



Phytoremediation potential of *Vachellia nilotica* under cadmium contaminated soil by using organic amendments



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Abstract

Cadmium (Cd) is a non essential element for plants and humans but is present in many soils in excessive amounts. When it enters into the food chain, it poses a major threat to the living biota. The control of Cd accumulation in plants is complicated by the fact that most of the essential nutrient transporters, such as copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn), also facilitate Cd uptake. In this study, the main objective was to examine the effect of *Vachellia nilotica* in cd contaminated soil by using organic amendments such as cow dung and compost. The pot experiment was carried out in a nursery of the Department of Forestry and Range Management, University of Agriculture, Faisalabad under natural conditions. Plants were grown in pots having soil with 20 mg kg⁻¹ of Cd stress with CD @ 3% and CM. Plants were also grown in pots with uncontaminated soils as a control treatment (T₁). Cd is highly toxic to the plant and with 20 mg kg⁻¹ of applied Cd, the mortality of the plant occurs. The Cd concentration increased in *Vachellia nilotica* with the use of compost (T₃). The lowest concentration of Cd was found in cow dung (T₂) treatment. The result indicates that the plant height and fresh and dry weight significantly decreased as the level of Cd increased. The result also revealed that the use of cow dung in combination with compost was found superior in enhancing the phytoremediation potential of Cd by *Vachellia nilotica*.

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1. Introduction

Heavy metals naturally occur at lower concentrations in soils. However, heavy metals are considered soil contaminants due to their widespread occurrence, and acute and chronic toxicity. Common heavy metal contaminants include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc, which present numerous health dangers to higher organisms and are also known to decrease plant growth, and ground cover and harm soil microflora (Prasad *et al.*, 2003, Qiao *et al.*, 2013). Heavy metals can be classified into two categories regarding their need in a biological environment. They may be essential or non-essential metals, those heavy metals which are indispensable for the proper bio-functioning of life of organisms in very small quantities are called essential heavy metals. They include Ni, Fe, Zn, Mn, and Cu. Those heavy metals whose deficiency has not affected on proper bio-functioning of life of organisms include Cr, Cd, Hg, Pb, and As (Ohashi *et al.*, 2006). The stable performance of the living system is disturbed when the concentrations of heavy metals increase from a specific acceptable range (Pandey *et al.*, 2019). Cadmium (Cd) is a non-essential element for plants and humans but is present in many soils in excessive amounts (Nikolopoulou *et al.*, 2009). When it enters into the food chain, it poses a major threat to the living biota. The control of Cd accumulation in plants is complicated by the fact that most of the essential nutrient transporters, such as copper (Cu), manganese (Mn), iron (Fe), and zincs (Zn), also facilitate Cd uptake (Waseem *et al.*, 2014). Chemical remediation is a mechanical process used for leaching the contaminated soil by using liquids enriched with solvents, freshwater, and chelating agents (Warren *et al.*, 2003, Upadhyay *et al.*, 2019). Researchers found that ethylene diamine tetra acetic acid (EDTA) is an effective chelating agent for soil washing. Recent studies have shown that biosurfactants, such as sophorolipids, saponins, and rhamnolipids, can efficiently remove Cd from contaminated soils (Rong *et al.*, 2020). Phytoremediation is an economically feasible method that uses plants, which have exceptional metal accumulating capabilities, to restore contaminated soils (Thiau *et al.*, 2018). The method is less disruptive to the environment and more acceptable to surrounding communities (Warren *et al.*, 2003).

Farmyard manure (FYM) is being used as the major source of organic manure in field crops. Limited availability of this manure is, however, an important constraint on its use as a source of nutrients. FYM positively controls crop production and recovers properties of soil and it can be used to decrease heavy metal stress in plants. FYM positively influences crop production and improves soil's physical, chemical and biological properties (Yang *et al.*, 2009).

