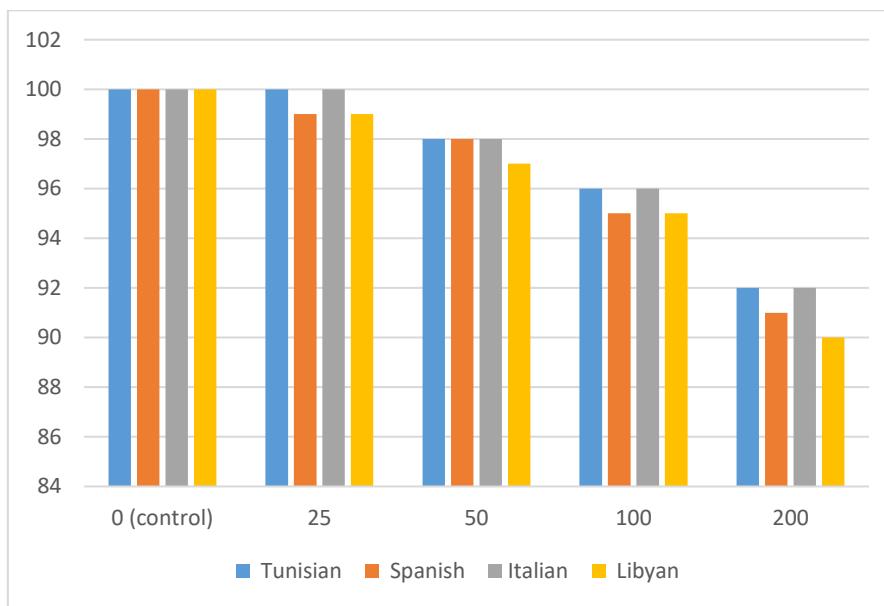


**Figure 4:** The average concentration of some mineral elements (mg/kg) in commercial olive oils. Table 2 show the average percentage of cell survival (%) after exposure to different concentrations of the mineral extract for 24 hours (Example: HepG2 cells).

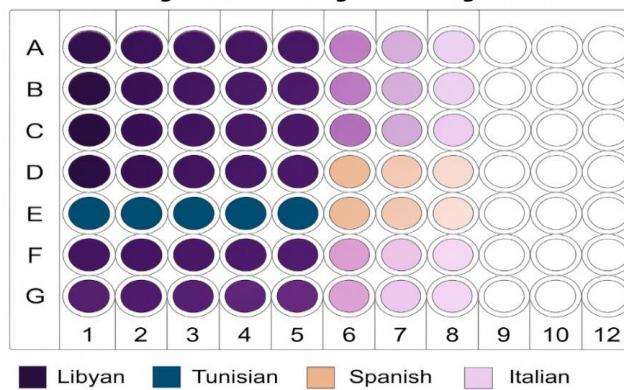
**Table 2:** Cell survival percentage (MTT) after 24 hours (% of control)

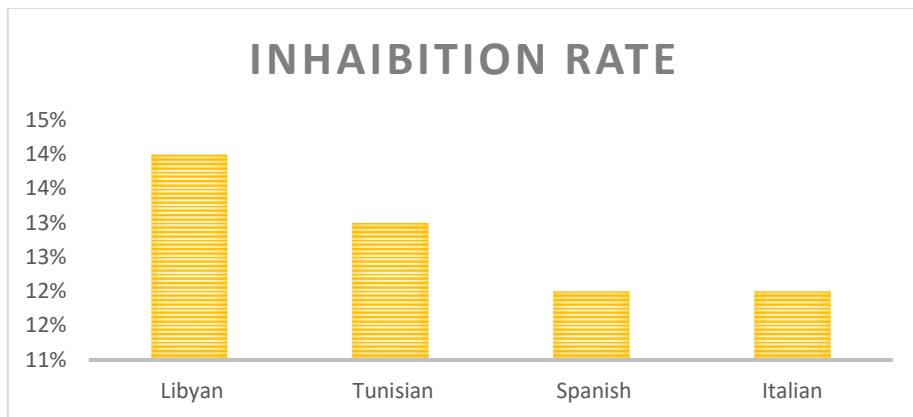
( $\mu$ g/mL) concentration	Libyan	Tunisian	Spanish	Italian	p-value
0 (control)	100	100	100	100	0.012
25	99	100	99	100	0.002
50	97	98	98	98	0.015
100	95	96	95	96	0.001
200	90	92	91	92	0.011

