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The effectiveness of bluemink (*Ageratum houstonianum* Mill.) and French marigold (*Tagetes patula* L.) in removing Zn and Cu from contaminated growing media

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Abstract: Utilizing ornamental plants for phytoremediation provides multiple benefits: they enhance the visual attractiveness of their surroundings and are predominantly non-edible, thus decreasing the chances of bioaccumulation in the food chain. To assess the effectiveness of bluemink (*Ageratum houstonianum* Mill.) and French marigold (*Tagetes patula* L.) in removing Zn and Cu from artificially contaminated substrates, a 6-week pot experiment was conducted in a greenhouse. The experiment consisted of four contamination treatments for each heavy metal examined, specifically 0, 100, 250, and 500 mg kg^{-1} for Zn, and 0, 50, 100, and 200 mg kg^{-1} for Cu. The Zn and Cu levels in the plant samples were determined by atomic absorption spectrophotometry. The bioaccumulation factor (BF) and translocation factor (TF) were used to evaluate the phytoextraction potential of the plants. BAF values for Zn ranged from 0.739 to 1.089 in bluemink and from 0.534 to 1.047 in French marigold, suggest that both plants analysed could be regarded as potential hyperaccumulators of Zn, particularly in the case of their long-term cultivation on contaminated soil. The BAF and TF values for Cu in both studied plants, bluemink and French marigold, were consistently below 1, indicating their limited capacity to remove Cu from the soil.

Key words: heavy metals, ornamental plants, phytoremediation, pollution

Učinkovitost modrika (*Ageratum houstonianum* Mill.) in rjavkaste žametnice (*Tagetes patula* L.) pri odstranjevanju Zn in Cu iz kontaminiranih rastnih medijev

Izvleček: Uporaba okrasnih rastlin za fitoremediacijo prinaša več prednosti: povečajo vizualno privlačnost okolice in so pretežno neužitne, s čimer se zmanjšajo možnosti bioakumulacije v prehranski verigi. Za oceno učinkovitosti modrika (*Ageratum houstonianum* Mill.) in rjavkaste žametnice (*Tagetes patula* L.) pri odstranjevanju Zn in Cu iz umetno onesnaženih substratov je bil v rastlinjaku izveden 6-tedenski lončni poskus. Poskus je obsegal štiri obravnavanja onesnaževanja z vsako preučevano težko kovino in sicer 0, 100, 250 in 500 mg kg^{-1} za Zn ter 0, 50, 100 in 200 mg kg^{-1} za Cu. Vsebnost Zn in Cu v vzorcih rastlin je bila določena z atomsko absorpcijsko spektrofotometrijo. Bioakumulacijski faktor (BF) in translokacijski faktor (TF) sta bila uporabljena za oceno fitoekstrakcijskega potenciala rastlin. Vrednosti BAF za Zn so se gibale od 0,739 do 1,089 pri modriku in od 0,534 do 1,047 pri rjavkasti žametnici, kar kaže na to, da bi lahko obe analizirani rastlini obravnavali kot potencialni hiperakumulatorki Zn, zlasti v primeru njunega dolgotrajnega gojenja v onesnaženih tleh. Vrednosti BAF in TF za Cu v obeh proučevanih rastlinah, modriku in rjavkasti žametnici, so bile dosledno pod 1, kar kaže na njuno omejeno sposobnost odstranjevanja Cu iz onesnaženih tal.

Ključne besede: težke kovine, okrasne rastline, fitoremediacija, onesnaženje

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1 INTRODUCTION

Soil heavy metal pollution is a significant environmental issue that poses substantial risks to plant life, human health, and the food supply on a global scale (Angon et al., 2024). Arsenic (As), mercury (Hg), cadmium (Cd), chromium (Cr), and lead (Pb) are considered priority metals of public health importance because of their high toxicity. However, essential heavy metals such as zinc (Zn) and copper (Cu) can also be toxic to plants, humans and animals when present in the environment above threshold concentrations (Kolesnikov et al., 2021).

