Environment and Ecology 42 (4) : 1495—1503, October—December 2024
Article DOI: https://doi.org/10.60151/envec/SIZG5023
ISSN 0970-0420

# Toxicity Assessment of Tannery Sludge Extract During Early Seedling Growth in *Brassica nigra, Vigna radiata, Vigna mungo, Raphanus sativus* and *Capsicum frutescens*

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Received 22 April 2024, Accepted 8 August 2024, Published on 18 October 2024

## ABSTRACT

Tannery sludge (TS) contains a number of useful components like Organic carbon, Organic Matter, $PO_4^{3-}$, $Ca^{2+}$, $Mg^{2+}$, and other nutrients essential for plant growth. However, presence of water-soluble toxic metals in TS may cause phytotoxicity. Primary objective of this work was to adopt a bio-chemical strategy for assessing the potential consequences of agricultural application of tannery sludge, via sludge characterization and subsequent bioassay investigations. Heavy metal concentration in the TS was found to be in the order of: Cr (19576±2065.52) > Pb (16.98±2.54) > Cu (1.76±0.20) > Ni (0.98±0.00) > Cd (0.19±0.00) mg $kg^{-1}$. Further, bioassay tests were performed in terms of germination index (GI), relative seed germination (RSG) and relative root growth (RRG) on *Brassica nigra, Vigna radiata, Vigna mungo, Raphanus sativus,* and *Capsicum frutescens.* RSG and RRG were found to be more than 80% whereas, GI was > 66% in all the tested crops exposed to TS extract at 1:5 and 1:10 (w/v) dilutions. Results revealed that the toxicants present in the TS were failed to cause toxicity at early seedling growth and may be used as a soil conditioner after appropriate dilution. However, long term field trails are essential before agricultural utilization.

**Keywords** Germination index, Heavy metals, Phytotoxicity, Relative root growth, Relative seed germination, Tannery sludge.

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## INTRODUCTION

Soil is an essential, non-renewable resource for seed germination and plant growth (Keesstra *et al.* 2016). Due to rapid industrialization, unplanned urbanization and improper waste management, soil has been subjected to increasing constraints that limit the vegetal cover and agriculture produce (Rahaman *et al.* 2023). Sludge is the final output of biological treatment of industrial effluents (Melo *et al.* 2018). Proper disposal of sludge is a global environmental concern (Seleiman *et al.* 2020). Sludge is frequently disposed of in landfills or incinerated, however, these disposal methods are costly and detrimental to the environment (Melo *et al.* 2018). Further, useful components like trace elements and other nutrients present in industrial sludge are wasted (Scotti *et al.* 2015). Ascribable to the presence of useful components such as organic matter, nitrogen, phosphorus, and other nutrients for plant growth, use of sludges as fertilizer or soil conditioner in agricultural farms, woodlands, and gardens appears to be an attractive solution of sludge management (de Matos Barbosa *et al.* 2021). Use of organic waste can enhance soil fertility by improving the physical, chemical, and biological properties of the soil (Scotti *et al.* 2015). However, the heavy metals present in the sludge may pose a serious environmental concern being non-biodegradable and persist in the environment for generations (Yan *et al.* 2020).

Rapid expansion in tanning industry in the last century has remarkably increased the amount and complexity of toxic substances into the environment (Patel *et al.* 2015). Globally around 6945.3 Mt of raw leather is processed each year with more than 60% of it discharged as waste (Zhou *et al.* 2021). India is one of the largest producers of leather in the world and about 700,000 tons wet salted hides and skin are processed annually in about 3000 tanneries (Chand *et al.* 2015). These industries produce large amount of sludge which need to be disposed safely. Being rich in various inorganic nutrients and organic components they are frequently used by farmers in agriculture to boost the production (Seleiman *et al.* 2020). However, presence of high concentration of toxic metals and hazardous substances in tannery sludge (TS) may pose toxicity to the plants and subsequently to the secondary consumers (Melo *et al.* 2018). Several phytotoxic effect can be induced by a number of organic and inorganic contaminants like heavy metals, ammonia, polycyclic aromatic hydrocarbons (PAHs), antibiotics, phenols (Gupta *et al*. 2020, Raklami *et al.* 2021). Further, significant quantity of toxic metals available in soil can limit seed germination, plant growth and biomass production (Raklami *et al.* 2019). There are numerous mechanisms involved during the initial germination stage where heavy metals directly impact seed germination and normal plant development. However, after proper processing, sludge containing bioavailable nutrients can be applied in agriculture field to enhance agricultural produce sustainably (Seleiman *et al.* 2017).