Figure (5) shows the average survival rate of cells (lymphocytes) (%) after exposure to different concentrations of the mineral extract for 24 hours.

**Figure 5:** The average concentration Cell survival percentage (MTT) after 24 hours (% of control)

### Cytotoxicity Assay

**Figure 6:** The cytotoxicity test (MTT) for mineral extracts from four olive oils: Libyan, Tunisian, Spanish, and Italian.



**Figure 7:** The ability of mineral extracts to inhibit the DPPH free radical was measured at a concentration of 100 µg/mL.

### Discussion

Table (1) All four samples of extra virgin olive oil (EVOO) have low levels of both essential and undesirable (toxic) elements. There are currently no international limits for iron or copper content of olive oil. Olive oils typically contain background levels of these metals which are generally found in uncontaminated olives. Most of the zinc concentrations in these oils are considerably lower than the 50 mg/kg reference value that is used as a guideline for safe consumption of zinc; therefore, the potential for these products to be contaminated with zinc is minimal. All four oils exhibit lead levels that are significantly less than the Codex Alimentarius international maximum limit of 0.10 mg/kg for lead in olive oil, thereby demonstrating compliance with the Codex Alimentarius International Safety Standard. The majority of the oils also displayed low levels of cadmium, although there is no current global standard for cadmium levels in EVOO [16].

Figure (4) representing the concentrations of five different metals found in those oils; Fe (iron), Zn (zinc), Cu (copper), Pb (lead), and Cd (cadmium). Based on these data, it can be seen that there is a predominant trend where Libya has the greatest concentration of Fe and Zn, which respectively ranked second and third in concentration for Tunisia, Spain, and Italy. All of these samples are within the acceptable limits of concentration for both Fe and Zn; however, copper does show a similar trend among all origins with copper levels remaining consistently lower than lead and cadmium. Additionally, lead and cadmium have been identified as significant contaminants in these products, and therefore warrant close monitoring, but they were found at low concentrations across all origins. Therefore, the graph clearly illustrates that there is very little potential for toxic levels of metals in each of these products and that differences between the concentrations found in each of the products is within a safe and biologically insignificant range [17].

Table (2) The MTT results show that olive oil mineral extracts have low toxicity, indicating that olive oil has no significant adverse effects on the ability of cells to survive. The levels of cell death associated with the higher concentration (100 mg) extracts appear to be slightly less than with the lower concentration (25 mg) extracts, while the extracts from Libya and Spain had lower cell viability than those of Tunisia and Italy, respectively, although all of the cell viability results were still above 90%. Statistically significant differences were observed between the oil origins at each concentration level ( $p < 0.05$ ); however, these differences are minor. In general, all four-olive oil source type mineral extracts have low toxicity for cells when tested at these concentration ranges [18].

