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# Phytoavailability of chromium in *Triticum Aestivum* in natural and synthetically fertilized soil irrigated with hudiara drain wastewater, Lahore

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**Key words:** *Arachis hypogaea*, fly ash, growth promoter, aerial part.

## Abstract

The present study compares the phytoavailability of chromium (Cr) in the presence of natural (kitchen compost) and synthetic (commercially available urea) fertilizers in *Triticum aestivum*. To achieve this, the contaminated water was collected from selected sites of Hudiara drain following random sampling technique. The collected water samples were combined to give homogeneous mixture of representative sample. The germination of seeds was evaluated after irrigation with different concentrations of this representative sample of Hudiara drain. Results indicated that higher concentration of Hudiara water (< 70%) affected the germination of plants where severe inhibition was observed when seeds were allowed to grow in the soil with 80% of Hudiara drain water. Further it was observed that concentration of metals is significantly ($p<0.01$) higher in the plants (roots and shoots) grown in fertilized soil compared to the control. The concentration of Cr was significantly ($p<0.01$) higher in the plants grown in synthetically fertilized soil as compared to the one in natural fertilized soil. The translocation factor showed that the movement of Cr from roots to shoot was positively correlated and was in the order of root > stem > leaves. The study concluded that the wheat plants (*Triticum aestivum*) used for food purpose if get irrigated with contaminated water can accumulate metals to toxic levels. This accumulation can potentially get intensified through application of synthetic fertilizers.

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**Introduction**

The build-up and concentration of toxic chemicals, which ultimately reduces the capability of the affected air, water and land to support life is referred to as environmental pollution (Gleick, 2001). The main causes of such pollution are natural as well as anthropogenic activities, which consequently have many adverse effects on plants and animals- the biodiversity that can alternatively affect the humans (Bañuelos *et al.*, 1997).

Heavy metals and metalloids accumulation into the soil are usually emitted from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition, are the main cause of soil contamination (Khan *et al.,* 2008). For the remediation of such highly contaminated soils, there can be many options, such as their removal and their replacement by uncontaminated soils (Hajdu and Licsko, 1999; Lanphear *et al.*, 2003; Nielsen and Kristiansen, 2005; Douay *et al.*, 2007). Some ex-situ techniques are also based on soil washing (Reddy and Chinthamreddy, 2000; Kos and Lestan, 2003; Tandy *et al.*, 2004; Makino *et al.*, 2007; Lestan *et al.*, 2008; Zou *et al.*, 2009) or on solidification (Alpaslan and Yukselen, 2002; Liao *et al.*, 2003; Yin, 2002; Pinto, 2008). The use of such technologies comes with an environmental cost as these technologies degrade the physico-chemical and biological parameters of remediated soils and make them unsuitable for plant production (Kos and Lestan, 2003).

A more promising solution to the soil remediation involves the in-situ immobilization of metals, which has received a growing amount of interest (Guo *et al.*, 2006; Ruttens *et al.*, 2006a). In this technique, the risk of groundwater contamination is alleviated, along with plant uptake and exposure of other living organisms, by the inactivation of metals through metal immobilizing amendments (Boisson *et al.*, 1999a). Many amendments have been used, such as addition of lime (Geeblen *et al.*, 2003), phosphate (Melamed *et al.*, 2003) and organic matter (Farfel *et al.*, 2005), for immobilization of heavy metals. This concept for the use of metal accumulating plants for removing heavy metals and other compounds was first introduced in 1983, but the idea has actually been implemented for the past 300 years (Henry, 2000) after which the concept of phytoremediation was introduced (Salt *et al.*, 1998). Phytoremediation includes mitigation of large pollutant concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them, however, this may lead to phyto-accumulation (Raskin *et al.*, 1994). This accumulation can get concerning if staple crop starts absorbing metals to toxic levels.

