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POTENT INDIGENOUS WEEDS FOR SOIL CHROMIUM REMEDIATION AND PUBLIC HEALTH RISK ASSESSMENT WITH POT-GROWN EDIBLE PLANTS

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Abstract

Chromium, naturally present in trace amounts in the environment, poses a significant risk as both a carcinogen and a contaminant at elevated concentrations. The study explores the chromium accumulation potential of seven indigenous weeds of Hazaribagh, Bangladesh. It further investigates the threat and toxicity associated with vegetables, crops, and aromatic plant cultivation in chromium-laden soil. The study revealed that the study area contains alarmingly high levels of Cr (III), i.e., 2328–34,536 mg Cr/kg as demonstrated in a spatial GIS map. The weeds accumulated 27.8 – 2496.6 mg Cr/kg and 11.4 –506.9 mg Cr/kg in roots and shoots respectively. Among the seven weeds, *Phyllanthus niruri, Cyperus* sp., and *Vernonia patula* met at least one phytoremediation criteria and can be used for soil chromium remediation purposes. In the pot study, five species, *Brassica nipus* (mustard), *Helianthus annuus* (sunflower), *Ocimum sanctum* (holy basil), *Capsicum annuum* (green chili), and *Abelmoschus esculentus* (okra), were exposed to chromium (III) sulfate spiked soil for forty days at 100, 500, and 20000 mg Cr/kg soil concentrations. Green chili, sunflower, mustard, and okra demonstrated high chromium uptake in roots (13.3- 195 mg/ kg) and shoots (13.2-63.7 mg/kg) exceeding permissible and toxicity threshold for plants. These findings highlight the importance of not cultivating these species in chromium-contaminated areas. However, basil showed chromium tolerance by limiting the uptake in the shoot along with excellent growth reducing the risk of chromium transferring through the food chain, and therefore, is safe to grow in Hazaribagh for producing metal-free aromatic oil.

Introduction

The soil and water of Hazaribagh, an abandoned tannery area amidst Dhaka metropolitan, are dangerously polluted with chromium, predominantly Cr (III), that those tanneries released for decades. The highest chromium concentration recorded in this area was 172,792 mg/kg near the main disposal point during the dry season (Mondol *et al.* 2017). Cr (VI) is more bioavailable to plants and thus poses a greater threat of contaminating the food chain (McLean 1992) while Cr (III), conversely, is sparingly soluble in the soil at pH > 4.5 with predicted low mobility in soils, plants, animals, and natural water (Elleouet *et al.* 1992). Surface Cr accumulation in Hazaribagh was reported mostly to be Cr (III) with very irregularly distributed Cr (VI) (highest 1 mg/kg) (Shams *et al.* 2009).

Baker and Brooks (1989) specified hyperaccumulators of Cr as plants containing over 1000 mg/kg of Cr in the dry matter after phytoremediation. Due to the lack of suitable plants with such capacity, a threshold value of 300 mg/kg of Cr in dried foliage has been proposed (van der Ent et *al.* 2013). Hyperaccumulators of Cr remain largely unconfirmed (van der Ent *et al.* 2013), although a few species have been identified with good Cr accumulating capacity (Table 2). Considering the invasive nature, excellent tolerance, and survival capacity, weed species can colonize rapidly under extreme environmental conditions and play a vital role in the successful remediation of soil contaminants. Naturally growing medicinal plants and aromatic plants are

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tolerant to heavy metals, and the final products extracted are metal-free as well (Lal *et al.* 2013, Jaison and Muthukumar 2017). There is a spontaneous growth of indigenous weeds in the Cr-contaminated soil of Hazaribagh. It is, therefore, imperative to evaluate the Cr (III) phytoremediation ability of these native weeds.

Heavy metals from soil can enter the human body via two pathways: soil-human (direct) and soil-crop-human (indirect). Since vegetative species grown in chromium-contaminated soils accumulate higher levels of Cr in their upper body parts, they can contaminate the food chain (Islam and Hoque 2014). While investigating the study area, few vegetable and fruit plants were spotted which may exert a direct toxic effect on inhabitants. Therefore, it is of interest to simulate a pot study to assess Cr uptake by food crops and vegetative plants from the soil. The present study was conducted to identify efficient chromium-accumulating weeds in the tannery waste-contaminated soil of Hazaribagh. Also, a pot study was conducted with chromium spiked soil to assess the risk associated with cultivating vegetative plants and food crops in terms of chromium retained in aerial parts.