It has been reported that the phytotoxicity tests such as seedling growth, biomass production, and seed germination are useful to assess initial toxic effect on plants (Bona *et al.* 2023). Seed germination stage has been reported as a key period in an individual's plant life cycle to examine phytotoxic effects (Bao *et al.* 2022). Various crop species have been recommended for phytotoxicity test such as, *Brassica nigra* (Mustard), *Cyamopsis tetragonoloba* (Guar), *Cajanus cajan* (Pigeon pea), *Vigna radiata* (Mung bean), *Vigna mungo* (Urad), *Trigonella foenum-greacum* (Fenugreek), *Solanum melongena* (Brinjal), *Abelmoschus esculentus* (Okra), *Raphanus sativus* (Radish) and *Capsicum frutescens* (Chili) (Raklami *et al.* 2021).

The main objective of this work was to establish a bio-chemical approach for assessing the possible consequences of agricultural application of tannery sludge, through sludge characterization and subsequent bioassay studies.

## MATERIALS AND METHODS

Sample collection Tannery sludge (TS) was collected from common effluent treatment plant (CETP) Banther, Unnao, Uttar Pradesh, India. Collected TS was air dried and homogenized to remove the large size stone, plastics, and other particles. Subsequently sieved to less than 165µm and stored in PEP containers for further analysis. Garden soil (GS) collected from horticulture garden of Babasaheb Bhimrao Ambedkar University, Lucknow (UP) India, served as control. Equipment, reagents and analysis of the experiment are shown in Table 1.

### Heavy metal analysis

Sludge samples as well as control were processed for heavy metals analysis following Iticescu *et al.* (2021). One gram of homogenized samples were

**Table 1.** Techniques and instruments used for analysis of various physico-chemical parameters of TS and GS.

| Parameters | Techniques | Instruments | References |
|---|---|---|---|
| EC | Digital water analyzer | Systronics- T371 | Kumar and Maiti (2015) |
| pH | | | |
| Salinity | | | |
| Moisture | | | |
| Organic carbon | Rapid titration method | | Walkley and Black (1934) |
| Organic matter | | | |
| $Ca^{2+}$ | EDTA titration methods | | Karunanidhi *et al.* (2021) |
| $Mg^{2+}$ | | | |
| $Na^{+}$ | Flame photometer | Systronics flame photometer 130 | Karunanidhi *et al.* (2021) |
| $K^{+}$ | | | |
| $PO_4^{3-}$ | Stannous chloride method | UV-visible scanning spectrophotometer | Murphy and Riley (1962), Sletten and Bach (1961) |
| SAR | | | |
| Cr | Acid digestion methods | ICPMS, iCAP RQ-RQ01013 | McGrath and Cunliffe (1985) |
| Cd | | | |
| Pb | | | |
| Ni | | | |
| Cu | | | |

taken in five replicates (n=5) and digested overnight in the solution of $HNO_3$:$HClO_4$ (4:1 v/v) at 70 to 80 ºC. The solution was allowed to evaporate by raising the temperature to 105 ºC until the sludge samples were digested completely and the remaining solution become transparent. Final volume of the digested samples were makeup to 25 ml with distilled water and analyzed on ICPMS.

## Seed germination and phytotoxicity test

Seeds of Mustard (*Brassica nigra*), Moong bean (*Vigna radiata*), Urad (*Vigna mungo*), Radish (*Raphanus sativus*) and Chili (*Capsicum frutescens*) as recommended by USEPA (1996) and Radovich (2018) were used for seed germination and root elongation study. The seeds were purchased from the Yadav Beej Bhandar and Keet Nashak Kendra CRPF, Chauraha, Bijnaur, Lucknow and Punjab Beej Bhandar, Kanpur-Lucknow Road Sardari Khera, Alambagh, Lucknow, (UP) India. All the seeds were surface sterilized with 0.1% sodium hypochlorite (Disinfectant) solution for 10 minutes then repeatedly cleaned with deionized water before performing germination studies (Pan and Chu 2016).

