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Bioremediation of the Soils Contaminated with Cadmium and Chromium, by the Earthworm *Eisenia fetida*

Biorremediação de Solos Contaminados por Cádmio e Cromo, pelo Anelídeo *Eisenia fetida*

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Abstract

One of the most important environmental problems in the world is the soils contamination by heavy metals in the industrial areas, and especially the contamination of the agricultural lands. The use of earthworms to bioremediate the soils results in reducing the pollutants concentration through a bioaccumulation mechanism on the contaminants in the earthworm's body. Hence, the present study aimed to prove the biological effectiveness of *Eisenia fetida* earthworms in bioremediation the soils contaminated with chromium and cadmium. Concentration of chromium and cadmium pollution in soil was determined to be 0.04 mg/g and 0.08 mg/g respectively. 30 worms were added to 500 g soil samples. Chromium and cadmium concentration in soil and in the body of worms was measured at two time periods of 21 and 42 days. To measure the concentration of chromium and cadmium we used ICP spectrometry. Software in usage was SPSS version 17. There was a significant correlation between the reduction of chromium and cadmium metals in the soils and the accumulation of chromium and cadmium metals in the worm's body. A significant decline of chromium levels of the soil was observed in the days 21 and 42 during the study compared to initial amount of 0.1 mg/g. on the other hand chromium concentration of the soil decreased from 0.14 mg/g to 0.1 mg/g after 42 days. Comparison of mortality in two different time periods showed that by passing the time and by increase in soil chromium and cadmium concentrations the death toll of worms rises. The increased mortality of worms in the soil at a concentration of 0.08 mg/g of chromium, say that using the worms for bioremediation is not recommended at such concentration of chromium but using the worms for the removal of cadmium at concentrations of 0.04 mg/g and 0.08 mg/g in the soil is recommended.
Keywords: Chromium; Cadmium; *Eisenia fetida*; bioremediation

Resumo

Um dos problemas ambientais mais importantes do mundo é a contaminação dos solos por metais pesados nas áreas industriais e, principalmente, a contaminação das terras agrícolas. O uso de anelídeos para biorremediar os solos resultam na redução da concentração de poluentes por meio de um mecanismo de bioacumulação dos contaminantes no corpo dos anelídeos. Assim, o presente estudo teve como objetivo comprovar a eficácia biológica dos anelídeos *Eisenia fetida* em biorremediação dos solos contaminados com cromo e cádmio.

A concentração de cromo e cádmio em solo poluídos foi determinadao como sendo 0,04 mg/g , e de 0,08 mg/g respectivamente. Trinta anelídeos foram adicionados a 500 g de amostras de solo. A concentração de cromo e cádmio no solo e no corpo dos anelídeos foi medida em dois períodos, de 21 e 42 dias . Para medir a concentração de cromo e cádmio usamos espectrometria de ICP . O software em uso foi o SPSS versão 17. Houve uma correlação significativa entre a redução dos metais cromo e cádmio nos solos e da acumulação de metais de cromo e cádmio no corpo dos anelídeos. Também houve uma diminuição significativa na concentração de cromo no solo após 21 e 42 dias em que a concentração inicial de 0,1 mg / g de solo e foi observada após 42 dias a 0,14 mg / g de concentração de crómio no solo diminuiu para 0,1 mg / g . A comparação de mortalidade em dois períodos de tempo diferentes mostraram que há o aumento de mortalidade com o aumento da concentração de cromo e cádmio no solo. O aumento da mortalidade de anelídeos no solo ocorre a uma concentração de 0,08 mg/g de crómio, o que indica que utilização dos anelídeos para a biorremediação não é recomendado em tal concentração de cromo, todavia seu uso é recomendado para a remoção de cádmio em concentrações de 0,04 mg / g e 0,08 mg / g no solo.
Palavras-chave: cromo; cádmio; *Eisenia fetida*; bioremediação

