**Assessment of heavy metal analysis on the medicinal plant *Stylochaeton hypogaeus* used for the treatment of prostate diseases in Casamance (Senegal): Implication for Human health**

**ABSTRACT**

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| --- |
| Monitoring the presence of minerals in medicinal plants has become a health emergency due to the widespread consumption of herbal products and the risk of long-term bioaccumulation of heavy metals.This study aimed to determine the amount of heavy metals in the medicinal plant *Stylochaeton hypogaeus*, used in Casamance (Senegal) to treat hemorrhoid and prostate diseases. Minerals concentrations in roots, stems and leaves of *Stylochaeton hypogaeus* were in the range of 401.1 to 3505.4 mg/kg for sodium, 9370.1 to 63359.9 mg/kg for potassium, 1824.1 to 6074.3 mg/kg for magnesium, 2136.8 to 11530.0 mg/kg for calcium, 0.1 to 4.6 mg/kg for chromium, 96.8 to 345.3 mg/kg for iron, 0.00 mg/kg for nickel, 3.5 to 3.7 mg/kg for copper, 19.6 to 38.9 mg/kg for zinc, 0.2 to 0.3 mg/kg for cadmium and 1.0 to 2.4 mg/kg for lead. The levels of toxic heavy metal such as Cr, Fe, Cu, Zn, and Pb are higher in the roots with respective values ​​of 4.600±0.001 mg/kg, 345.300±0.019 mg/kg, 3.700±0.001 mg/kg, 38.900±0.005 mg/kg and 2.400±0.002 mg/kg. For cadmium, a value of 0.300±0.001 mg kg-1 is found for stems and leaves, while the roots have a content of 0.200±0.001 mg/kg. The average concentrations of some of the heavy metals analyzed are below the authorized limit in the roots, stems and leaves. The target hazard quotient (THQ) was less than 1 for all heavy metals in roots, stems, and leaves, suggesting that there are no obvious non-cancer health risks associated with the consumption of these medicinal plants, although prolonged use may lead to health risks. The overall hazard factor (HI) is greater than 1 for roots, indicating that the combined effects of heavy metal contaminants in preparation made from the roots of this plant pose a long-term health risk. However, the overall HI for leaves and stems remains less than 1, indicating no health risk for the consumption of preparations made from stems or leaves of this plant. Our current results suggest that medicinal plants contain an acceptable amount of heavy metals and that their use is therefore beneficial in addressing micronutrient deficiencies. Due to the high consumption of plant extracts and the risk of long-term bioaccumulation of heavy metals, monitoring heavy metal concentrations in medicinal plant preparations consumed by patients of traditional practitioners is a health emergency. |

*Keywords: Stylochaeton hypogaeus, medicinal plants, heavy metal, health risk, potassium–sodium ratio, calcium–magnesium ratio.*

# Introduction

Human activities such as agriculture, chemical industry, and major communication routes generate significant pollution that contributes to environmental degradation [1–4] . This pollution causes negative changes in humans, animals, and plants. Depending on the type of activity carried out in a given area, the impact of pollutants can be more or less serious. The nature of the pollutants and their concentration determine the severity of the harmful effects on human health [5, 6]. Persistent pollutants have specific characteristics that are manifested by their persistence, their harmfulness through bioaccumulation, and their ability to be transported by air or water [7–9]. Among these pollutants, heavy metals, which are non-biodegradable, can accumulate in soils, crops, and animals and end up in the food chain, thus affecting the health of consumers [10, 11]. Several sources of pollution are listed. Indeed, wastewater discharge areas, busy roadsides, industrial areas and public landfills are often heavily loaded with heavy metals and medicinal plants grow there in an uncontrolled manner. Added to this is the use of phosphate fertilizers, sewage sludge and organic compost which contain heavy metals. Toxic elements such as chromium, cobalt, nickel, copper, zinc, cadmium, lead and arsenic are often found in cultivated soils on which plants used by traditional practitioners also grow [12–14]. These heavy metals are not biodegradable and persist strongly in contaminated soils and waters long after their introduction and are bioavailable for absorption by plants. Harvests can be significantly affected by the presence of heavy metals and consequently, human and animal health can be seriously impacted by the consumption of these products [15–17]. The high tolerance of certain plants to heavy metals gives them a special status for use in the purification of contaminated water or soil through phytoremediation [18, 19]. Indeed, these plants can absorb large quantities of heavy metals without affecting their health. Daily practice shows that plants are widely used in the treatment of certain diseases by the pharmaceutical industry, paramedical professionals, and traditional practitioners [20–22]. In recent decades, consumers have shown a growing interest in so-called natural medicine, consuming plants alone or in mixtures of plants without any precautions. However, the quality of these plant products can be significantly affected by the quality of the soil and water used to grow them. Several types of pollutants, such as heavy metals, pesticides, herbicides, and fertilizers, can significantly alter the quality of products placed on the market. Several studies have reported the effects of heavy metal toxicity on medicinal plants used by traditional practitioners around the world [23–26]. In Senegal, where medicinal plants are used by all segments of the population, careful analysis must be conducted to rigorously monitor the concentrations of heavy metals present in these plants. In this context, our work aims to assess the heavy metal levels in the medicinal plant *Stylochaeton hypogaeus*, used in the treatment of prostate diseases. The frequent use of medicinal plants to improve health, in the current context of pollution, requires special attention, as they may contain heavy metals, which can pose hazards and risks to human health when contaminated medicinal plants are subsequently consumed, such as teas, other beverages, and cosmetics.