An excess of Zn in plants leads to a range of morphological and physiological disorders, including reduced growth, smaller leaves, and a weakly developed root system. Furthermore, an excess of Zn in plants adversely affects Fe absorption and is also one of the causes of nitrogen deficiency in plants (Balafrej et al., 2020). Excessive intake of Zn can lead to health complications in humans, with symptoms that may include nausea, dizziness, headaches, stomach pain, vomiting, and a lack of appetite (Schoofs et al., 2024).

The functioning of plants is also compromised due to elevated levels of Cu accumulation. Excessive Cu can damage root cells, inhibit the formation of root hairs, and hinder nutrient and water uptake, interfering with essential physiological processes in plants, particularly photosynthesis (Chen et al., 2020). Additionally, high levels of Cu in plants lead to oxidative stress by catalysing oxidation-reduction reactions that produce reactive oxygen species, including singlet oxygen (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). Cu can also bind to sulfhydryl groups in enzymes, inhibiting their activity and disrupting the metabolic pathways in which these enzymes are involved (Permyakov, 2021). Excessive copper in the human body can also be harmful, resulting in liver damage, kidney disorders, and other serious health risks (Binesh & Venkatachalam, 2024).

Considering the adverse effects of Zn and Cu on both plant and human health, there has been a notable increase in ecological and global public health concerns in recent years regarding environmental contamination by these heavy metals. In light of the above, the effective remediation of soil contaminated with Zn and Cu is of great importance for protecting human health, maintaining ecosystems, and ensuring sustainable land use.

Currently, phytoremediation is recognized as the most cost-efficient and environmentally sustainable technique for the remediation of soil contaminated by heavy metals (Yan et al., 2020). This is mainly because physical and chemical remediation techniques are complex regarding implementation and costs (Sánchez-Castro et al., 2023).

Among phytoremediation processes, phytoextraction is regarded as the most efficient method for removing heavy metals from polluted soils. Typically, it utilizes plants characterized by rapid growth, large biomass, a well-developed root system, tolerance to heavy metal toxicity, and a high capacity for accumulating heavy metals in their above-ground parts (Rosariastuti et al., 2019). Plant species exhibiting these characteristics are called hyperaccumulators (Sharma et al., 2023).

The plants' ability to remove heavy metals from polluted soils varies among plant species, depending mainly on their genetic background and on the soil's physical and chemical properties that influence heavy metal availability (Park & Oh, 2023). Despite the major progress in phytoextraction research, the implementation of this technique with ornamental plants has not been extensively investigated. Using ornamental plant species for phytoremediation offers numerous advantages: they enhance the beauty of the area they occupy, and are mostly non-edible, thus reducing the risk of biomagnification and bioaccumulation in the food chain (Al-Sayaydeh et al., 2022). In light of the above, this study aims to explore the potential of two ornamental plants: bluemink (*Ageratum houstonianum* Mill.) and French marigold (*Tagetes patula* L.) in removing Cu and Zn from artificially contaminated growing media.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

The pot experiment was performed from mid-May until June 2025 in a greenhouse of the Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina. On May 12, 2025, one-month-old seedlings of bluemink (*Ageratum houstonianum* 'Blue Hawaii') and French marigold (*Tagetes patula* 'Aurora Orange') were placed into plastic pots with dimensions of 12 cm x 10.8 cm and 11 cm x 9.5 cm, respectively. These pots were previously filled with a commercial growing substrate (Garden Centre 'Flora') artificially contaminated with Zn and Cu. Only healthy seedlings of similar height were chosen for transplantation. The substrate composition selected was a mixture of coconut fibre, black and white peat, composted plant material, organic matter, and perlite, incorporating 1 g of fertilizer N-P-K 15-15-15 per litre of substrate. According to the manufacturer's specification, the main chemical properties of the growing substrate before adding the contaminants were as follows: pH (KCl): 6.0, organic matter content: 56 % by mass, available phosphorus: 37.6 mg 100 g^{-1}, and available potassium: 43.2 mg 100 g^{-1}. The total forms of Zn and Cu were 56.2 mg kg^{-1} and 18.7 mg kg^{-1}, respectively.