V. nilotica is a valuable member of the plant family Fabaceae, under the kingdom Plantae (Qurat-ul-Ain, 2017; Rahman *et al.*, 2015). Common names of *V. nilotica* are known as Kikar, Babul, Arabic tree and Indian gum. It is a multipurpose tree and is distributed worldwide and also in Pakistan, it is widely spread. *V. nilotica* has the potential to tolerate saline toxicity. Keeping in view the above facts, there is a need to develop a suitable technique for soil remediation by enhancing the phytoremediation of Cd from contaminated soil.

1. To examine the effect of *V. nilotica* with the use of cow dung in Cd contaminated soil.
2. To examine the effect of *V. nilotica* with the use of compost in Cd contaminated soil.
3. To find out the best organic amendment to reclaim the Cd contaminated soil by using *V. nilotica*.

2. Materials and Methods

A pot experiment was carried out to find out the phytoremediation potential of *V. nilotica* for cadmium-contaminated soil. Pot experiments were conducted in the nursery area of the Department of Forestry and Range Management, University of Agriculture, Faisalabad (UAF) (31° 25'57" N, 73° 04' 21" E). The average annual temperature ranges between 22°C and 9°C respectively. Uniform-sized seedlings of these species were collected from the nursery of the Department of Forestry and Range Management, UAF. Due consideration was given to the fact that seedlings should be healthy and free of disease. For the pot experiment, a 4 mm sieve was used and a 2 mm sieve was used for the analysis of physicochemical properties. Cow dung and compost were collected from the farm area of the UAF. The soil was treated with cadmium metal as cadmium chloride (CdCl₂) in 20 mg kg⁻¹ concentration. Each pot contained 3-5 kg of soil. The treatment table is shown in Table 1.

2.1 Heavy metal analysis

Heavy metals were analyzed by atomic absorption spectrophotometer (AAS) through the wet digestion method. The oven-dried samples were ground, and sieved at 1mm and about 0.5 g of the sample were digested in 10 ml of strong di-acid solution (70 % high purity HNO₃ and 65 % HClO₄, 4:1 v v-1) (Allen *et al.*, 1986). Samples were digested at about 80°C till the solution became transparent. The solution was cooled down before filtering through the Whatman no.42 filter paper. Then the metal Cd concentrations in the roots, shoots and branches of *V. nilotica* digested samples were examined using an AAS (Hitachi Polarized Zeeman AAS, Z-8200, Japan) in the Hi-Tech Lab of the University of Agriculture, Faisalabad, Pakistan. During these analyses sterilized glass apparatus and highly purified deionized water were used (Amon, 1990). Commercially available aqueous stock solutions were used to prepare calibrated standards for this analysis.

Table. 1. Treatments table

Treatments	Amendments	Dosage
T1	Control	(0) Cd stress
T2	CD + Cd	3% + 20 mg/kg
T3	CM + Cd	3% + 20 mg/kg
T4	Organic matter + Cd	3% + 20 mg/kg
T5	Cd stress	20 mg/ kg

2.3 Statistical analysis

Statistical analysis was done to determine the significance between the means and draw a valid conclusion. The “Analysis of Variance” method was adopted as the statistical tool for the raw data observed during the whole experiment. The difference in the treatment means was verified using the critical difference (CD) at a 1% level of probability following the Complete Randomized Design (CRD) to find a valid and authentic difference among the treatments.