# Materials and methods

## Instrumentation

All experiments were carried out using a Thermo Fischer Atomic Absorption 3000. The samples were run in triplicates and the values reported are mean of triplicates.

## Reagents and standards

All solutions were prepared with distilled-deionized water (18 MΩcm, Milli-Q, Millipore, Bedford, MA, USA). Sulfuric acid (H2SO4, 98%), perchloric acid (HClO4, 70%) and nitric acid (HNO3 65%), from Sigma-Aldrich France, were used in the procedure of digestion of the samples. The analytical solutions were prepared from standard solution dilutions 1000 μgmL-1 of Na, K , Mg, Ca, Cr, Fe, Ni, Cu, Zn, Cd and Pb (Aldrich, France). All materials used were decontaminated in nitric acid solution 10% v/v by 24 h. The medicinal plant samples were purchased from the market of Dakar situated in the center of the town.

## Extraction of minerals from roots, stems or leaves of *Stylochaeton hypogaeus*

Five grams of roots, stems or leaves of *Stylochaeton hypogaeus* were weighed dried in the shade for two days before completing the drying in an oven at 50°C for two days. The vegetal are then finely ground using a blender. For each sample, 5 g of vegetal are mixed with 10 mL of a 1:1 mixture of ethanol and glycerol before being calcined at 500°C for 24 hours. After cooling, the ashes are collected and then treated with concentrated HNO3 to dissolve the different metal ions present in the matrix. The suspensions were filtered into a graduated flask before making up to 100 mL with ultrapure water [27].

**Table 1 : Analytical conditions and calibration curves for Na, K, Mg, Ca, Cr, Fe, Ni, Cu, Zn, Cd and Pb analysis by Atomic Absorption Spectroscopy.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mineral | Wavelength (nm) | Range of detection (mg/L) | Correlation coefficient (R2) | Calibration curve equation |
| Sodium | 598 | 0.02-0.8 | 0.9960 | y = 123.66x +14.486 |
| Potassium | 766.5 | 0.03-1.6 | 0.9969 | y = 0.1621x +0.0096 |
| Magnesium | 285.2 | 0.003-0.6 | 0.9955 | y = 0.5648x +0.0109 |
| Calcium | 422.7 | 0.005-4 | 0.9918 | y = 0.0052x +0.0018 |
| Chromium | 357.9 | 0.03-10 | 0.9966 | y = 0.0133x +0.0029 |
| Iron | 248.3 | 0.05-0.8 | 0.9979 | y = 0.031x +0.0042 |
| Nickel | 232 | 0.09-8 | 0.9906 | y = 0.0451x +0.0173 |
| Copper | 324.8 | 0.01-4 | 0.9975 | y = 0.1418x +0.0078 |
| Zinc | 213.9 | 0.005-1.6 | 0.9958 | y = 0.2175x +0.0040 |
| Cadmium | 228.8 | 0.004-1.8 | 0.9971 | y = 0.1947x +0.0006 |
| Lead | 217 | 0.1-12 | 0.9992 | y = 0.0253x +0.0005 |

## Human Health Risk Assessment

Consumption of herbal preparations containing heavy metals may pose a risk to human health. This risk can be assessed based on the estimated daily intake (EDI) (equation 1) of heavy metals, the target hazard quotient (THQ) (equation 2) and the hazard index (HI) (equation 3). To estimate the daily metal burden in the body of a consumer of a given body weight, the estimated daily intake (EDI) was calculated using the following equation 1 [28, 29].