Before the initiation of the experiment, the substrate with a moisture content ranging from 60 % to 65 % was divided into eight equal parts. Subsequently, 2 kg from each part of the substrate was transferred into individual polystyrene containers. Each container was then spiked with 200 ml of an aqueous solution of $ZnSO_4 \cdot 7H_2O$ or $CuSO_4 \cdot 5H_2O$, formulated to reach final concentrations of 0, 100, 250, and 500 mg kg^{-1} for Zn, and 0, 50, 100, and 200 mg kg^{-1} for Cu in the substrate. Thorough mixing was then conducted to ensure an even distribution of the heavy metal solution. Following this, the contaminated substrate was transferred into plastic pots, each with a capacity of approximately 0.5 l, which were utilized in the experiment.

The experiment consisted of four contamination treatments for each heavy metal examined, specifically 0, 100, 250, and 500 mg kg^{-1} for Zn, and 0, 50, 100, and 200 mg kg^{-1} for Cu, with three replications for each treatment. Each contamination treatment included four groups, each containing three pots, resulting in 72 pots for every plant species analysed. During the experimental period, the air temperature varied between 18 °C and 33 °C, while the relative humidity fluctuated from 55 % to 95 %. Air circulation was maintained by opening the roof vents and the main entrance throughout the day, while a green shade cloth was used to avoid overheating on hot days. Each plant/pot was watered every 2-3 days to maintain a steady moisture level in the substrate. At the end of the experiment, 40 days post-setup, the selected plant morphological parameters were measured: plant height, leaf number, inflorescence number, and fresh and dry mass of root and aerial parts. Following this, the plants were harvested and divided into their underground and aerial parts to assess the bioaccumulation and translocation factors. The plant's height was measured using a ruler, whereas the number of leaves and inflorescences was determined by counting. The fresh mass of both the root and above ground parts was measured right after harvesting, while the dry mass was determined after drying in an oven at 60 °C to constant mass.

2.2 EXTRACTION AND ANALYSIS OF ZINC AND COPPER FROM PLANT SAMPLES

For the extraction of Zn and Cu from plant samples, the dry ashing digestion method was utilized (Lisjak et al., 2009). In brief, a 2 g dry plant material was placed in a crucible and subjected to ignition in a muffle furnace at 550 °C for six h. The ash obtained was then digested with 10 ml of a mixture of HNO_3 and H_2SO_4 in a 2.5:1 ratio, and subsequently heated on a hot plate under a fume hood for one h at 60 °C. After cooling to room temperature, the digested plant sample was filtered through Whatman quantitative filter paper No. 42 into a 50 ml volumetric flask and then diluted to the mark with deionized water. Concentration of Zn and Cu in the digested plant samples was measured using a Shimadzu atomic absorption spectrometer (Model 7000-AA, Tokyo, Japan) in accordance with the ISO 11047 method (ISO, 1998). The working standard solutions containing Zn and Cu were prepared by diluting the stock solutions (1000 mg l^{-1}) with deionized water as necessary.

2.3 BIOACCUMULATION FACTOR AND TRANSLOCATION FACTOR

The Zn and Cu concentrations recorded were used to estimate the bioaccumulation factor (BAF) and the translocation factor (TF). BAF was calculated from the metal concentration ratio of the plant's harvestable part to the growing substrate (Ladislas et al., 2012):

$$BAF = \frac{C\ plant}{C\ soil}$$

TF was evaluated from the ratio of heavy metals in the plant's above-ground part to that in the plant root (Bonanno et al., 2018):

$$TF = \frac{C\ aboveground\ plant\ parts}{C\ roots}$$

Plants with BAF and TF values both higher than 1 have the potential to be used for phytoextraction (Alghamdi & El-Zohri, 2024).

2.4 STATISTICAL ANALYSIS

Statistical analyses were carried out using SPSS 20 software. The data were subjected to analysis of variance (ANOVA), and Fisher's LSD post hoc test determined differences in means at a 5 % significance level ($p \leq 0.05$). Results were expressed as mean ± standard deviation (SD).

3 RESULTS

The study's findings demonstrated that the contamination of the growth substrate with Zn and Cu had no negative impact on the morphological traits of bluemink and French marigold plants, regardless of the level of substrate contamination. There were no considerable differences in the morphological characteristics of the heavy metal-treated plants compared to the control plants (Tables 1 and 2).