The present study is therefore focused on absorption of heavy metal Cr in wheat (used as staple crop) when irrigated with contaminated water. One such a drain, surrounded by industrial belt one side and wheat crops on the opposite side is Hudiara drain. Hudiara drain is a natural storm water channel, which originates from Batala in Gurdaspur District, India and after flowing nearly 55 km on Indian side at village Laloo enters Pakistan at Hudiara village on Pakistan side. It joins the river Ravi, after flowing for nearly 63 km inside Pakistan. The river Ravi has been facing serious pollution problems, due to the fact that there are around 100 industries located adjacent to the Hudiara drain on the 55 kilometers Indian side, so it is already quite polluted when it enters Pakistan (Kirkham, 1983; Ghafoor *et al.* 1999). On the Pakistan side, there are 112 small industries located next to the drain as it travels 63 kilometers through the Punjab into the Ravi. The water of the drain is used for irrigation of fields along its length. Such untreated water seeps into the soil and facilitates the entry of a number of pathogens and heavy metals into the food chain, when used for irrigation. As a result, crops grown with polluted water may cause diseases when consumed by the people raw or cooked (Ibrahim *et al.*, 1998).

The main objective of this research work is to observe the bioavailability of chromium in wheat when fertilized with natural and synthetic fertilizers, study and compare the concentrations of chromium in soil and plants with WHO Permissible Limits and finally determine the potential rate of chromium entering in human body through consumption of this contaminated food.

## Materials and methods

Sampling of wastewater and soil

The water samples were collected from the twelve selected locations on Hudiara drain (focusing few km from the leather tanneries and pharmaceutical industries) through random sampling. The samples were mixed together to give one homogenous representative sample for further analysis.

An agricultural soil was selected as a control, which was located away from any urban area, main road or industrial site. The control soil was sampled using a hand auger at different points, following random sampling. The samples were mixed and then sieved through a 10 mm stainless steel sieve to give one representative sample for further analysis.

Both the soil and water were analyzed for parameters, such as pH, total dissolved solids (TDS), electrical conductivity (EC), salinity, soil particle size distribution, moisture content (MC), carbonate content and cation exchange capacity (CEC) (Table 1).

*Pre-experimental analysis for Cr concentration*

Both the representative samples for soil and water were analysed for their Cr onetration before starting the experiment. For this purpose the soil was crushed and passed through a 0.250-mm sieve for total dissolution. As described by the NF X 31-147 standard (1996), the soil was burnt at 450˚C and a mixture of hydrofluoric (HF) and per chloric acids ($HClO_4$) were used for the total chromium dissolution. Chromium concentration was determined by Atomic Absorption Spectrophotometer (AAS).

The water samples were characterized for available Cr following the standard digestion procedure, in which 20 ml of water was taken in a beaker and stirred vigorously with a magnetic stirrer for 2 minutes. The solution was then filtered and 10 ml of deionized water was added in the filtrate. Further 5 ml of HCl was added and the solution was heated on a hot plate until dryness. Afterwards 15 ml of deionized water and a few drops of HCl were added and the solution was filtered again to run on Atomic Absorption Spectrophotometer (AAS).

*Seed materials*

Seeds of wheat *(Triticum aestivum* L.*)* were obtained commercially through lab reference. After stratification for two days, they were allowed to germinate. The seedlings at two cotyledon stage with uniform size and shape were selected for experimental purpose, and allowed to germinate, by watering the seeds with different concentrations of distilled water and Hudiara drain water (20%, 40%, 60%, 80% and 100%).

*Soil amendment*

The amendment used in this study were synthetic fertilizer (Urea [$CO(NH_2)_2$], Merck Germany) and natural compost (formed from organic kitchen waste-consisting of fruit and vegetable peels, papers, paper towels and tea etc). The soil was divided into ten sections, five of which were amended with natural compost, whereas the other five were amended with synthetic fertilizer, urea. The amendment and soil (1.6 kg) were thoroughly mixed with a hand auger for 10 min.

The five sections of soils amended with natural fertilizer were watered with different concentrations of Hudiara drain water (20%, 40%, 60%, 80% and 100%). Similarly, the other five sections of soils amended with synthetic fertilizer were watered with different quantities of Hudiara drain water (20%, 40%, 60%, 80% and 100%).

*Assessment of Chromium mobility*

Selective extractions (calcium chloride, acetic acid, citric acid) were performed using non-amended and amended soils. These extractions were conducted before and after 4 weeks of incubation period to evaluate the effects of the nitrogenous amendment on chromium mobility.