*EDI* = $\frac{Cn\*IR\*EF\*ED}{BW\*AT\*1000}$ equation 1

(*EDI* is the estimated daily intake of heavy metals ingested from a medicinal plant in mg/kg day, *Cn* is the concentration of heavy metal in medicinal plants measured in mg/kg, *IR* is the ingestion rate which is measured in mg/day, *EF* is the exposure frequency in days/year, *ED* is the exposure duration over years, *BW* is the body weight of the exposed individual in kg, *AT* is the time period over which the dose is averaged in days as seen in Table 2).

**Table 2. Exposure parameters for health risk assessment through various exposure pathways for plants** [30].

|  |  |  |
| --- | --- | --- |
| Parameter | Unit | Adult |
| Body weight | Kg | 70 |
| Exposure frequency (EF)  | days/year | 350 |
| Exposure duration (ED) | year | 30 |
| Ingestion rate (IR) | mg/day | 100 |
| Plant adherence factor (AF) | mg/cm2 | 0.07 |
| Dermal absorption factor (ABS) | None | 0.1 |
| Dermal exposure ratio (FE) | None | 0.61 |
| Average time (AT): For carcinogens  | Days | 365 x 70 |
| For Non-carcinogens | Days | 365 x ED |

## Target Hazard Quotient (THQ)

Prolonged exposure to heavy metals from medicinal plants may pose a carcinogenic risk to consumers. This risk is assessed using the target hazard quotient (THQ) method calculated, according to equation 2, as a percentage of the determined dose relative to the reference dose (RFD) [31]. If the THQ is less than 1, no risk to human health is expected; if the THQ is greater than 1, adverse health effects could occur. The THQ is calculated as the ratio of the average daily intake (EDI) to the reference dose (RFD, Table 3) (equation 2) :

$THQ= \frac{EDI}{RFD}$ equation 2

**Table 3. Reference doses (RFD) used for Cr, Ni, Cu, Zn and Pb** [32]**.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Metal** | Cr | Ni | Cu | Zn | Cd  | Pb |
| **RFD** | 0.003 | 0.02 | 0.04 | 0.3 | 0.001 | 0.004 |

**Hazard Index**

The consumption of medicinal plants containing several heavy metals can pose a significant risk to the consumer's health. Indeed, the effects of these different toxic metals can be additive. The hazard index (HI) is a tool for assessing the total non-carcinogenic risk induced by these metals on human health. The HI is calculated according to equation 3 using the sum of the individual THQ of each metal hazardous to human health. The health risk level is low if HI < 1, while the health risk is high if HI > 1.

$HI=\sum\_{}^{}THQ$ equation 3

# Results and Discussion

### Concentration of Metals in roots, stems, and leaves of Stylochaeton hypogaeus

Table 4 summarizes the mean concentrations of Na, K, Mg, Ca, Cr, Fe, Ni, Cu, Zn, Cd and Pb (mg/Kg) in the different parts of the plant studied in this work.

The Na concentration in the samples varied from 401.1 to 3505.4 mg/Kg. The stems show higher concentration while the roots show lower concentration. The K concentration in samples ranged from 9370.1 to 63359.9 mg/Kg. As observed by the Na, the stems show higher concentration of K, while the roots show lower concentration of K. The Na and K concentrations are comparable to those reported for several plant drugs examined by Szentmihályi *et al*. [33] and Damame *et al*. [34]. Sodium and potassium are important minerals needed to maintain the osmotic balance of body fluids, body pH, proper functioning of the muscular and nervous systems, glucose absorption, and normal protein retention during growth [35]. The sodium/potassium ratio is an important factor in preventing high blood pressure and the recommended K/Na ratio should be greater than 1. The potassium-sodium ratio calculated by the recommended dietary allowances (RDA) which are 23.6:1 (roots), 18.07:1 (stems) and 18.12:1 (leaves), suggest that the consumption of the different parts of this plant can have a beneficial effect on high blood pressure.