In addition, throughout the entire experimental period, no visual signs of heavy metal toxicity were found in the tested plants, suggesting that bluemink and French marigold can grow successfully in growth substrates enriched with Zn and Cu.

For a plant to be deemed to have significant phytoremediation potential, it should not only be able to successfully grow in soils that are contaminated with heavy metals but also have a strong ability to accumulate one or more heavy metals in large amounts from these polluted soils. The average values of Zn and Cu in the roots and above-ground parts of bluemink and French marigold plants grown in substrates with varying degrees of Zn and Cu contamination are presented in Tables 3 and 4.

The data presented in Tables 3 and 4 indicate that the accumulation of Zn and Cu in both the above-ground and below-ground parts of the bluemink and French marigold plants increased with higher concentrations of these elements in the growing substrate. In addition, Zn and Cu levels have consistently been higher in the roots than in the above-ground parts of the plant, regardless of the level of substrate contamination to which the

Table 1: Morphological characteristics of bluemink depending on the contamination of the growth substrate with Zn and Cu

Treatment	Plant height (cm)	Above-ground fresh mass (g)	Fresh root mass (g)	Number of inflorescences per plant	Number of leaves per plant
Zn 100 mg kg^{-1}	13.4 ± 1.1	10.6 ± 0.8	1.2 ± 0.4	36.4 ± 3.5	246.7 ± 25.2
Zn 250 mg kg^{-1}	13.2 ± 1.0	10.5 ± 0.8	1.3 ± 0.2	37.2 ± 3.2	232.3 ± 47.6
Zn 500 mg kg^{-1}	12.9 ± 1.1	10.0 ± 0.9	1.1 ± 0.3	34.6 ± 4.2	218.2 ± 27.8
Control	13.5 ± 0.9	10.6 ± 0.7	1.3 ± 0.2	37.1 ± 3.2	241.9 ± 34.8
F test	n.s.	n.s.	n.s.	n.s.	n.s.
Cu 50 mg kg^{-1}	14.1 ± 1.9	11.2 ± 1.5	1.2 ± 0.4	35.7 ± 4.1	241.3 ± 14.9
Cu 100 mg kg^{-1}	13.1 ± 1.5	10.5 ± 1.1	1.2 ± 0.3	36.2 ± 4.5	234.4 ± 13.1
Cu 200 mg kg^{-1}	12.7 ± 2.6	10.3 ± 1.4	1.0 ± 0.4	32.7 ± 5.3	227.4 ± 23.5
Control	13.6 ± 1.3	10.8 ± 1.1	1.1 ± 0.2	35.5 ± 3.7	242.2 ± 13.9
F test	n.s.	n.s.	n.s.	n.s.	n.s.

Results were expressed as mean ± standard deviation (SD); n.s. - non significant

Table 2: Morphological characteristics of French marigold depending on the contamination of the growth substrate with Zn and Cu

Treatment	Plant height (cm)	Above-ground fresh mass (g)	Fresh root mass (g)	Number of inflorescences per plant
Zn 100 mg kg^{-1}	9.2 ± 1.4	9.8 ± 0.5	1.2 ± 0.3	6.3 ± 1.5
Zn 250 mg kg^{-1}	10.4 ± 2.5	9.5 ± 0.9	1.0 ± 0.3	5.3 ± 1.5
Zn 500 mg kg^{-1}	10.4 ± 1.4	9.5 ± 0.6	1.1 ± 0.2	5.1 ± 1.7
Control	10.6 ± 1.0	10.1 ± 0.9	1.1 ± 0.3	6.0 ± 2.0
F test	n.s.	n.s.	n.s.	n.s.
Cu 50 mg kg^{-1}	9.5 ± 1.0	10.6 ± 0.8	1.1 ± 0.2	6.0 ± 1.1
Cu 100 mg kg^{-1}	9.9 ± 1.3	10.0 ± 1.6	1.1 ± 0.2	5.4 ± 1.3
Cu 200 mg kg^{-1}	9.2 ± 0.7	8.9 ± 1.3	0.9 ± 0.3	5.3 ± 1.1
Control	10.5 ± 1.1	10.4 ± 1.0	1.1 ± 0.1	6.1 ± 0.9
F test	n.s.	n.s.	n.s.	n.s.