The evaluation of the effects of natural and synthetic amendments on bioavailability of chromium was carried out, using 0.01 M calcium chloride ($CaCl_2$), 1 M acetic acid, and 0.11 M citric acid following the single extraction procedure. Three grams of air dried soil sample was taken and the suspensions with a 1/10 (w/v) soil/extractant ratio were shaken for 2 h, using a rotor disc (10 rpm), for $CaCl_2$ and 16 h when acids were used. Through centrifugation (4530 rpm) for 20 min, the extract was separated from the solid residue, at room temperature. The solution was then filtered over a Millipore membrane (Millipore, 0.45 μm porosity). The filtrate was poured into a container, with 92 μl of nitric acid solution (65%) for acidification, and stored at 4˚C for analysis.

To determine the fractionation of chromium in amended soils, a three-step sequential extraction procedure was used (Rauret *et al.*, 1999). These steps were noted as fractions A, B or D. The fourth step (fraction R) was added for total chromium dissolution in the residual fraction.

*Phytoavailability of Chromium*

Wheat was sown into ten sections containing soil, five amended with natural compost, and five amended with synthetic fertilizer. The plants were grown with temperature ranging between 15 and 25˚C. The plants were regularly watered with different concentrations of Hudiara drain water (20%, 40%, 60%, 80%, and 100%).

After 4 weeks, when the plants matured, they were removed, and weighed at the beginning and end of the uptake experiments. The plants were then washed with deionized water to remove the soil particles. Each plant was then dissected into roots (cutting at the root neck), stem and leaves with a sharp razor. The roots of dissected plants were soaked in 0.01 M $H_2SO_4$ for 30 sec after excision and then washed in deionized water.

The plants were dried at 70˚C for 3 hours, milled, and one half gram samples were digested in 4 ml boiling concentrated $HNO_3$ on a hot block (Huang and Schulte, 1985). After dilutions with double distilled water, the digests were analyzed for chromium uptake by plants through Atomic Absorption Spectrophotometer (AAS).

*Soil and wheat moisture contents*

The residual moisture contents were measured by weighing three replicates of individual soils (amended naturally and synthetically) or wheat grass before and after drying at 105˚C in an oven until it reached a constant mass according to the NF ISO 11465 standard. A precise dry mass was thus obtained and was applied to the reported analytical values.

*Analytical technique*

The chromium concentration in the wheat plants was determined using a flame atomic absorption spectrophotometer (AAS) (Thermo Scientific- M-GF95Z), with an air-acetylene flame. For chromium, hollow cathode lamp modulated with low and a high current mode was used to avoid spectral interferences (Oppermann *et al.*, 2003; Bidar *et al.*, 2006, Douay *et al.*, 2007). For the lowest chromium concentrations, the AAS was fitted with a graphite furnace atomizer (Zeeman Furnace) and an autosampler in combination with an auto- diluter.

*Statistical analysis*

Chromium concentrations in soils and in wheat, shoots and plant biomass, were expressed as mean and standard deviation and were compared to percentages of Hudiara water given to plants, by applying statistical Correlation. The uptake values were obtained and compared to WHO Standards.

## Results and discussion

*Effect of amendments and uptake pattern of plants*

The uptake pattern of Cr in all the three parts of wheat plants- roots, shoots and leaves; (both amended with natural as well as synthetic fertilizers) was observed to be in ascending order, this means that as the concentration of Hudiara drain water increased (from 20% to 100%) the uptake of chromium increased as well. The percentage uptake of chromium by plants amended with natural fertilizer has been calculated to be 31% of total chromium. Whereas, the percentage uptake of chromium by plants amended with synthetic fertilizer is calculated to be 55%. The percentage uptake of chromium in control plants has been calculated to be 1.6% of the total chromium in water and soil. The overall uptake of chromium by plants amended with natural fertilizer, synthetic fertilizer and control plants has been compared with WHO Permissible Limit (1.0 mg/kg). Both the plants amended with natural as well as synthetic fertilizer have the exceeded amount of chromium when compared with WHO standard of 1.0 mg/kg (Fig. 1 and 2), whereas the amount of chromium in control plants is quite low, due to the absence of any amendments (Fig. 3).