The Ca concentrations in the roots (11530 mg/Kg) is higher than the calcium concentrations in the stems (4596.1 mg/Kg) and the leaves (2136.8 mg/Kg). The Mg concentrations in the roots (1824.1 mg/Kg) is lower than the magnesium concentrations in the stems (2746.6 mg/Kg) and the leaves (6074.3 mg/Kg). The Ca and Mg concentrations are lower than there reported for *Urticae folium* medicinal plant which shows Ca and concentrations of 41210 mg/Kg and 6275 mg/Kg, respectively [36]. The calcium and magnesium levels in different parts of the plant can contribute significantly to ensuring the necessary daily intake of these two micronutrients, which are essential for the development and maintenance of normal bones and teeth, normal muscle function, and energy and cellular metabolism. A Ca/Mg ratio of between 2 and 1 is recommended for a good balance. The Ca/Mg ratios are outside the recommended value for the roots (6.32) and leaves (0.35). These two parts of the plant are not suitable for human consumption. The stems have a Ca/Mg ratio of 1.67, which is within the recommended range for this ratio, suggesting that the consumer can use *Stylochaeton hypogaeus* stem preparations without risk.

The Cr concentrations ranged from 0.1 to 4.6 mg/Kg. The roots contained the highest values of 4.6 mg/Kg of chromium. Chromium is a micronutrient necessary for the proper functioning of the body at low concentrations below 0.03 mg/kg. Its bio accumulation can lead to dysfunctions and lead to chronic hypoglycemia, cardiovascular problems or alimentary disorders, dermatitis, lung cancer and permanent nose damage [37, 38]. The permissible limit of chromium in plant raw materials is 2.0 ppm and that of finished products is 0.02 mg/day [39~~]~~. Our results show that Cr levels are higher than the reference values ​​defined by the WHO, for all organs of the plant studied.

Iron is a vital component of hemoglobin and also plays a fundamental role in the metabolism of living organisms [40, 41]. The iron content permitted by WHO (2007) in edible plants is 15 mg/Kg [39, 42, 43]. Consumption of medicinal plants with high iron content can lead to iron accumulation in the human body and cause serious damage to the pancreas, heart, liver, or lungs [44]. The recommended dietary intake of iron for human consumption is 8 to 11 mg/day [45]. Iron concentrations in the roots, stems, and leaves of this medicinal plant range from 96.8 to 345.3 mg/kg and are well above the WHO guideline values. These high levels of iron are found in medicinal plants such as: *T*. *heterophylla* (391 mg/Kg), *R. vomitoria* (574 mg/Kg), *C. anisata* (639.5 mg/Kg), *G. sylvestre* (105 mg/Kg) [46].

**Table 4. Metal level in triplicate (mg/Kg) and Provisional Tolerable Weekly Intake (PTWI) values for metals** [47, 48]**.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Element (mg/Kg) | Roots | Stems | Leaves | PTWI for a 70-kg Individual (mg/week) | LOD (mg/l) |
| Na | 401.1±0.0171 | 3505.4±0.0088 | 2283.5±0.0090 | 14000  | 0.030 |
| K | 9370.1±0.0001 | 63359.9±0.4445 | 41378.7±0.1979 | 24500 | 0.2 |
| Mg | 1824.1±0.000 | 2746.6±0.0096 | 6074.3±0.0027 | 2940 | 0.0008 |
| Ca | 11530.00±0.001 | 4596.1±1.0475 | 2136.8±0.001 | 8400 | 0.0007 |
| Cr | 4.600±0.001 | 0.1±0.000 | 0.9±0.000 | 1.631 | 0.005 |
| Fe | 345.300±0.019 | 96.8±0.00 | 204.8±0.021 | 392 | 0.001 |
| Ni | 0.000 | 0.000 | 0.000 | 2.450 | 0.0012 |
| Cu | 3.700±0.001 | 3.500±0.002 | 3.600±0.001 | 245 | 0.001 |
| Zn | 38.900±0.005 | 31.400±0.003 | 19.600±0.003 | 490 | 0.001 |
| Cd | 0.200±0.001 | 0.300±0.001 | 0.300±0.000 | 0.490 | 0.0004 |
| Pb | 2.400±.002 | 1.600±0.002 | 1.000±000 | 1.750  | 0.005 |