Materials and methods

The field study was undertaken at the Hazaribagh tannery area in Dhaka, Bangladesh, and samples were collected from July 2020 to February 2021. Eight locations were selected in an area of 26,861 m^2 within latitudes 23°43'59"N - 23°44'6"N and longitudes 90°21'43"E - 90°21'50"E as represented with symbols S1-S8 in (Fig. 1). At each point, soil samples were collected within a 10×10cm quadrant from a maximum depth of 20 cm using a spatula. Eight representative plant samples, each growing extensively in those locations were collected, wrapped in poly bags, and transported to the laboratory. Department of Botany, University of Dhaka, Bangladesh helped with the identification of indigenous species. Samples were carefully cleaned, separated into roots and shoots, and preserved in the refrigerator until further processing.

Five species, i.e., sunflower, Indian mustard, basil, okra, and green chili, were selected for indirect estimation of toxicity in terms of chromium uptake with pot study. The pot experiment involved three $Cr_2(SO_4)_3$ doses, i.e., 100, 500, and 20000 mg Cr/ kg dry soil, administered to the soil in solution form. Garden soil was collected, air-dried, fertilized with N-P-K fertilizer, mixed with chromium salt solution, and equilibrated for at least ten days for better absorbance of Cr into the soil matrix. Dried seeds of selected species were germinated in wet paper towels inside a closed box for a week. Healthy seedlings were transferred into a growth bed. A week later stronger plants were selectively transplanted in spiked soil one plant per pot (1 kg soil/pot). Each species for each soil concentration was planted in triplicates. The plant with the best growth among each triplet was retained after 40 days of chromium exposure. The leaf count and height of the plants were measured each week.

Both weeds from Hazaribagh and pot plants were digested for chromium analysis. Oven-dried plant samples were digested according to Juel *et al.* (2016). Oven drying temperature, HNO_3 digestion temperature, and HNO_3 -$HClO_4$ digestion temperature were 80℃, 90℃ and 125℃ respectively. Finally, distilled water was added up to a final solution volume of 200 mL. The solution was filtered and diluted 10, 100, and 1000 times for analysis using atomic absorption spectrophotometry with AAS (Shimadzu, AA6800).

Samples collected from the sites in Fig. 1 were manually cleaned from debris, and non-soil materials, and analyzed for pH, moisture content, and Cr. Moisture content was measured according to Jamal (2017). Soil sample digestion was done according to Juel *et al.* (2016). Filtration and dilution were done as previously mentioned for analysis with AAS.

Plants having TI and BCF >1 can be used in phytoextraction, whereas, plants having a combination of BCF < 1 and TI> 1 or BCF > 1, and TI< 1 show phytostabilization potential (Srivastava *et al.* 2021). BCF and TI were calculated using Eqn. (1-2) (Diwan *et al.* 2010)

$$\text{BCF} = \frac{\text{Cr in the plant tissue } (\frac{\text{mg}}{\text{kg}}\text{ DW})}{\text{Cr in the soil } (\frac{\text{mg}}{\text{kg}}\text{ DW})} \quad (1)$$

$$\text{TI} = \frac{\text{Cr in plant shoots } (\frac{\text{mg}}{\text{kg}}\text{ DW})}{\text{Cr in corresponding plant roots } (\frac{\text{mg}}{\text{kg}}\text{ DW})} \quad (2)$$

The spatial distribution map is generated using the kriging tool in ArcGIS using the eight data points (Fig. 1). Ordinary kriging is used to generate the map as it is the most appropriate one among the several kriging methods (Suh *et al.* 2016). The correlation between the soil Cr concentration, moisture content, pH, plant root BCF, shoot BCF, and TI was examined using bivariate analysis. For pot studies, the interaction effect was tested by one-way ANOVA with soil concentrations as factors and plant dry weight as dependent variables.

Results and Discussion

Alkaline soil (pH 7.2-8.9) contamination by Cr (III) found in the study area ranged from 2,327-34,536 mg/kg; significantly exceeding the permissible limit of 100 mg/kg (WHO 1996). The moisture content of the soil ranged from 18-27%. The spatial distribution of Cr (III) in the study area is displayed in Fig. 1.