**Table 1.** Soil properties of ten samples collected from reference agricultural site.

| Soils | pH | TDS (mg/l) | EC (μS/ cm) | Salin-ity | Sand (%) | Silt (%) | Clay (%) | MC (%) | $CaCO_3$ (%) | CEC ($cmol^+$/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| $S_1$ | 8.8 | 449 | 476 | 0 | 5 | 30 | 65 | 18.1 | 32.7 | 56.5 |
| $S_2$ | 8.7 | 452 | 480 | 0 | 5 | 30 | 65 | 18.1 | 32.5 | 56.6 |
| $S_3$ | 8.8 | 446 | 465 | 0 | 5 | 30 | 65 | 18.4 | 32.8 | 56.5 |
| $S_4$ | 8.6 | 448 | 467 | 0 | 5 | 30 | 65 | 18.2 | 32.7 | 56.3 |
| $S_5$ | 8.9 | 451 | 474 | 0 | 5 | 30 | 65 | 18.1 | 32.4 | 56.8 |
| $S_6$ | 8.7 | 456 | 478 | 0 | 5 | 30 | 65 | 18.3 | 32.7 | 56.6 |
| $S_7$ | 8.8 | 447 | 468 | 0 | 5 | 30 | 65 | 18.5 | 32.6 | 56.9 |
| $S_8$ | 8.9 | 443 | 477 | 0 | 5 | 30 | 65 | 18.1 | 32.5 | 56.6 |
| $S_9$ | 8.5 | 448 | 483 | 0 | 5 | 30 | 65 | 18.0 | 32.6 | 56.5 |
| $S_{10}$ | 8.6 | 449 | 476 | 0 | 5 | 30 | 65 | 18.1 | 32.4 | 56.4 |

**Table 2.** Concentration of Chromium in wheat dry mass of naturally amended soil.

| Soil Section | Roots | Stem | Leaves |
|---|---|---|---|
| | Mean $\pm$ Std. Dev. | Mean $\pm$ Std. Dev. | Mean $\pm$ Std. Dev. |
| 20% Hudiara Water | 0.64 $\pm$ 0.1528 | 0.42 $\pm$ 0.1528 | 0.28 $\pm$ 0.1 |
| 40% Hudiara Water | 0.98 $\pm$ 0.1528 | 0.87 $\pm$ 0.0577 | 0.76 $\pm$ 0.0577 |
| 60% Hudiara Water | 1.29 $\pm$ 0.1155 | 1.1 $\pm$ 0.1 | 0.93 $\pm$ 0.0577 |
| 80% Hudiara Water | 1.43 $\pm$ 0.1 | 1.19 $\pm$ 0.2 | 1.0 $\pm$ 0.1 |
| 100% Hudiara Water | 1.6 $\pm$ 0.1026 | 1.38 $\pm$ 0.2082 | 1.27 $\pm$ 0.1 |

The addition of compost and fertilizer to the plants enhanced the uptake of heavy metals in the plants. But when compared statistically, the plants amended with synthetic amendment were found to have more concentration of chromium in them (Fig. 4) as compared to those amended with kitchen compost (Fig. 5). The synthetic fertilizers had been observed to be more efficient in enhancing the bioavailability to about 24%, as compared to natural fertilizers. This study shows that plants that had been amended with synthetic amendment have a large amount of chromium in them, as compared to those amended with natural amendment.

**Table 3.** Concentration of Chromium in wheat dry mass of synthetically amended soil.

| Soil Section | Roots | Stem | Leaves |
|---|---|---|---|
| | Mean $\pm$ Std. Dev. | Mean $\pm$ Std. Dev. | Mean $\pm$ Std. Dev. |
| 20% Hudiara Water | 1.29 $\pm$ 0.1527 | 1.18 $\pm$ 0.1 | 1.1 $\pm$ 0.1 |
| 40% Hudiara Water | 1.47 $\pm$ 0.1 | 1.33 $\pm$ 0.5774 | 1.24 $\pm$ 0.1154 |
| 60% Hudiara Water | 1.68 $\pm$ 0.2577 | 1.59 $\pm$ 0.1527 | 1.48 $\pm$ 0.1154 |
| 80% Hudiara Water | 2.1 $\pm$ 0.2 | 1.84 $\pm$ 0.0577 | 1.71 $\pm$ 0.1527 |
| 100% Hudiara Water | 2.32 $\pm$ 0.1 | 2.15 $\pm$ 0.1 | 2.0 $\pm$ 0.1 |