Ni is completely absent from the roots, stems and leaves of the studied plant. Nickel is toxic at high concentrations and high exposure has been associated with various pathologies such as high blood pressure, neurological deficit, cardiovascular diseases and developmental disorders in children [49, 50]. The WHO permissible limit of Ni for medicinal plants remains to be established but some authors have estimated the daily dietary intake of nickel between 70 μg and 400 μg [51]. The Ni concentration in this study was lower than that reported by Sulaiman et al. which gives values ​​of 0.96 to 1.95 mg/Kg [52].

The concentration of Cu for roots, stems and leaves of ***Stylochaeton hypogaeus*** are, respectively, 3.700 mg/Kg, 3.500 mg/Kg and 3.600 mg/Kg. Copper is an essential component of many enzymes. It plays an important role in metabolism. It controls energy production, connective tissue formation, neurotransmitter synthesis, and iron metabolism. Despite its important roles, excess of Cu can cause significant disorders such as respiratory irritation, dermatitis, liver damage, vomiting, and other damage [53–55]. The results of our study show that roots, stems, and leaves have copper concentrations below the WHO limit (10 mg/kg) [56]. These values are lower than the limit value of 10 mg/Kg, defined by WHO /FAO [57] for medicinal plants. Copper concentration is not of concern from the point of view of toxicity for roots, stems and leaves of ***Stylochaeton hypogaeus*** consumption.

Zinc concentrations in roots, stems and leaves of Stylochaeton hypogaeus are in the range of 19.6-38.9 mg/Kg. The leaves are richer in zinc with a concentration of 38.9 mg/Kg while the stems have a content of 31.4 mg/Kg. The stems have a content of 19.6 mg/Kg. These different levels are below the authorized limit for edible plants which is set by FAO/WHO at 50 mg/Kg [58]. Zinc is a micronutrient essential for metabolism. It is involved in the activation of enzymes and proteins, and in the absorption of vitamins A and E [59–61]. It is toxic at high doses. It can cause ataxia, lethargy or gastrointestinal disturbances [62]. In young children, excess zinc can lead to growth retardation [63].

The highest Cd concentration is recorded in stems and leaves with a value of 0.300 mg/Kg, while the roots have a content of 0.2 mg/Kg of Cd. The maximum limit authorized by WHO for Cd in traditional herbal products is 0.3 mg/Kg [64]. The Cd concentrations detected in all parts of this plant studied are in accordance with the limit authorized by WHO for medicinal plants and are lower than the values ​​reported for the plants *Gynostemma pentaphyllum* (1.57 mg/Kg) and *Stevia rebaudiana* (0.68 mg/Kg) [65]. Consumption of herbal preparations containing high doses of Cd is recognized as extremely toxic and carcinogenic. Bioaccumulation can cause severe damage to the kidneys, skeleton, and lungs [66, 67].

Lead concentrations in the roots, stems and leaves of Stylochaeton hypogaeus are 2.4 mg/Kg, 1.6 mg/Kg and 1.0 mg/Kg respectively. All parts of the plant have a Pb concentration below the WHO limit of 10 mg/Kg [64]. Lead is one of the most toxic heavy metals that can cause brain and kidney damage, digestive, hearing and vision disorders, following high exposure [68]. Even regular consumption of low-dose herbal preparations of lead can cause serious damage due to bioaccumulation of this element in biological tissues [69, 70]. Other authors have reported lead concentrations higher than the maximum limit permitted by WHO in medicinal plants [71, 72].

### Health Risk Assessment of Heavy Metals Analyzed

The determination of the daily intake (EDI) of heavy metals is one of the essential tools for assessing health risk associated with the consumption of medicinal plant preparations. It integrates several parameters such as the consumer's body weight, the frequency of intake and the duration of consumption. In Table 5, the estimated daily intakes (EDI) of the different elements are listed. The values ​​found for the heavy metals Cr, Fe, Ni, Zn Cd and Pb are lower than the reference doses, thus suggesting that the consumption of preparations based on roots, stems or leaves of ***Stylochaeton hypogaeus*** does not pose any significant risk to the consumer's health.