3. Results

3.1 Growth parameters

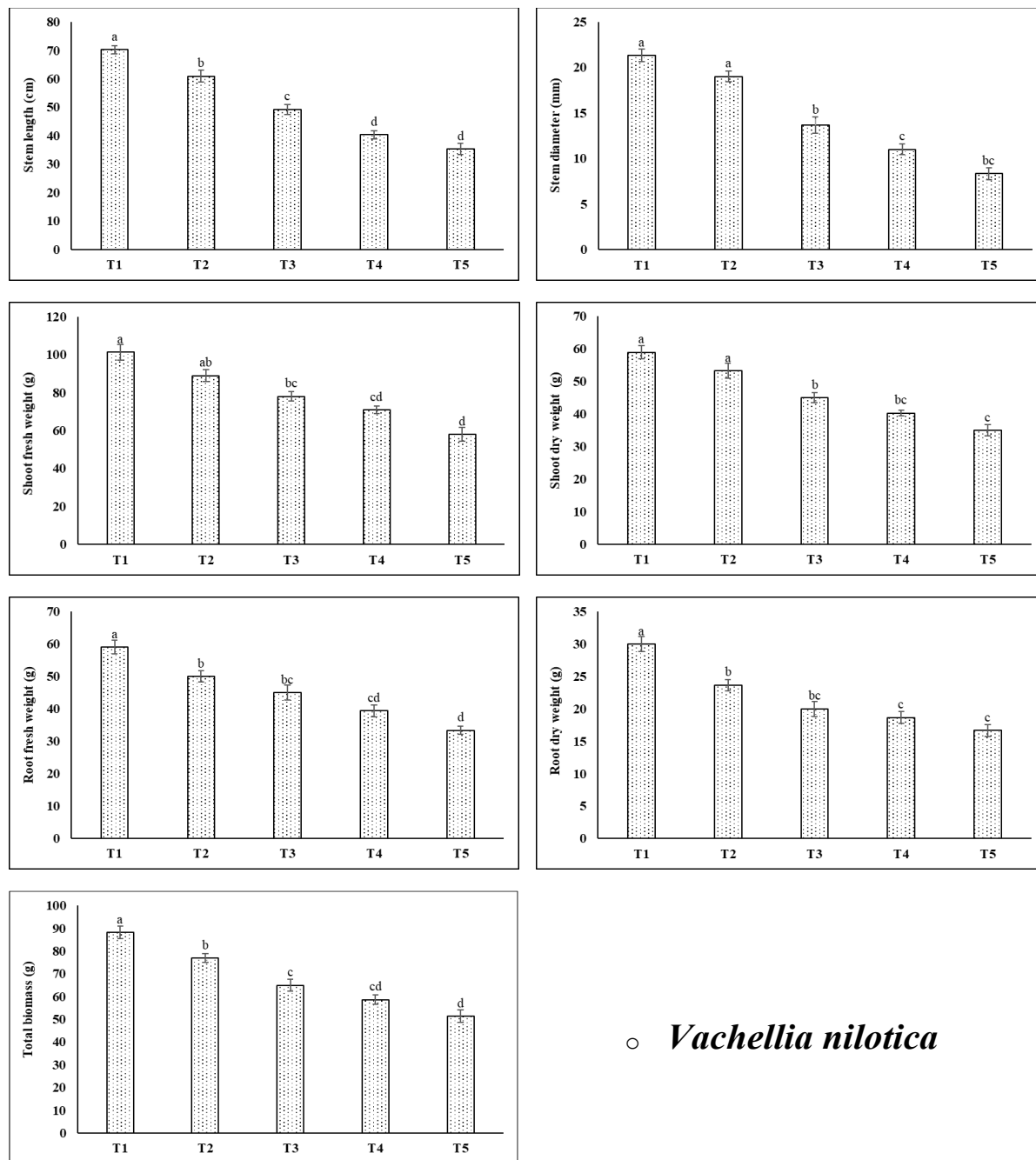
Results showed that T₂ significantly increased stem length, stem diameter, fresh and dry weight of shoot and root and total biomass as compared to T₃ treatment in *V. nilotica* (Fig.1). All of the growth parameters showed better results in under two organic amendments especially cow dung (CD) T₂ treatment as compared to compost treatment (T₃). In cow dung (CD) treatment, the maximum height of the plant was recorded in the plants of *V. nilotica* (70.33 cm), whereas the minimum stem height was recorded (35.33 cm) in compost treatment (T₃). In this study, the general trend for treatments was T₁>T₂>T₃>T₄>T₅.

3.2 Biochemical parameters

The results showed that the biochemical parameters such as SOD, POD, CAT, MDA and Phenolics were significantly different in different treatments as compared to the control. Results of SOD, POD, CAT, MDA and phenolics in *V. nilotica* have been shown in Fig. 2.