Sludge extract was derived in ground water at 1:5 and 1:10 ratio (w/v), and used for germination study, whereas, the similar strengths of garden soil extracts taken as control. 20 seeds of each crop species (in five replicates i.e., n = 5) were put on a sheet of filter paper in a petri-dish with 5 milliliters of each extract (ASTM 2003), and kept at 25±0.5 ºC, 80% humidity and in complete darkness (Pan and Chu 2016). 1mm emergence of radicle from the seeds were considered as germination. Seed germination (%) and root length (cm) was calculated after 72 hours of germination. The relative seed germination (RSG), relative root growth (RRG), and the germination index (GI) were calculated following Pinho *et al.* (2017).

$$\text{RSG (\%)} = \frac{\text{Number of seed germinated in treatment}}{\text{Number of seed germinated in control}} \times 100 \quad (1)$$

After 72 hours of the seed sowing, the number of germinated seed exposed with sludge sample extract and the number of germinated seeds in control exposed with garden soil extract were counted for each experimental set. The relative seed germination (RSG) was determined according to Eq. 1 (Pinho *et al*. 2017, Kumar *et al*. 2022).

$$\text{RRG (\%)} = \frac{\text{Treatment (average root length in cm)}}{\text{Control (average root length in cm)}} \times 100 \quad (2)$$

The average root length of the treated and control were measured for each experimental set. The relative root growth was expressed in percentage.

Germination index (GI) % = RSG×RRG/100 (3)

GI was used to assess the detrimental effects on seed germination. It was calculated following equation 3 (Pinho *et al.* 2017).

## RESULTS AND DISCUSSION

### Characterization of the sludge and garden soil

Physico-chemical properties of TS and GS are summarized in Table 2. pH of tannery sludge (TS) and garden soil (GS) was found to be (8.2 ± 0.74) and (8.6 ± 0.82) respectively and considered as alkaline. Slightly alkaline pH favors the availability of several elements and nutrients to the plants (Karwal and Kaushik 2020). Electrical conductivity (EC) of the TS (3316 ± 184) was higher than the GS (525 ± 48.6) samples. High value of EC of TS can be assigned to salts used during the processing of tanning (Chand *et al.* 2015). Moisture content of the TS (55.9 ± 6.54%) was several folds higher than the GS (15.6 ± 1.88%). Tannery sludge samples contained higher level of organic carbon (OC) and organic matter (OM) compared to garden soil. Higher OM may reduce the capability of metals to be phytotoxic in the soil due to metal-organic complexation (Rehman *et al.* 2023). It was reported that the copper strongly bounds with OM and released slowly over the time as the OM of the sludge is decomposed, (Zamulina *et al.* 2022). It has been reported that the addition of OM in soil can establish a more stable soil structure, increase water dispersion and flow in the soil and reduce surface runoff. Further, it may also be supportive for fertility maintenance, which can improve crop productivity and biomass generation (Xu and Wu 2022). Metals like calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and phosphorus (P) were also present in the sludge (Table 2). All these components are crucial for plant growth (Adedeji *et al.* 2023). Tannery sludge was found to have a high content of Cr: 19576±2065.52, while its level was very low in garden soil; 0.57±0.03 mg kg$^{-1}$. The use of chromium during the tanning is the main reason for the high-level Cr in tannery sludge and which makes it questionable for agricultural use (Rigueto *et al.* 2020). It has been reported that the presence of high concentration of Cr in the growing medium may affect seed germination. Thus, the potential of the seeds to germinate in the presence of Cr may indicate their degree of tolerance to Cr (Moreira *et al.* 2020). Cd is one of the heavy metal with known toxicity in biological systems due to its non-essentiality, high solubility and accumulation in biological system (Kato *et al.* 2020). The presence of high level of Cd in growing medium may causes reduction in root and shoot growth, leaf area, dry biomass content and oxidative stress (Abdal *et al.* 2023). Cd stress also disrupt the water content in leave, stomatal regulation, N-fixation potential and abscisic acid content (Mittal *et al.* 2023). It has been reported that the presence of high levels of Pb can affect the enzyme function especially in photosynthesis (Amin *et al.* 2018). Lead concentration in the tannery sludge was found to be on higher side; 16.98±2.54 mg kg$^{-1}$. Several researchers have reported that high concentration of Cu causes reduced biomass and inhibition of photosynthesis which may leading to plant death, Mir *et al.* (2021). Ni is considered as an essential micronutrient at very low concentration, but could be toxic to germinating seeds when present at higher level (Kebrom *et al.* 2019).