1 Introduction

Over the past few centuries the human activities for exploitation of natural resources had a negative impact on the global balance of heavy metals and have resulted to the increase of metals concentration in the soil's ecosystem (Kızılkaya, 2005). Heavy metals have features such as bioaccumulation, toxicity, high chemical stability, low biodegradability, and high solubility in water, which features are causing a widespread pollution on the soil surface (Robinson *et al.*, 1998; Rajaei *et al.*, 2012). Chromium and Cadmium are two of the said heavy metals existing in the soil. Cadmium is a metal causing kidney lesions, mutagenic, carcinogenic, and increase in blood pressure. Chromium is an essential element for humans and animals health, but in excessive amounts, especially in the form Cr (VI) it will be Harmful for health causing lung and colon cancers (Kaplan *et al.*, 2011; Alidadi *et al.*, 2014). In the field of environmental risk assessment, the earthworms are considered as the major component of the soil, and are important bio- indicators to measure the environmental health and quality of the soil (Nahmani *et al.*, 2009; Sizmur & Hodson, 2009). These organisms can accumulate high concentration of heavy metals in the body (Li *et al.*, 2010). They are normally in contact with the soil and are therefore used, in recent years for the treatment of soil to remove the insecticide used in agriculture, heavy metals and petroleum pollutants out of the soil (Schreck *et al.*, 2008). The use of earthworms on soil Bioremediation is a biological method, so that the pollutant concentrations in the soil are reduced through bioaccumulation mechanisms in the body of the earthworms (Matscheko *et al.*, 2002; Slizovskiy & Kelsey, 2010). Earthworms form a large part of the terrestrial vertebrates' diet and play an important role in the ecology of the earth (Paul & Ghosh, 2011). In general, the presence of earthworms in soil can improve the bio-availability to the contaminants as well as the microbial activity which give rise to microbial decomposition of the most soil contaminants, also one of the most important applications of these organisms consists of the treatment in the wastes management process acting as a biosorbent in soil detoxification and Bioremediation out of heavy metals, persistent hydrocarbons and some organic pesticides, also it causes the population growth of the beneficial microorganisms in the soil and strengthen their enzyme activities as well as improving the soil quality and agricultural development (Sun *et al.*, 2011; Yousefi *et al.*, 2012). A lot of researches were carried out in different countries using earthworms, including the study of Darling and Thomas. (2005) which showed that the concentration of soluble lead compounds in earthworms body are more than the low soluble compounds. In a study it was found that the rate of absorption of essential metals in *Eisenia fetida* earthworms has less value and less diversity than non-essential metals (Nahmani *et al.*, 2007). Spurgeon and Hopkin. (1995) showed that the presence of cadmium, lead, copper and zinc in the soil caused a decreased in the growth of the earthworms and increased the mortality of the *Eisenia fetida* earthworms. Previous studies have shown that there was a significant correlation between the viability, weight and level of Earthworm cocoons production with Chromium and Cadmium pollution and the contact time (Haghparast *et al.*, 2013; Jamshidi & Golchin, 2013).

As today one of the most important environmental problems in the world is the soils contamination in the industrial areas, and especially in agricultural lands by heavy metals such as lead, cadmium, arsenic, mercury, chromium, etc., and the use of earthworms can be a suitable biological method to these contaminants, Hence, we carried out the present study to prove the biological effectiveness of *Eisenia fetida* earthworms in bioremediation the soils contaminated with cadmium and chromium.

2 Methods and Materials

This study was investigated in batch experiments.

2.1 Earthworm

The earthworm used in this study is *Eisenia fetida* which is belonging to the ring worms group- Lumbricide family- *Eisenia gender* - and *fetida* species. These worms were prepared by Salaneh Company producing worms and vermicompost fertilizers in Kashan city.

2.2 Soil

The soils required were obtained from Kashan farmlands in the soil depth of 0-30 cm. from the city of Kashan. The amount of chromium, cadmium and some physicochemical properties of the soils were measured according to the conventional procedures of Iran Soil & Water Research Institute (Haghparast *et al.*, 2013) (Table 1).

Cr (mg/g)	Cd (mg/g)	SP %	EC Ds/m	PH	TKN %	OC %	N %	K (PPM)	P (PPM)	Physical Tests			
										S %	Si %	C %	Texture
0.06	0	22.15	15.90	7.95	20.96	0.13	0.01	86.46	3.05	75	16	9	Sandy Loam

Table 1 Some physicochemical properties of the soil under test physical Tests. Dry soils were passed through sieve 2 mm (sieve mesh 50) and were kept in the shade till the start of the experiment.