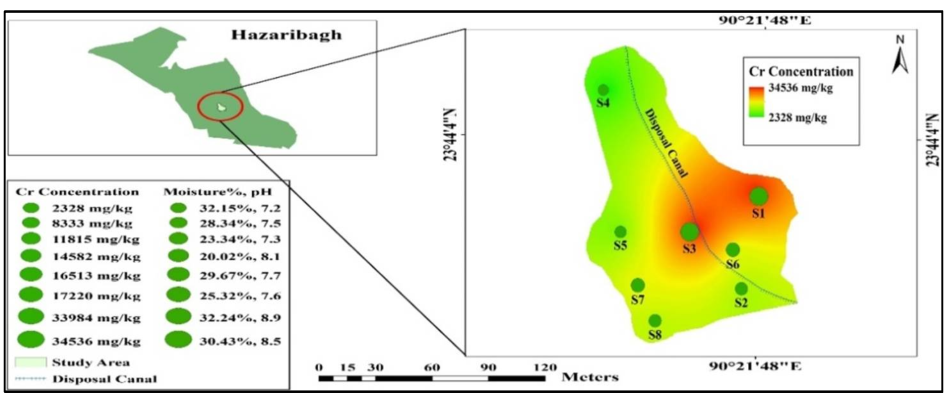

Fig. 1. Spatial distribution of trivalent chromium in the study area.

The correlation values obtained for soil parameters and weed species uptake parameters are presented in Table 2. Soil pH is strongly correlated with Soil Cr concentration (P < 0.01), previous studies in Hazaribagh Tannery have found similar findings (Mondol *et al.* 2017; Alam *et al.* 2020). Higher concentrations were found near the canal but decreased as the sampling locations moved further away from the canal (Fig. 1). An earlier study in the Hazaribagh tannery area reported concentrations of Cr ranging from 113-15,519 mg/kg DW and suggested insignificant lateral mobility of chromium (Alam *et al.* 2020).

Among eight collected species identified in Fig. 2, S1 (*Spilanthes acmella*) and S3 (*Cyperus* sp.) grew in abundance whereas S5 (*Ludwigia perennis*) and S6 (*Helitropium indicum*) had widespread occurrence. All collected species were weed type except S4 (*Carica papaya*) hence S4 will be excluded from further analysis.

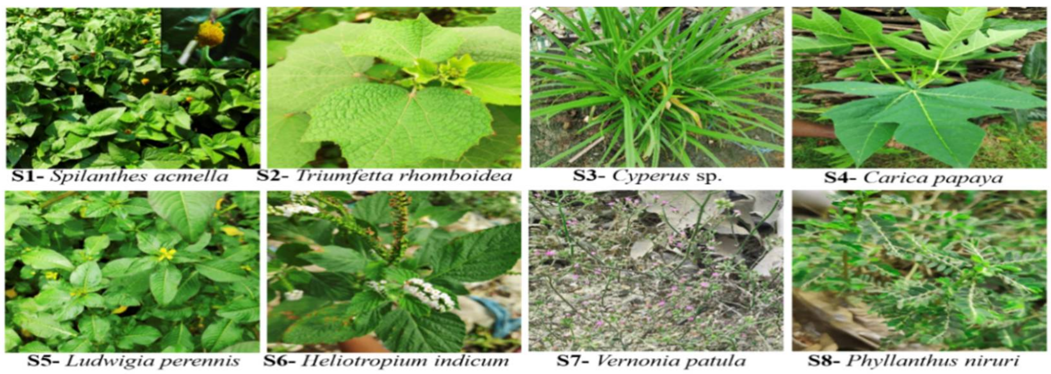

Fig. 2. Endemic plant species existing in the Hazaribagh tannery area.