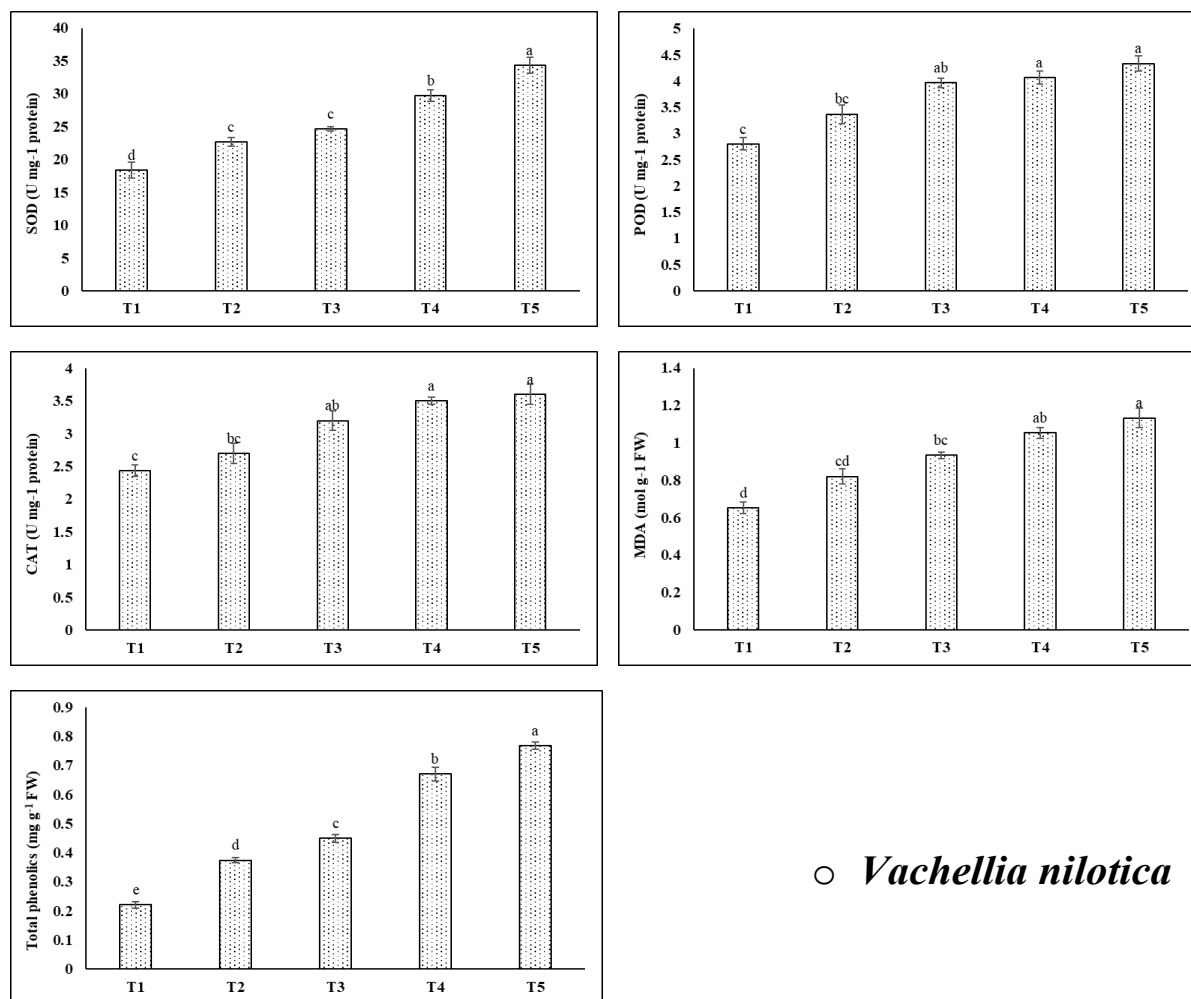
3.3 Photosynthetic Parameters

The results showed that the photosynthetic parameters such as carotenoids, chlorophyll a, chlorophyll b and total chlorophyll were significantly different in different treatments as compared to control treatment (T5). Results of carotenoids, chlorophyll a, chlorophyll b and total chlorophyll in *V. nilotica* have been shown in Fig. 3.