2.3 Providing the Required Levels of Chromium and Cadmium Concentrations

Analyzed concentrations for chromium and cadmium were 0.04 mg/g and 0.08 mg/g soil. To obtain these concentrations first the solution 40 g/lit and 80 g/lit chromium and cadmium were made from salts of potassium chromate and cadmium sulfate. Given that the primary soil had 0.06 mg/g of chromium, so the concentrations of 0, 0.04 and 0.08 mg/g of chromium changed to 0.06, 0.1 and 0.14 mg/g.

2.4 Preparation of Worm

In order to adapt the worms to the new environment and avoid any stress, before the start of the experiment, the worms were kept in the soil under test for 10 days. Water was added to the soils so that their moisture content is preserved in the range of 50%. After the 10 days the worms are taken out of the adaptability environment and washed with distilled water and then put them on a wet filter paper for 24 hours so that their gut excrements are excreted out.

2.5 Preparing the Soil

For preparing the soil contaminated with chromium, 0.5 ml and 1ml of a solution of 40 g/lit chromium was uniformly added to pots containing 500g of soil and thus a concentration of 0.04 mg/g and 0.08 mg/g were obtained. Also for the preparation of soil contaminated with cadmium we did the same. To add heavy metals to the soil, the soils of each pot were flatted on a plastic sheet and watered manually by a handy sprinkler so that the soils moisture is adjusted to 50%. Then 30 worms were added to each pot. Pots temperature and humidity were maintained at respectively 25 ± 2 ° C and 70%. To determine the removal of heavy metals from the soil and bioaccumulation of heavy metals in the body of the worm, samples of soil and earthworm were taken after 21 and 42 days.

2.6 Preparing Soil to Measure the Chromium and Cadmium

For the extraction of chromium and cadmium from soil, about 4 grams of soil in each pot was sampled and after drying and grinding them, 1 g of each sample, was weighed with a digital scale with an accuracy of 0.0001 g and the digestion and extraction were performed with a mixture of concentrated nitric acid and hydrochloric acid 50% on the electric oven at 95°C. The samples were filtered by Whatman filter paper, Grade 1, and were stored in polyethylene containers until the measurement time. Also to evaluate possible errors, control samples were prepared with each series of samples (Amouei & Naddafi, 2012).

2.7 Preparing Earthworms' Body to Measure the Chromium and Cadmium

To assess the ability of earthworms *Eisenia fetida* in removing the chromium and cadmium from the contaminated soil by bioaccumulation, earthworms added at the end of each experiment step (day 21 and day 42) were isolated from the soil and washed by water and gently dried with a tissue and then placed on a moist filter paper in a glass plate for 24 hours without any food so to evacuate their gut contents, then the worms were collected and re-washed again, dried gently and put in vials with caps. Acid digestion method was used for the determination of elements (Li *et al*., 2009). In this method, the earthworms tissue after being freezed, were dried in oven and then 0.5g of them weighed and poured into a test tube and 5ml of concentrated nitric acid and 1ml of hydrogen peroxide were added to and then they were heated at 180-220 °C until a clear solution is obtained. The samples were filtered after cooling.

2.8 Method of Measurement

To measure the concentration of chromium and cadmium ICP instrument, Model Optima 2100 DV

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Perkin Elmer factory make belonging to the Laboratory of Kashan Faculty of Medical Sciences was used. **The definition of death:** If all the worms in a pot are gone then that pot is considered as a lost one and to calculate the percentage of deaths, the number of lost pots will be divided to the total number of pots.

2.9 Analysis of Data

For data analysis, the frequency of worms' death in each of the levels of chromium and cadmium concentration was calculated. Also one-sample of t test was used to compare the observed values with the initial values. The software used was SPSS version 17. Significant level was $P<0.05$.