Results were expressed as mean ± standard deviation (SD); n.s. - non significant

Table 3: Zn and Cu levels (mg kg^{-1} dry mass) in the bluemink grown in contaminated substrates.

Treatment	Zn content		Treatment	Cu content	
	Aerial parts	Roots		Aerial parts	Roots
Zn 100 mg kg^{-1}	101.7 ± 5.5c	173.8 ± 8.3c	Cu 50 mg kg^{-1}	7.8 ± 2.0bc	20.3 ± 4.6b
Zn 250 mg kg^{-1}	164.5 ± 21.6b	366.5 ± 20.6b	Cu 100 mg kg^{-1}	10.2 ± 2.7b	45.4 ± 4.9a
Zn 500 mg kg^{-1}	339.9 ± 19.1a	1457.6 ± 75.9a	Cu 200 mg kg^{-1}	16.9 ± 2.8a	51.4 ± 4.1a
Control	51.2 ± 8.6d	51.8 ± 4.5d	Control	4.7 ± 1.4c	8.3 ± 2.5c
F test	s.	s.	F test	s.	s.
LSD0.05	28.8	74.6	LSD0.05	4.3	7.7

Results were expressed as mean ± standard deviation (SD); s. - significant; Different letters in the same column indicate statistically significant differences ($p \leq 0.05$)

Table 4: Zn and Cu levels (mg kg^{-1} dry mass) in the French marigold grown in contaminated substrates.

Treatment	Zn content		Treatment	Cu content	
	Aerial parts	Roots		Aerial parts	Roots
Zn 100 mg kg^{-1}	62.4 ± 2.8c	255.7 ± 6.1c	Cu 50 mg kg^{-1}	6.0 ± 1.3b	49.3 ± 3.9c
Zn 250 mg kg^{-1}	116.9 ± 6.1b	540.4 ± 13.6b	Cu 100 mg kg^{-1}	8.2 ± 1.7b	68.5 ± 4.9b
Zn 500 mg kg^{-1}	260.4 ± 14.8a	1946.2 ± 55.6a	Cu 200 mg kg^{-1}	17.5 ± 2.3a	87.7 ± 6.8a
Control	29.1 ± 1.7d	31.6 ± 7.7d	Control	2.9 ± 0.9c	10.3 ± 2.7d
F test	s.	s.	F test	s.	s.
LSD0.05	15.6	54.7	LSD0.05	2.6	9.1

Results were expressed as mean ± standard deviation (SD); s. - significant; Different letters in the same column indicate statistically significant differences ($p \leq 0.05$)

Table 5: Bioaccumulation factor (BAF) and translocation factor (TF) for Zn and Cu determined in the cultivation of bluemink

Treatment	BAF	TF	Treatment	BAF	TF
Zn 100 mg kg^{-1}	1.089	0.585	Cu 50 mg kg^{-1}	0.181	0.384
Zn 250 mg kg^{-1}	0.739	0.449	Cu 100 mg kg^{-1}	0.137	0.225
Zn 500 mg kg^{-1}	0.903	0.233	Cu 200 mg kg^{-1}	0.102	0.199
Control	0.918	0.988	Control	0.271	0.566

Table 6: Bioaccumulation factor (BAF) and translocation factor (TF) for Zn and Cu determined in the cultivation of French marigold

Treatment	BAF	TF	Treatment	BAF	TF
Zn 100 mg kg^{-1}	0.785	0.244	Cu 50 mg kg^{-1}	0.207	0.122
Zn 250 mg kg^{-1}	0.689	0.216	Cu 100 mg kg^{-1}	0.142	0.119
Zn 500 mg kg^{-1}	1.047	0.134	Cu 200 mg kg^{-1}	0.123	0.199
Control	0.534	0.921	Control	0.195	0.282

tested plants were exposed.Bioaccumulation (BAF) and translocation (TF) factors for Zn and Cu determined in bluemink and French marigold plants are given in Tables 5 and 6.