Significant root Cr uptake in the collected weeds was found in descending order as follows: *Cyperus* sp.> *Phyllanthus niruri* (529 mg/kg)>*Vernonia patula* (348 mg/kg)>*Triumfetta rhomboidea*>*Spilanthes acmella*> *Ludwigia perennis*> *Heliotropium indicum* (Fig. 3A), *Cyperus* sp. accumulated highest Cr in the root (2496 mg/kg) while the shoot uptake is 191 mg/kg indicating Cr immobilization in the roots of this weed. Higher root accumulation of Cr in Cyperus esculentus was also observed in earlier studies (Chandra and Yadav 2011), rendering them less likely to transfer Cr through the food chain. The highest shoot Cr content was found in *V. patula* (506 mg/kg) followed by *P. niruri* (471 mg/kg) (Fig. 3A). Similar results were obtained from analysis of a chromite mine area where a higher Cr accumulation in shoots compared to roots of *Vernonia cinerea* was observed (Mohanty and Kumar Patra 2020). The weed species *Phyllanthus reticulatus* originating from the same genus as *P. niruri* has reportedly shown higher Cr accumulation at pH > 4.5 (Sampanpanish *et al.* 2006). Although *S. acmella* and *T. rhomboidea* did not meet the criteria, they hold great potential for Cr accumulation as they can produce significant biomass.

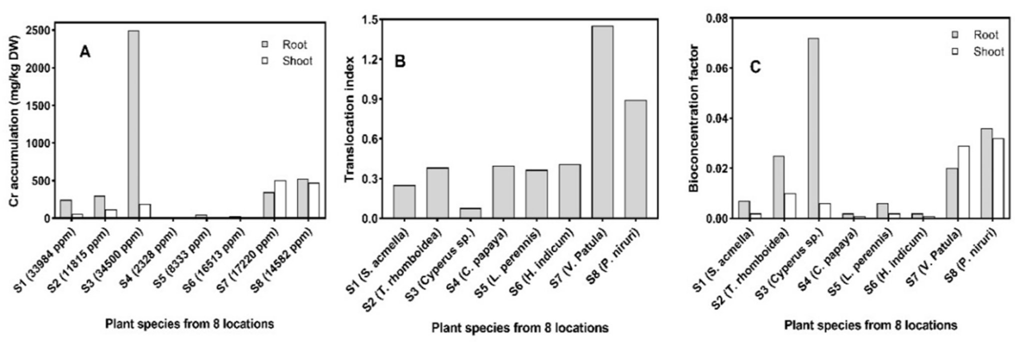

Fig. 3. Cr accumulation and phytoremediation indices of samples S1 to S8 (A: Plant Cr uptake with soil concentration in parentheses, B: Translocation index, C: Bioconcentration factor).

Differences in Cr uptake by weed species can be linked to their root system. Shams *et al.* (2009) reported that toxicity of Cr (III) is limited to surface and subsurface levels and weeds with a shallow developed root system, i.e., fibrous root and taproot with fibrous rootlets, can access those regions. However, a fibrous root system is not critical for phytoremediation (Banks *et al.* 2000). S1 (*S. acmella*) had a complex and developed adventitious nodal stem root suspectedly developed in response to heavy metal-induced stress and burial under layers of waste (Steffens and Rasmussen 2016), showed moderate uptake (root: 245 mg Cr/kg). S2 (*T. rhomboidea),* S7 (*V. Patula),* and S8 (*P. niruri*) had a taproot system with numerous fibrous rootlets, providing a high contact surface area for high Cr uptake. S6 (*H. indicum*) accumulated the lowest among all weeds (Fig. 3A) probably due to the taproot system without rootlets.

Table 1. Correlation matrix between Hazaribagh soil concentration, moisture content, pH, plant root BCF, shoot BCF, TI.

	Soil Cr Conc.	Moisture content	Soil pH	Root BCF	Shoot BCF	TI
Soil Cr Conc.	1.00	0.27	0.905**	0.53	-0.05	-0.26
Moisture Content	0.27	1.00	0.23	-0.25	-0.821*	-0.57
Soil pH	0.905**	0.23	1.00	0.37	-0.02	-0.28
Root BCF	0.53	-0.25	0.37	1.00	0.32	-0.11
Shoot BCF	-0.05	-0.821*	-0.02	0.32	1.00	0.842**
TI	-0.26	-0.57	-0.28	-0.11	0.842**	1.00

Level of Significance: **P < 0.01 (two-tailed), *P < 0.05 (two-tailed).