3 Results

3.1 Effect of Different Concentrations of Chromium, Cadmium and Time on the Death Percentage of the Worms

This study was an experimental study with 108 subjects conducted at a laboratory scale. The results showed that the percentage of worm's deaths 21 and 42 days after intervention start in concentration of 0.06 mg/g chromium in soil was zero percent. These numbers on the initial concentration of 0.1 mg/g chromium in soil were respectively, 11.1 and 55.6%, and at a concentration of 0.14 mg/g chromium in soil reached to 44.4 and 77.8 percent. The mortality rate at zero mg/g concentration of cadmium in days 21 and 42 was zero percent. Also the mortality rate in the initial concentration of 0.04 mg/g in the sampling days were 0 and 11.1%, respectively, and at a concentration of 0.08 mg/g cadmium, 0% and 33.3% respectively, which shows a clear increase in the mortality level of worms with the increase of chromium concentration in soil, while little change was observed in the mortality of worms by increasing the concentration of cadmium in soil Table 2).

Type of Mineral	Concentration in soil(mg/g)	Time of contact(day)	
		21	42
Chromium	0.06	0	0
	0.1	11.1	55.6
	0.14	44.4	77.8
Cadmium	0	0	0
	0.04	0	11.1
	0.08	0	33.3

Table 2 Percentage of mortality of worms in different concentrations of chromium and cadmium per mg/g in two different time periods.

3.2 Effect of Chromium and Cadmium Concentration as well as Time in the Removal of Metals out of Soil and their Accumulation in the Worms' Body

Table 3 shows that there was no significant statistical hanged observed in the level of initial chromium concentration in soil after 21 and 42 days of bioremediation process by worm ($P = 0.317$). In the initial concentration of 0.1 mg/g, the soil's chromium after 21 days of Bioremediation was 0.064 mg/g ($P=0.014$) and after 42 days it reached 0.065 mg/g ($p = 0.048$), which was approximately 0.04 mg/g reduction in soil chromium. Also in the concentration of 0.14 mg/g, chromium after 21 days was 0.131 mg/g ($P = 0.745$) and after 42 days it was 0.1 mg/g ($P = 0.01$), which in this concentration there is no significant change in the chromium level of soil. The initial concentration of 0.04 mg/g of soil cadmium after 21 days of Bioremediation was 0.024 mg/g and after 42 days reached 0.025 mg/g ($P = 0.0001$), which about 0.02 mg/g deletion was observed. About the concentration of 0.08 mg/g cadmium, this amount after 21 days, was 0.062 mg/g and after 42 reached to 0.057 mg/g which shows approximately 0.02 mg/g and 0.03 mg/g of deletion ($P = 0.0001$).

Concentration of chromium in the Soil (mg/g)		0.06		0.1		0.14	
		$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P
Time of contact (day)	21	0.061±0.003	0.317	0.064±0.030	0.014	0.1319±0.0517	0.745
	42	0.061±0.003	0.317	0.065±0.021	0.048	0.099±0.006	0.01
Concentration of cadmium in the Soil (mg/g)		**0**		**0.04**		**0.08**	
		$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P
Time of contact (day)	21	0.0001±0.00001	0.0001	0.0248±0.0028	0.0001	0.0622±0.0071	0.0001
	42	0.0001±0.00001	0.0001	0.0254±0.0026	0.0001	0.0574±0.0022	0.0001

Table 3 Average and standard deviation of soil's chromium and cadmium per chromium and cadmium initial concentrations in the soil, and the measurement time.

Concentration of chromium in the worms' body (mg/g)		0.06		0.1		0.14	
		$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P
Time of contact(day)	21	0.0044±0.0001	0.0001	0.0282±0.020	0.0001	0.0424±0.020	0.0001
	42	0.0045±0.0001	0.0001	0.0283±0.0129	0.002	0.0416±0.0107	0.049
Concentration of cadmium in the worms' body (mg/g)		**0**		**0.04**		**0.08**	
		$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P	$\bar{x} \pm SD$	P
Time of contact(day)	21	0.0025±0.00022	0.0001	0.1718±0.0565	0.0001	0.1484±0.085	0.043
	42	0.0025±0.00008	0.0001	0.2921±0.0469	0.0001	0.357±0.096	0.001

Table 4 Average and standard deviation of chromium and cadmium level in the worms' body per chromium and cadmium initial concentrations in the soil, and the measurement time.