4 DISCUSSION

Despite the high concentrations of Zn and Cu in the growth substrates, and the fact that the accumulation of Zn and Cu in the studied plants generally correlated positively with their concentrations in the growth substrate, no visual symptoms of Zn and Cu toxicity were observed. This suggests that blumink and French marigold belong to Zn- and Cu-tolerant species. The successful growth of these two species in soils enriched with Zn and Cu has also been confirmed in several studies (Mkumbo et al., 2012; Zhou et al., 2017; Fu et al., 2021). From the standpoint of using bluemink and French marigold in landscape architecture, this data is especially important, particularly in relation to landscaping in regions where the soils are polluted with Zn and Cu. The study's results also showed that the levels of Zn and Cu in the roots of both plants analysed were up to several times higher than in the above-ground parts. These outcomes are unexpected, particularly because Zn and Cu are essential elements for the plant‘s growth and development. However, high concentrations of Zn and Cu can disrupt normal metabolic activities, prompting plants to activate mechanisms that restrict the translocation of these metals from the roots to the above-ground parts (Behtash et al., 2022). Hu et al. (2024) reported that plants have evolved various mechanisms for this purpose, including heavy metal sequestration in root cell walls or vacuoles, production of heavy metal-binding polypeptides, and the activation of barriers that limit the influx of heavy metals from roots to the xylem. The relatively low TF values for Zn and Cu determined in this study (Tables 3 and 4) support the hypothesis that bluemink and French marigold possess some specific mechanisms. The TF values for Cu were generally much lower than Zn in both examined plants: bluemink and French marigold, indicating that the mechanisms preventing the transport of Cu from the roots to the aerial parts of the plant are more pronounced.

The ability of bluemink and French marigold to remove Zn and Cu from contaminated substrates was evaluated by estimating the BAF. The BAF values for Zn in bluemink plants ranged from 0.739 to 1.089. In contrast, in French marigold plants it varied from 0.534 to 1.047, indicating that both plants possess a relatively high capacity for removing Zn from contaminated sites. Accordingly, both plant species, bluemink and French marigold, could be regarded as potential hyperaccumulators of Zn, particularly in the case of their long-term cultivation on contaminated soil. Two other studies have also demonstrated a relatively high capacity of French marigold to accumulate Zn from contaminated sites (Mónok et al., 2018; Nair et al., 2019). However, it is important to note that the phytoextraction potential of bluemink and French marigold for Zn primarily relies on the accumulation of Zn in the roots, which should be considered when conducting phytoextraction on contaminated soil.

In this study, the BAF values for Cu in bluemink plants ranged from 0.102 to 0.271, while in French marigold plants it varied from 0.123 to 0.207, depending on the substrate contamination level. In both examined plants, the BAF values for Cu were significantly lower compared to those for Zn. These results support the hypothesis that bluemink and French marigold should not be classified as hyperaccumulators for Cu. Similar findings for French marigold were observed by Lacerda et al. (2025). On the other hand, several studies have indicated that French marigold could be effective in the remediation of Cu-contaminated soils (Mónok et al., 2019; Roshanfar et al., 2024). The variations in the ability of French marigold to remove Cu from contaminated soils, as presented in scientific literature, strongly imply that its effectiveness in remediating Cu-polluted soils is contingent upon the physico-chemical properties of the soil and other factors that affect Cu phytoavailability (Biswal et al., 2022). In contrast, there is insufficient research on the ability of bluemink to serve as Cu-hyperaccumulator plants; thus, additional studies are required to confirm the low ability of bluemink to remove Cu from the contaminated substrate noted in this study.

5 CONCLUSIONS

This study‘s outcomes imply that both plants analysed, bluemink and French marigold, can tolerate high levels of Cu and Zn in the growing media. No visible Zn and Cu toxicity symptoms were observed in either plant, regardless of the level of substrate contamination. From the Zn and Cu accumulation viewpoint, bluemink and French marigold exhibit a similar behaviour pattern when grown in substrates enriched with Zn and Cu. The levels of Zn and Cu in the roots of both plants analysed were up to several times higher than in the above-ground parts. BAF values determined in this study suggest that both plant species could be regarded as potential hyperaccumulators of Zn, particularly in the case of their long-term cultivation on contaminated soil. On the other hand, the results from this study revealed that none of the plants investigated exhibited significant phytoextraction potential for Cu.

DATA AVAILABILITY STATEMENT

The author confirms that all data generated or analysed during this study are included in this published article.

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