**TABLE 5. Estimated daily intake (mg/kg/day) according to the average concentration of each metal in roots, stems and leaves of the medicinal plant for adults**

|  |  |  |  |
| --- | --- | --- | --- |
| **Part of Plant**  | **Roots** | **Stems** | **Leaves** |
| Na | 0.23 | 2.06 | 1.34 |
| K | 5.50 | 37.19 | 24.29 |
| Mg | 1.07 | 1.61 | 3.57 |
| Ca | 6.77 | 2.70 | 1.25 |
| Cr | 2.71 x 10-3 | 5.87 x 10-5 | 5.28 x 10-4 |
| Fe | 2.0 x 10-1 | 5.68 x 10-2 | 1.20 x 10-1 |
| Ni | 0 | 0 | 0 |
| Cu | 2.17 x 10-3 | 2.05 x 10-3 | 2.11 x 10-3 |
| Zn | 2.28 x 10-2 | 1.84 x 10-2 | 1.15 x 10-2 |
| Cd | 1.17 x 10-4 | 1.76 x 10-4 | 1.76 x 10-4 |
| Pb | 1.41 x 10-3 | 9.39 x 10-4 | 5.87 x 10-4 |

### Non-Carcinogenic Risk Assessment

Table 6 presents the non-carcinogenic risk quotient (THQ) and the non-carcinogenic risk index (HI). For all heavy metals Cr, Fe, Ni, Cu, Zn, Cd, and Pb in the various organs of the medicinal plant ***Stylochaeton hypogaeus***, the THQ values ​​are all less than 1. These findings suggest that the consumption of preparations made from the roots, stems, or leaves of ***Stylochaeton hypogaeus*** poses no health risks to consumers. The HI values ​​are less than 1 for stems and leaves. This indicates that the combined effects of the various heavy metals in stem and leaves preparations pose no long-term health risks to consumers. However, the HI of the roots is greater than 1, indicating that the combined effects of heavy metals present in a preparation based on roots of ***Stylochaeton hypogaeus*** present a major risk to the health of the consumer in the long term. Our results are similar to those reported by other authors who report THQ values ​​lower than 1 and HI values ​​higher than 1 for some medicinal plants [73].

**Table 6. HQ and HI (mg/kg/day) of each metal in roots, stems and leaves of the medicinal plant for adults**

|  |  |  |  |
| --- | --- | --- | --- |
| **Element** | **THQ (Roots)** | **THQ (Stems)** | **THQ (Leaves)** |
| Cr | 9.00 x 10-1 | 1.96 x 10-2 | 1.76 x 10-1 |
| Ni | 0.00 | 0.00 | 0.00 |
| Cu | 5.43 x 10-2 | 5.14 x 10-2 | 5.28 x 10-2 |
| Zn | 7.61 x 10-2 | 6.14 x 10-2 | 3.84 x 10-2 |
| Cd | 1.17 x 10-1 | 1.76 x 10-1 | 1.76 x 10-1 |
| Pb | 3.52 x 10-1 | 2.35 x 10-1 | 1.47 x 10-1 |
| **HI** | **1.50** | **5.43 x 10-1** | **5.90 x 10-1** |

# Conclusion

The present study, based on the comparison of the results with the concentration limits authorized by WHO, showed that the levels of heavy metals in the plants were within the permitted limits for medicinal plants, except for chromium and iron in ***Stylochaeton hypogaeus roots***, which may pose a health risk to the consumer. Iron was also present in stems and leaves. The health risk assessment indicated that the consumption of these plants may not pose a health risk to consumers, with non-carcinogenic target risk quotient (THQ) values ​​less than 1 for all metals in all samples. The non-carcinogenic hazard index (HI) is less than 1 for stems and leaves, but it value is 1.5 for roots. Therefore, vulnerable people with chronic diseases, who may consume ***Stylochaeton hypogaeus*** root preparations for long periods, should be warned of the risks to their health, as they are more susceptible to toxicities. Public health officials are advised to enforce regulations for the safety control of herbal products before they are placed on the market to protect consumer health.

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