The concentration of chromium in the body of worm, after 21 days at a concentration of 0.06 mg/g soil cumulative chromium were not found (0.0044 mg/g) at concentration of 0.1 mg/g it was 0.0282 mg/g and at the concentration of 0.14 mg/g reached to 0.0424 mg/gr (Table 4). After 42 days, in a concentration of 0.06 mg/g again no aggregation was observed (0.0045 mg/g), while at the concentration of 0.1 mg/g and 0.14mg/g, respectively, 0.0283 mg/g and 0.0416 mg/g were reached. (Results are given in Figures 1 and 2).

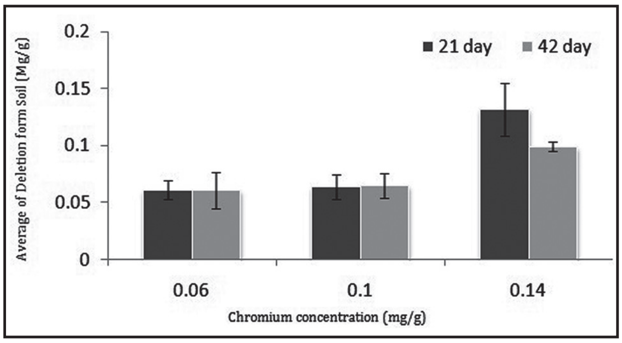

Figure 1 Comparison of soil's chromium average based on the initial concentrations in different times of study.

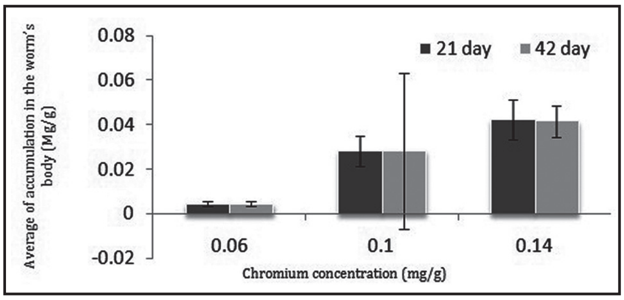

Figure 2 Comparison of chromium accumulation average in the worm's body based on the initial concentrations in different times of study.

About the Cadmium accumulation in the body of the worm, at the concentration of 0.04 mg/g after 21 and 42 days the accumulation respectively reached to 0. 171 mg/g and 0.292 mg/g and at the concentration of 0.08 mg/g it reached to 0.148 mg/g and 0.357 mg/g respectively. (Results are given in Figures 3 and 4).

4 Discussion

The purpose of this study was to evaluate the effectiveness of earthworms Eisenia Fetida in Bioremediation of the soils contaminated with

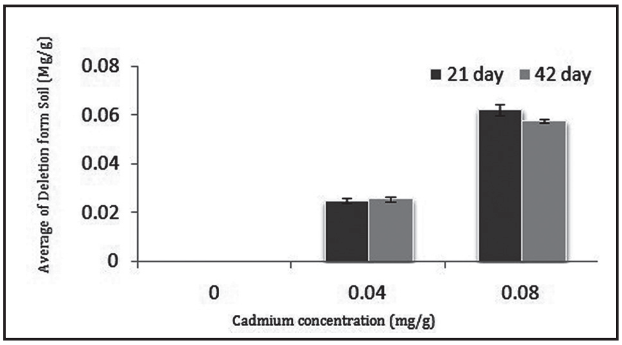

Figure 3 Comparison of soil's cadmium average based on the initial concentrations in different times of study

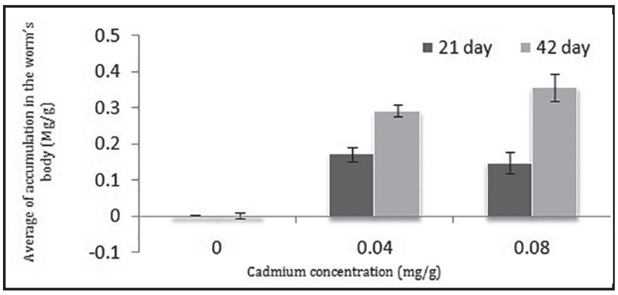

Figure 4 Comparison of cadmium accumulation average in the worm's body based on the initial concentrations in different times of study

cadmium and chromium, and was investigated in batch experiments.