**Table 2.** Physico-chemical characteristics of tannery sludge and garden soil. Results are expressed as mean of 5 replicates (i.e. n=5) ± SD.

| Parameters | Unit | Tannery sludge | Garden soil |
|---|---|---|---|
| pH | | 8.2 ± 0.74 | 8.6 ± 0.82 |
| EC | (ms cm$^{-1}$) | 3316 ± 184 | 525 ± 48.6 |
| Salinity | (psu) | 2.79 ± 0.18 | 0.25 ± 0.03 |
| Moisture | (%) | 55.9 ± 6.54 | 15.6 ± 1.88 |
| Organic carbon | (g kg$^{-1}$) | 8.25 ± 0.12 | 0.68 ± 0.84 |
| Organic matter | (g kg$^{-1}$) | 14.22 ± 1.68 | 1.16 ± 0.84 |
| $Ca^{2+}$ | (me kg$^{-1}$) | 510 ± 47.42 | 152 ± 18.44 |
| $Mg^{2+}$ | (me kg$^{-1}$) | 540 ± 38.49 | 56 ± 6.12 |
| $Na^{+}$ | (mg kg$^{-1}$) | 1380 ± 178.48 | 790 ± 88.16 |
| $K^{+}$ | (mg kg$^{-1}$) | 400 ± 46.56 | 300 ± 38.22 |
| $PO_4^{3-}$ | (mg kg$^{-1}$) | 0.23 ± 0.03 | 0.56 ± 0.07 |
| SAR | (me kg$^{-1}$) | 2.12 ± 0.31 | 2.73 ± 0.38 |
| Cr | (mg kg$^{-1}$) | 19576 ± 2065.52 | 0.57 ± 0.03 |
| Cd | (mg kg$^{-1}$) | 0.19 ± 0.00 | 0.09 ± 0.00 |
| Pb | (mg kg$^{-1}$) | 16.98 ± 2.54 | 0.09 ± 0.01 |
| Ni | (mg kg$^{-1}$) | 0.98 ± 0.00 | 0.94 ± 0.02 |
| Cu | (mg kg$^{-1}$) | 1.76 ±0 .20 | 2.28 ± 0.35 |

### Germination indices

Bioassay tests were performed using different concentrations of tannery sludge and garden soil extracts