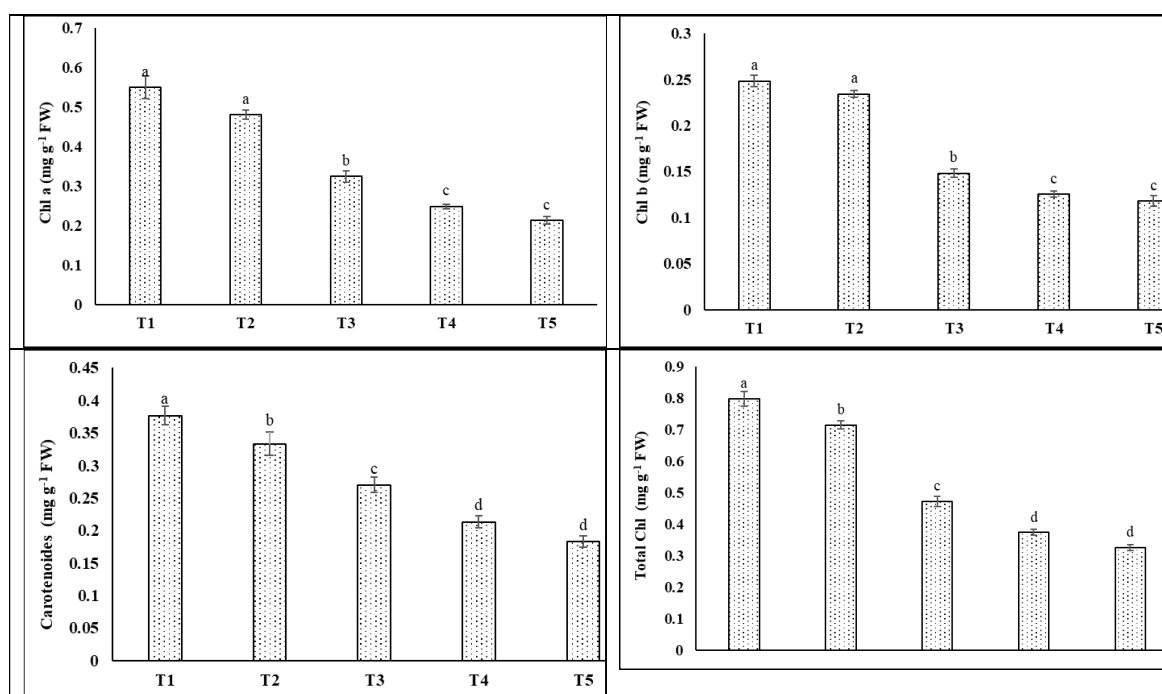
○ *Vachellia nilotica*

Fig.1. Growth response of *Vachellia nilotica* under organic amendments treatment



○ *Vachellia nilotica*

Fig.2. Biochemical response of *Vachellia nilotica* under organic amendments treatment.



○ *Vachellia nilotica*

Fig.3. Photosynthetic pigments of *Vachellia nilotica* under organic amendments treatment.

Discussion

This study was originated to check the effects of two soil organic amendments on cadmium (Cd) bioavailability, Cd uptake and growth of *V. nilotica* under cadmium (Cd) contaminated soil. The cow dung (CD) and compost (CM) were selected for this study. Treatments used in this study are T₁, T₂, T₃, T₄ and T₅. Cow dung (CD) was performed a very good and effective results in increasing plant growth against another amendment (Compost) in this study (Figure 1). Growth and Cd concentration of plants with organic amendments based on species specific, cow dung (CD) was showed effective and good results to enhancing the growth of *V. nilotica* when compared with compost amendments. On the other hand, compost (CM) was showed a best results and great potential against Cd stress in all the treatments in *V. nilotica* as compared to control (Cd).