Studies have shown that earthworms have a high potential for biological bioremediation of contaminated soils (Suthar *et al.*, 2008; Nahmani, Hodson *et al*., 2009; Li, Xu *et al*., 2010; Hirano & Tamae, 2011). However, it has been found that the accumulation of heavy metals in the body of earthworm is partly due to the heavy metal concentrations in soil, Ma *et al*. (1983) reported. According to the results obtained in this current study, the accumulation of cadmium in the earthworm's body was greater than Chromium. Aleagha & Ebadi (2013) indicated in their study that the rate of accumulation of Cadmium during 14 days is higher than the concentration of chromium in the earthworm's body. Previous studies have also confirmed that as well, according to the results those previous studies; earthworms have a high capacity in accumulating cadmium biodegradability (Brewer & Barrett, 1995; Lapinski *et al*., 2002; Li, Xu et al., 2010). Of course in some cases, the death of earthworms due to their incompatibility with the environment or the extra toxification of the metals during the bioremediation, the accumulation rate of a given metal in the earthworm's body is less than its removal rate from the soil. And on the contrary, in some other cases the accumulation rate of a given metal in the earthworm's body is more than its removal rate from the soil; which was due to the higher volume of the soil than the number of the earthworms.

In the present study, we can learn that in the 0.06 mg/g concentration of the soil's Chromium no removal occurred which the reason was probably the low concentration of Chromium in the soil and the low contact of the earthworms with the soil. In other hand, in the concentration of 0.1 mg/g during 21 days about 35% decrease in the concentration of soil's Chromium was seen that this increase of the performance was due to the accessibility of more metal Ions in the contaminated soil. According to the results, with increasing concentration of chromium in soil from 0.1 mg/g to 0.14 mg/g the removal efficiency was decreased by about 30%. Similar results were obtained for the removal of cadmium, so that with increasing cadmium concentration from 0.04 mg/g to 0.08 mg/g the removal efficiency was decreased by about 16% which can be due toxic effect of chromium and cadmium metals as well as the increase in the death rate of the earthworms.

The findings of this study showed that with increase in the contact time of the earthworms with cadmium and chromium in low concentration rates of these metals, no significant changes in the removal performance occurs but on the contrary, with increase in the contact time of the earthworms with these metals in high rate concentration the removal rate increases so that the removal efficiency of cadmium increases from 21 days to 42 days, about 6% and chromium about 23%; it has been confirmed by Davies *et al*. (2003) study.

The results of Zaltauskaite & Sodiene (2010) showed that by increasing the concentration of lead earthworms' mortality increases significantly but in the case of cadmium no significant deaths occurred. According to the statistical analyses carried out in this study, it was approved that there was a significant correlation between the concentration rate of the soil's chromium and the mortality rate of the earthworms ($p>0.05$) so that with increasing the chromium concentration the mortality rate increases as well that this is due to the high toxicity of chromium for the worms. On the other hand, according to the findings by increasing the concentration of cadmium in the soil, little change in Mortality of worms was observed which was due to the detoxification of cadmium by Metallothionein proteins existing in the posterior alimentary channels of the earthworms (Morgan & Morgan, 1992). Differences reported in the studies about the effect of the elements on the growth parameters of the earthworm can be due to the different contact times during which the earthworm face with the contaminants so that the test times differ from several hours to several weeks (Haghparast; Golchin *et al*., 2013). Concentration of the metals in the body of earthworms can reach the toxic level if the contact time of earthworm with them is prolonged. Hagparast *et al*. (2013) , Jamshidi and Golchin (2013) also confirmed that. Thus, the potential effects of metals may be overlooked or underestimated in the short-term experiments (Bengtsson *et al*. 1986), which may explain why the study Debra Kennett *et al*. (2002) showed that, despite the high levels of metal contamination in the soil, no significant earthworm mortality was seen in none of the soil samples tested.

5 Conclusions

The results of this study show that *Eisenia fetida* earthworms can remove the chromium and cadmium metals, but their effectiveness in removing cadmium is more than chromium. The relationship between increase in chromium and cadmium concentration and the viability there.

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