In table 1, shoot BCF is strongly correlated with TI (P < 0.01) which is expected. Also, there exists a negative correlation (P < 0.05) between soil moisture content and shoot BCF suggesting lower accumulation in the upper body part of plants grown in soils having higher water content. Singh *et al.* (2016) and Yuan *et al.* (2016) also found plants growing in soils with higher Cr concentrations accumulate higher levels of chromium in their roots. However, for very high heavy metal concentrations in soil, BCF values are expected to be very low (Sakakibara *et al.* 2011) and may not be very useful as a criterion for phytoremediation. Translocation Index (TI) was significant in *V. patula* (1.45) and *P. niruri* (0.89). TI greater than 1 indicates that the plant is favorable for phytoextraction whereas plants with high root accumulation are favorable for phytostabilization (Diwan *et al.* 2010). A list of naturally growing weed species as chromium hyperaccumulators and the results obtained from the present study are presented in Table 2.

The moisture content and pH of garden soil ranged between 15-23% and 6-7 respectively. The significant growth of sunflower (*Helianthus annuus*), basil (*Ocimum sanctum*), and mustard (*Brassica nipus*) over this forty days' exposure period can be ascribed to their shoot uptake being below the suggested toxicity threshold of 57 mg Cr /kg, as proposed by Sheppard *et al.* (1984). Indian mustard and okra survived with Cr levels in their shoots beyond the toxicity limit. Okra (*Abelmoschus esculentus*) and green chili (*Capsicum annuum*) accumulated Cr nearing 57 mg Cr/kg in shoots which may have contributed to their slow aerial growth.

The concentration of Cr in both roots and shoots of all five species was higher than the permissible limit for plants - 1.3 mg/kg (WHO 1996). Shoot uptake did not vary much for increasing Cr (III) concentration. Basil shoot accumulated the least Cr in their aerial part, indicating Cr tolerance to some extent. TI and BCF were almost always decreasing with increasing soil Cr concentration, which supports the diminished uptake mechanism based on the toxic exposure level (Terry 1981). The maximum value of TI was noted in Indian mustard for 100 mg/kg (TI - 2.20) and 500 mg/kg (TI - 2.37) soil concentration which drops to 0.45 for 20000 mg/kg soil. A higher translocation index increases the potential risk of contaminants traveling up the food chain. The BCF for all studied plants was <1 indicating lower Cr in root and shoot than that of soil (Fig. 4).

Table 2. Chromium accumulation potential of native plants and weed species.

Species	Family	Cr uptake ($mg\ kg^{-1}$)	Description	Study Location	Reference
Eichhornia crassipes	Pontederiaceae	1348[c]	Native plants of Hazaribagh tannery complex	Dhaka, Bangladesh	(Mondol *et al.* 2017)
Cynodon dactylon	Poaceae	684[c]			
Ipomoea aquatica	Convolvulaceae	564[c]			
Ricinus communis	Euphorbiaceae	1501[b]	Medicinal plants from tannery waste and sludge contaminated sites	Edayar, Kerala, India	(Jaison and Muthukumar 2017)
Amaranthus viridis	Amaranthaceae	1263[b]			
Phyllanthus reticulatus	Euphorbiaceae	1076[b]			
Cyperus rotundus	Cyperaceae	512[b]			
Vernonia cinerea	Asteraceae	>5000[a]	Weed diversity in a chromite mining area	South Kaliapani, Orissa, India	(Mohanty and Kumar Patra 2020)
Diectomis fastigiata	Poaceae	>2500[b]			
Kyllinga monocephala	Dryopteridaceae	>500[b]			
Linaria spartea L.	Scrophulariaceae	706[a]	Plants growing on serpentine soil of an old mining area	Pingarela, Portugal	(Freitas *et al.* 2004)
Cyperus sp.	Cyperaceae	2496[b]	Weeds naturally grown in tannery-waste contaminated area	Hazaribagh, Dhaka, Bangladesh	Present study
Vernonia patula	Asteraceae	506[a], 348[b]			
Phyllanthus niruri	Phyllanthaceae	471[a], 529[b]			

a, b, and c refer to accumulation in plant shoots or leaves, root, and whole biomass respectively