Toxic concentrations of Cd triggered various disorders in plants at morpho-physiological and biochemical levels leading to retarded growth, necrosis, reduced photosynthetic activities (Abbas *et al.*, 2018; Suriyagoda *et al.*, 2018; Jiang *et al.*, 2022), plant water status (Bhat *et al.*, 2021) and reactive oxygen species metabolism (Siddiqui *et al.*, 2020). Gunes *et al.* (2009) reported that Cd increased its concentrations in plant organs, reduced seedling growth, and induced oxidative stress through increasing ROS and MDA concentrations in plant cells. Singh *et al.* (2021) reported impaired root morphology and reduced growth and development in Arabidopsis plants. Similarly, working with Cicer arietinum, Kapoor *et al.* (2021) demonstrated that higher uptake of Cd resulted in retarded plant growth and development, and affected water potential and physio-biochemical events. Recently, Mishra *et al.* (2021) recorded that the toxic levels of Cd hindered plant height, root hair density, seedling growth, and photosynthetic pigments. Working with the wheat crop, Saeed *et al.* (2021) reported that Cd toxicity wholly affected seedling germination, root growth, and biomass production, seed productivity, and thus persuaded the oxidative stress. According to Khan *et al.* (2020), higher uptake of heavy metal ions by plant roots alters the synthesis of photosynthetic pigments. A similar was reported by Rahman *et al.* (2015) where authors stated that high Cd concentrations resulted in a substantial reduction in chlorophyll pigments and photo assimilate production. In the same context, Chaves *et al.* (2009) reported reduced seedling growth, photosynthesis, and gasses exchange parameters. Singh *et al.* (2019) studied the effects of two concentrations of arsenic viz. 100 and 200 μ M and reported that both levels resulted in a prominent reduction in photosynthetic and transpiration rates and stomatal conductance with a genotypic-dependent response.

Cadmium stress causes oxidative stress through the excessive accumulation of ROS in plant cells which causes injury to the cells and disrupts metabolic events (Ahammed and Yang, 2022; Nahar *et al.*, 2022). Under cadmium stress, alterations in antioxidative

enzymes have been well-reported in different crop species (Rai *et al.*, 2011; Suhel *et al.*, 2022; Dolui *et al.*, 2022). A similar work was reported in which tree plants under cadmium-contaminated sites recorded a significant reduction in growth, and physiological and biochemical traits. Higher uptake of cadmium by plant roots causes disorders in primary (such as fundamental chemical processes that control plant growth) and secondary (i.e., non-essential chemical compounds) metabolisms. For example, working with *Ceratophyllum demersum* (L.), Mishra *et al.* (2008) recorded a significant increase in amino acids activity under cadmium stress conditions. Srivastava *et al.* (2011) reported a significant increase in amino acids in *Hydrilla verticillata* seedlings when exposed to arsenic stress. A significant decrease in protein content in cadmium-treated seedlings was also demonstrated by various authors (Singh *et al.* 2006; Gupta and Ahmad, 2014).

Conclusions

Cadmium (Cd) contamination in soil is a global threat to food safety and human health. Intake of Cd contaminated water through irrigation of soil is one of the major pathways of Cd exposure in humans. Organic amendments can play a major role in the reclamation and amelioration of Cd-contaminated soil which absorbs the pollutants by binding and reduce the mobility of trace elements. This phenomenon is referred to as stabilization. It is observed that the use of organic amendments can enhance plant growth and reduce Cd phytoavailable in plant parts. In conclusion, the addition of two different organic amendments showed good results as compared to other treatments but cow dung reduced Cd translocation and phytoavailability and give the best growth in agroforestry species under Cd contaminated soil. On the other side, *V. nilotica* showed the best result among all the amendments and a significant reduction in Cd uptake with cow dung application as compared to another amendment, which can be grown in cadmium-contaminated soils. In the future, stakeholders can use organic amendments as an additive to improve the phytoremediation of Cd contaminated soil by agroforestry tree species. Moreover, they should consider phytoremediation to remediate Cd contaminated sites with the help of cheap on-site operations and byproducts of mines and industries.

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