*Estimated $LC_{50}$ value of Hudiara drain water for plants*

The $LC_{50}$ value of chromium for wheat as calculated was found to be about 70% of Hudiara water (Fig. 6). This means that beyond this percentage, the plants did not develop properly but are severely affected due to chromium accumulation in tissues. As the concentration of Hudiara drain water increased, the plants undergo structural damage (Fig. 7). The plant on the extreme left had been watered with least amount of Hudiara drain water (about 200 ml) and greater amount of distilled water (about 800 ml). This was probably the reason why it had grown normally without cholorosis. Contrarily, the plant on the extreme left wilted and turned yellow because the plant was given 1000 ml Hudiara drain water, but was not given distilled water.

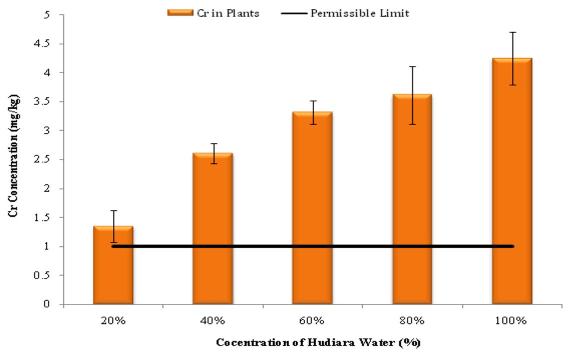


**Fig. 1.** Comparison of uptake of Chromium by soil and plants with WHO permissible limit in naturally amended soils.

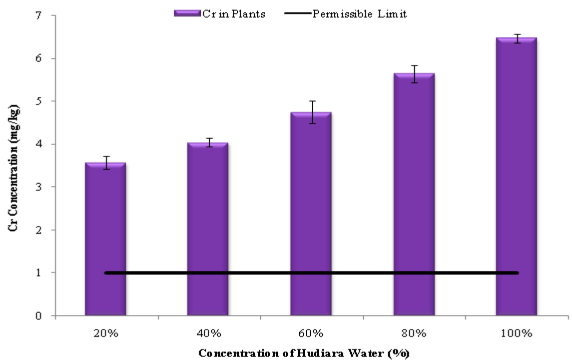


**Fig. 2.** Comparison of uptake of Chromium by soil and plants with WHO permissible limit in synthetically amended soils

*Role of organic matter*

A significant role was observed to be played by organic matter in two aspects in determining the availability and mobility of heavy metals in soils. Firstly, the bioavailability of heavy metals in soils was observed to reduce by organic matter, by adsorption or formation of stable complexes with humic substances (Liu *et al.*, 2009). Secondly, organic matter was also found to be the main supplier of organic chemicals to the soil solution by changing the pH, where acidic pH may increase metal availability to plants (Vega *et al.*, 2004; McCauley *et al.*, 2009). Evidence suggested that natural organic matter in the environment can form complexes with soluble chromium (Shrestha *et al.*, 2007; Luo *et al.*, 2010) and thus, chromium can become bio-available (Howe *et al.*, 2003).