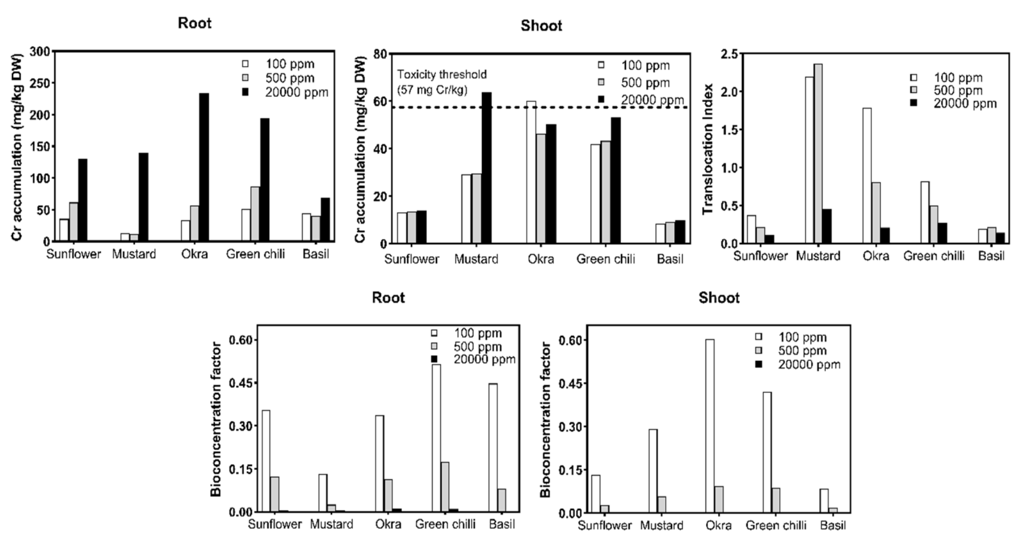

Fig. 4. Cr accumulation, BCF, and TI of species in the pot study.

ANOVA analysis shows that the soil Cr concentration does not affect average dry weights of sunflower and basil (Table 3). However, significant F values are found for mustard, okra, and green chili. Values presented in table 3 indicate that the dry biomass of okra and chili increased with increasing soil concentration, whereas the dry biomass of mustard slightly decreased with increasing soil concentration of 100 to 500 mg kg^{-1}. Plant uptake capacity decreases with decreasing soil concentration (Fig. 4), nonetheless, crop species will accumulate a significant amount of Cr in total as the dry biomass remains constant. As reported by Ahmed *et al.* (2022), the health Risk Index (HRI) values of chromium for six vegetables grown in Hazaribagh soil exceeded 1.

Table 3. ANOVA analysis for the effect of soil concentration on total plant dry weight.

Soil (mg/kg)	Sunflower		Mustard		Basil		Okra		Green Chili	
	F	Mean±SD	F	Mean±SD	F	Mean±SD	F	Mean±SD	F	Mean±SD
100		9.41±0.11		2.95±0.16		10.36±0.09		1.73±0.06		0.82±0.02
500	3.68	9.12±0.19	44.79*	2.96±0.11	6.85	10.11±0.10	51.405*	2.14±0.10	62.420*	0.79±0.12
20000		9.34±0.09		1.94±0.18		10.27±0.07		2.28±0.03		1.36±0.04

Level of Significance: *$P<0.001$

According to the present study, three weed species *Cyperus* sp., *V. patula,* and *P. niruri* can be considered chromium hyperaccumulators according to the new criteria (van der Ent *et al.* 2013). *Cyperus* sp. can be considered as a Cr hyperaccumulator that limits accumulation in the root (2496.6 mg/kg), displaying characteristics of phytostabilization. *V. patula,* and *P. niruri* with good translocation index, are more effective for phytoextraction of Cr. Apart from these, *S. acmella* and *T. rhomboidea* displayed good Cr accumulation potential. All species from pot study namely, sunflower, indian mustard, basil, okra, and green chili, accumulated Cr in roots and aerial parts more than the permissible limit of 1.3 mg/kg. Root uptake capacity increases with increasing soil Cr dosage, while shoot uptake is consistent, in general. Hence, vegetable and crop species are not suitable for cultivation in chromium-contaminated soils. Hazaribagh residents are at risk of developing health complications from eating vegetables or crops grown in that area.. But basil, with remarkable tolerance to Cr, can be cultivated to produce metal-free aromatic essential oil since shoot uptake is much lower.

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