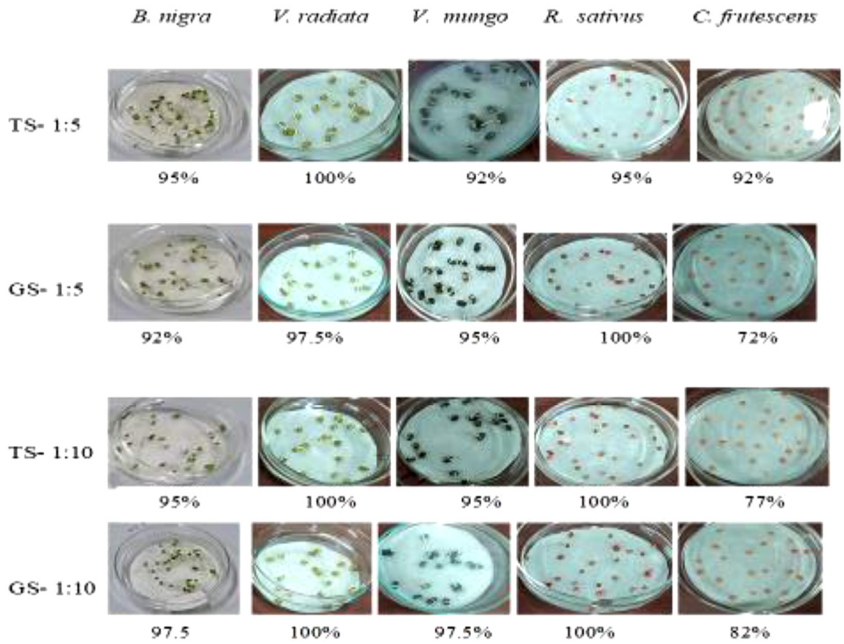


**Fig. 1.** Germination (%) of various crop seeds exposed to tannery sludge extract.

to examine the phytotoxicity during early seedling growth (Fig. 1). The seed germination in *Brassica nigra, Vigna radiata, Vigna mungo, Raphanus sativus* and *Capsicum frutescens* exposed to different strength (1:5 and 1:10) of TS and GS extract was found between 72-100%. The above results confirm that the heavy metal levels in the treatments had no adverse effects on the physiological development of the plants at this early stage (Fig. 1). The germination percentage and GI were better in all repetitions, however variation in pattern were seen in each of the five replicates at the beginning of the experiment. Ecotoxicological bioassay studies showed that the application of contaminated sludge in soil increases seed germination and root elongation (de Moraes *et al.* 2020). Further, it has been reported that the bioassay test can be considered as effective, fast and low-cost method for evaluation of phytotoxicity of biosolids (Lutterbeck *et al.* 2015).

Germination index (GI) for selected crop seeds exposed to different concentration of TS and GS are provided in (Table 3). GI is a reliable and sensitive parameter for phytotoxicity assessment which is based on germination of seeds and root elongation (de Moraes *et al.* 2020). It has been reported that the GI greater than 80% indicates no toxicity while, GI lower than 30% indicates high toxicity (Tiquia *et al.* 1996). Whereas, GI less than 60% indicates that the residue is unsafe for use as a soil conditioner in agricultural field (de Moraes *et al.* 2020). GI of *Brassica nigra* exposed to 1:5 and 1:10 garden soil extract was found to be 90.3±7.4, and 113.0±9.1 respectively, while on exposure to tannery sludge extracts GI value was found as 112.2±8.2 and 80.2±4.2 respectively. GI in *Vigna radiata* exposed to GS extract at concentration of 1:5 and 1:10 of was recorded as 78.7±4.5; and 101.3±3.3; respectively, while on exposure to TS extract it was found to be 127.1±6.7 and 131.2±9.1 respectively. Similarly, it was observed that the GI in *Vigna mungo, Raphanus sativus* and *Capsicum frutescens* was more than 66% on exposure of GS and more than 76% on exposure of TS extract at strength of 1:5 and 1: 10 (Table 3).

Similarly, RSG and RRG experienced negligible effect of both the extracts. In *Brassica nigra*, the RSG at both extracted ratios (1:5 and 1:10) of TS

**Table 3.** RSG, RRG, and GI of various crop species exposed to tannery sludge and garden soil extract.

| Crops | RSG (%) | RRG (%) | GI (%) |
|---|---|---|---|
| Control | 100 | 100 | |
| *Brassica nigra* | | | |
| GS 1:5 | 97.4 ±3.7 | 92.4±7.5 | 90.3±7.4 |
| GS 1:10 | 100 ±7.4 | 113.0±0.6 | 113.0±9.1 |
| TS 1:5 | 100 ±0.1 | 112.2±8.2 | 112.2±8.2 |
| TS 1:10 | 100 ±0.1 | 80.2±7.2 | 80.2±4.2 |
| *Vigna radiate* | | | |
| GS 1:5 | 97.5±3.5 | 80.8±7.5 | 78.7±4.5 |
| GS 1:10 | 100±0.1 | 101.2±3.3 | 101.3±3.3 |
| TS 1:5 | 100±0.1 | 127.1±6.7 | 127.1±6.7 |
| TS 1:10 | 97.5±3.5 | 134.8±8.1 | 131.2±7 |
| *Vigna mungo* | | | |
| GS 1:5 | 95±0.01 | 70.0±4.1 | 66.6±3.9 |
| GS 1:10 | 97.5±3.5 | 88.4±22.3 | 85.8±4.6 |
| TS 1:5 | 92.5±3.5 | 111.8±8.2 | 103.8±6.8 |
| TS 1:10 | 95±0.1 | 98.3±7.4 | 93.5±7.9 |
| *Raphanus sativus* | | | |
| GS 1:5 | 100±0.1 | 97.2±4.0 | 97.2±4.0 |
| GS 1:10 | 100±0.1 | 74.1±3.3 | 74.1±3.3 |
| TS 1:5 | 95±7.1 | 81.0±0.2 | 76.9±5.9 |
| TS 1:10 | 100±0.5 | 97.3±2.0 | 97.3±2.0 |
| *Capsicum frutescens* | | | |
| GS 1:5 | 96.7±9.4 | 104.2±6.0 | 110.9±5.4 |
| GS 1:10 | 110±8.2 | 83.2±4.9 | 91.8±4.1 |
| TS 1:5 | 123.3±11.7 | 89.6±6.7 | 110.9±7.9 |
| TS 1:10 | 103.3 ±8.7 | 81.1±11.8 | 83.6±3.9 |