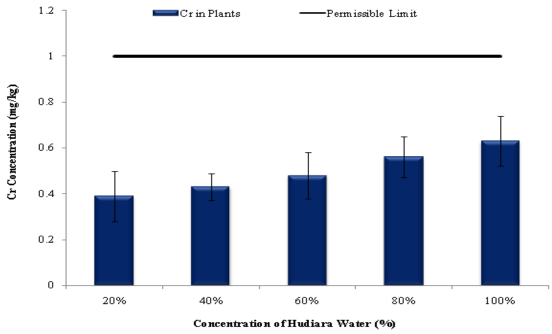


**Fig. 3.** Comparison of uptake of Chromium by soil and control plants with WHO permissible limit (unamended).

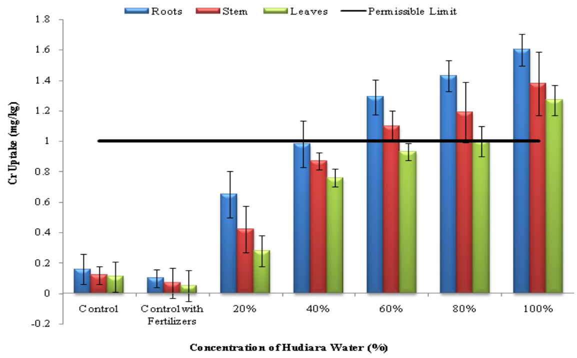


**Fig. 4.** Concentrations of Chromium in wheat dry mass of naturally amended soil.

Since the natural amendment- compost was entirely organic in nature, so it was quite clear that the organic matter in compost must had adsorbed to the soil particles by forming stable complexes. Also, the texture of the soil was very clayey, so the organic matter must have bound itself tightly to clay particles present in the soil.

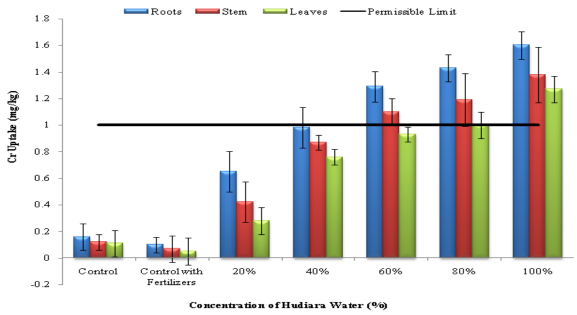


**Fig. 5.** Concentrations of Chromium in wheat dry mass of synthetically amended soil.

Also, by increasing CEC (cation exchange capacity) in soils, the organic matter may enhance nutrient availability to plants, providing metal chelates and increasing the solubility of nutrients in soil solution (McCauley *et al.*, 2009). As a result, the amount of chromium in soil solution would decrease, due to its complexation to the humified fraction of the organic matter, leading to limited plant availability and uptake, also influencing the processes responsible for translocation of chromium to grains. This was explained by the lower percentage of chromium uptake in wheat plants amended with natural fertilizer.

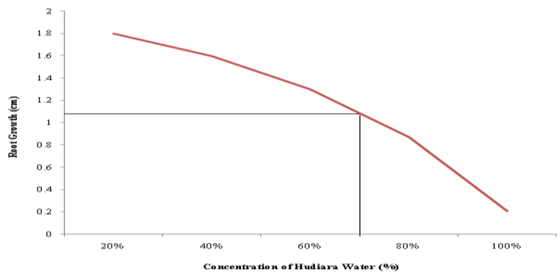


**Fig. 6.** Estimated $LC_{50}$ value of Chromium for wheat plants.


**Fig. 7.** Wheat plants after being removed from the soil (A=20%; B=40%; C=60%; D=80%; E=100% Hudiara drain water, F=20%; G=40%; H=60%, I=80%; J=100% Hudiara drain water).

*Highest concentration of Chromium in plant parts*

This study shows that among the roots, stems and leaves, the roots have been found to have most of the concentration of chromium. The maximum uptake of chromium in roots has been calculated to be 1.6 mg/kg for plants amended with compost (Table 2), whereas, for plants amended with fertilizer, the uptake is relatively higher, up to 2.32 mg/kg (Table 3).

This shows that the chromium in Hudiara drain water is present in excess amount, greater than the WHO Standard of chromium for wastewater effluents. There is a strict correlation of chromium between its concentration in soil and plant samples, thus implying an increase of its translocation when soil is amended with synthetic fertilizers.

## Conclusion

From this study, it can be concluded that the synthetic fertilizers are more effective in the uptake and bioavailability of heavy metals in plants, as compared to plants that have been amended with kitchen waste compost. The efficiency of synthetic fertilizers in enhancing the uptake and bioavailability is evident from the results and statistical analysis, which shows that the results calculated are highly significant of the obtained data. Also, the roots have been found to concentrate most amount of chromium in them, as compared to other plant parts- shoots and leaves.

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