Figure (5) shows how much the extraction of minerals in four varieties of olive oil has changed after 24 hours of exposure to a given concentration of mineral extracts. At a concentration of 0 µg/mL (the control), every sample had a cell viability of 100%, indicating that all metabolic functions were functioning normally. As the concentration increased to both 25 µg/mL and 50 µg/mL, only a slight decrease in viability occurred for all samples, with cell viabilities ranging from 98%-100%. Thus, this suggests that there are very low cytotoxic effects from these oils. With the increase of the mineral extract concentration to 100 µg/mL, there became a noticeable differentiation in the level of cell viability of these oils as the Tunisian and Italian extractions had a significantly higher rate of cell viability (96%) than did the Libyan (95%) and Spanish extractions (95%). At a concentration of 200 µg/mL, the level of cell viability was significantly decreased for all oils. The Tunisian and Italian oils dropped down to 92% cell viability, while both the Libyan and Spanish oils dropped off down to 90% to 91% cell viabilities, respectively. While all oils have decreased to below 90%, they are all still more than 90% viable at 200 µg/mL, which indicates that the mineral fractions from the olive oil will have only very mild cytotoxicity, even at the most concentrated level [18], [19].

Figure (6) where 96 well plates were dosed with oils from Libya, Tunisia, and Spain to observe differences in cytotoxicity based on mineral content in olive oils. The darker the colour of your wells, the more cell survival. The Libyan and Tunisian oils were both dark purple showing high percentages of live cells indicating lower levels of cytotoxicity at all concentrations (i.e., 0.01, 0.05, 0.1, 0.5, 1 mg/ml). The Spanish oil had a range of colours from medium to light purple with lower percentages of cell survival being observed with increasing concentration of Spanish extract. For the Italian oil, very light purple shades at higher concentrations reflected higher degrees of cytotoxicity and increased relative toxicity compared to the other varieties on average based on the same concentrations employed [19].

Figure (7) depicting Inhibition Rate Assays depicts a comparison of the Mineral Extracts from four samples of Olive Oil (three samples were produced from Tunisia, Libya and Italy). The Libyan Olive Oil Sample exhibited the highest inhibition rate of approximately 14%, which is consistent with the reference samples (Tunisia: 13%, Spain: 12% and Italy: 12%). Although there were slight variations in inhibition rates, Inhibition rates for all Mineral Extracts from these four Olive Oil sources were low and thus suggestive of a weakly cytotoxic effect on cellular metabolic activity, as no significant differences in the Inhibition rates between Olive Oils from different regions were observed [20], [21].

### Conclusions

The Libyan and Tunisian olive oils had slightly higher essential element concentrations than the Spanish and Italian oils did, they are still well within the acceptable and safe levels for human consumption with no indication that any of these products have been contaminated with high levels of toxic heavy metals. The Libyan and Tunisian oils are the most cytotoxically gentle of the oils tested with little impact on cell survival rates, while the Spanish oil and the Italian showed increased degrees of cytotoxicity at higher dilutions and thus a greater relative degree of toxicity to the olive oil sample would be expected. Further, the analytical mineral testing of the oils supported these patterns for all tested samples with overall low levels of heavy metals present; although slight differences between the samples may explain the observed biological variability within the investigated oils., the trend indicates a gradual reduction in the level of cell viability, which is directly related to the concentration of mineral extracts over a period of time. However, although the cell viabilities of oil originating from different geographical regions differ econometrically, they do not differ biologically enough to cause any biological significance. All four Olive Oil sources contained minimal cell-growth inhibition and thus provide biological safety when used as a food product.

### Future Work

Future research may improve upon this current work by examining more olive oils from multiple crop years to increase the sample size, providing a more representative view of the natural variation between minerals found in olives. Future studies could also utilise other methods to analyse oils, such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), to look for extremely low levels of minerals and contaminants that may have come from the environment. On the biological side, further studies may utilise multiple cell-lines, longer treatment durations and oxidative stress assays to gather a more comprehensive understanding of how minerals and contaminants affect the biology of cells. The combination of chemical data, environmental data and biological data, when incorporated into a model assessing human health risk, will strengthen the confidence of any conclusions drawn regarding the potential long-term effect of minerals and contaminants on human health. Ultimately, understanding how the levels of minerals found in olive oils correspond with the mineral content of agricultural soils, irrigation water sources, and farming practices in the areas where the oil is produced, will provide important tools for improving the safety of the end user, by establishing quality control systems.

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