Results are reported as mean of five replicates ± standard deviation (SD).

and GS was found to be 100±0.2%, 97.4±3.7% and 100±0.3%; 96.3 ±4.4% respectively. Whereas, RRG in *Brassica nigra* were 112.2±8.2%, 92.4±7.5% and 80.2±7.2%, 113.0±0.6% respectively. Similarly, RSG and RRG in *Vigna radiata, Vigna mungo, Raphanus sativus* and *Capsicum frutescens* were found to be more than 80% on exposure to TS and GS extracted solution (Table 3). Although relative seed germination is the most used assessment index, but it is not considered as the most reliable index to describe the germination ability of seeds (Leather and Einhelling 1988). It has been proven that the root length assessment is a more sensitive parameter, but difficult to measure as compared to the RSG (Enaime *et al.* 2020). Both RSG and RRG were recorded ≥80% on exposure to TS and GS extracted solutions for all five different crops. As other similar study reported that Rani *et al.* (2017), TS can ameliorate the micro-macro nutrient contain and heavy metal, when GS added in definite quantity. GS amended with 20% of TS had maximum percentage of seed germination and produced high biomass in *B. juncea* > *R. communis* > *N. Oleander* (Rani *et al.* 2017).

GI and RRG were found to be higher at 1:5 as compared to 1:10 TS extract in *Brassica nigra, Vigna mungo, Capsicum frutescens*. The seed of *Brassica nigra, Vigna mungo, Capsicum frutescens* have been able to germinate on exposure to tannery sludge extracted. Similar results were also recorded by Enaime *et al.* (2020) with olive mill waste water application to soil. Phytotoxicity is still high for some species; such as tomato (*Lycopersicon esculentum*), in this case a further dilution of water, which is considered the least expensive technique, is a good option for the minimizing phytotoxicity. Whereas, GI and RRG of *Vigna radiata* and *Raphanus sativus* were reduced with the higher the concentration (1:5) as compare to lower concentration (1:10) of TS extracted (Table 3). Previous study also reported the similar toxicity of TS effluents on *Vigna radiata* and *Trigonella foenum-graecum* Kumar *et al.* (2022). Overall, GI was recorded to be >76.9±5.9 at 1:5 TS extract that's proven the phytotoxic effect is negligible for all crops and can be safely applied to agriculture soils (de Moraes *et al.* 2020). In contrast, seed germination decreased after the use of solid sample of tannery sludge, signifying its phytotoxic effect on Lactuca sativa de Moraes *et al.* (2020). However, TS did not exhibit a phytotoxic impact on seed germination when solubilized sample had been used.

## CONCLUSION

Tannery sludge contained a high amount of Cr and Pb as compared to other heavy metals like Cd, Ni and Cu. Organic carbon and organic matter were also higher as compared to garden soil. The essential elements viz. $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$ and P were also found in significantly higher amount as compared to garden soil. The germination index value for each test crops viz- Mustard (*Brassica nigra*), Moong bean (*Vigna radiata*), Urad (*Vigna mungo*), Radish (*Raphanus sativus*) and Chili (*Capsicum frutescens*) were found more than 60% which indicated that the contaminants present in the TS are not much toxic at early seedling growth and may be used in agriculture field as a soil conditioner. Further, application of TS containing high nutrient level and organic matter can improve overall soil health which can improve crop productivity and biomass generation. However, field trials are required for corroboration of present findings.

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