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Full Length Article

Comparative Efficacy of Growth Media in Causing Cadmium Toxicity to Wheat at Seed Germination Stage

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Abstract

Cadmium (Cd) is toxic and negatively influences the crop growth. Growth medium may have central role in the expression of toxicity of heavy metals and these toxic effects may be less in soil as compared to other mediums. An experiment was conducted to determine the Cd toxicity on wheat in soil and filter paper media. Three levels of Cd (0, 5, 20 mg L^{-1}) were evaluated in these mediums in thermophore plates. Results revealed the toxic effect of different levels of Cd on wheat growth indices. Wheat cultivated on soil had better germination and growth in comparison to filter paper. Maximum reduction in plumule and radicle length 32 and 37%, respectively was observed at 20 mg Cd L^{-1} on filter paper whereas 16 and 12%, respectively in soil medium as compared to control. Germination percentage and index was relatively less affected than plumule and radicle growth on both media. Cd concentration in wheat radicle and plumule increased with increasing its concentration in growth media. Filter paper medium accumulated higher concentration (10-49 mg Cd kg^{-1}) in wheat tissues compared to soil medium (3-29 mg Cd kg^{-1}). Bioaccumulation factor ratio (BAF-plumule/BAF-radicle) was less than 1 implied root as major sink of Cd. Pearson correlation demonstrated the negative regulation of Cd on various indices of wheat. It could be concluded that soil provides relief to wheat against Cd stress as it has natural ability to sorb Cd on the exchange sites. Further, plumule and radicle length was good indicator to determine Cd toxicity.

Keywords: Metal stress; Embryonic tissues; Germination index; Correlation; Wheat

Introduction

Industrial uprising in the world brought intensive pollution problems thus their toxicity has led to threat for local community. Pakistan is an agriculture country where farmers use industrial effluents (grey water) for irrigation purpose. It has high capacity of nutrients but it also frequently contains toxic substances like heavy metals. Heavy metals released from industries caused toxic effects not only in terrestrial biota; it also disturbed marine life (Javed, 2012). Among those heavy metals Cd is easily taken up by the crops. Therefore, the ingestion of these crops as food induced ample amounts of Cd in humans (Wagner, 1993). There are intensive reports on Cd toxicity in vegetables and field crops grown on Cd contaminated soils (Wang *et al*., 2005; Li *et al*., 2006; Yang *et al*., 2009). Owing to its higher concentrations in soil, crop growth was inhibited on long or short term Cd exposure. Thus the people native to urban areas are prone to higher Cd toxicity (Hough *et al*., 2004; Datta and Young, 2005).

Cd toxicity in soil was determined by An *et al*. (2004) by using four sensitive plants wheat, maize, sorghum and cucumber. They concluded that root and shoot growth are good indicators of Cd toxicity. Similarly, AI-Rumaih *et al*. (2001) reported severe decreased root and shoot growth by the addition of Cd. Although metal accumulation is serious problem in vegetables, we cannot neglect their effects on other food crops like wheat, which is a staple food of the people in Pakistan. Therefore, it is look essential to investigate the Cd toxicity on wheat grown on different media. There are scanty reports on comparison of two growth media whereas several articles have been published regarding Cd effects on crops cultivated on contaminated soils (Alexander *et al*., 2006; Wang *et al*., 2006; An *et al*., 2004; Yang *et al*., 2009; Ahmad *et al*., 2012; Zulfiqar *et al*.,) and filter paper or solution medium (Aycicek *et al*., 2008; Ci *et al*., 2010). This research was unique in a sense that neither a single report on the determination of Cd toxicity using two growth media has been published yet. Here it was tried to explain the sensitivity of wheat seedlings on exposure to Cd being grown on different growth media.

The current study is aimed to investigate Cd toxicity using bioindicators (radicle and plumule growth) and to determine any role of growth medium (soil and filter paper medium) in inducing or alleviating Cd toxicity in wheat.

Material and Methods

Growth room trial was conducted to evaluate comparative efficiency of growth media on Cd toxicity and to find out its

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effects on radicle and plumule growth of wheat under axenic conditions at the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

Seed Disinfection

Seed of wheat cultivar (Inqlab-91) was kindly provided by the Cereal Section of Ayub Agriculture Research Institue, Faisalabad, Pakistan. The healthy and robust seeds of cultivar were suface sterilised with sodium hypochlorite (5%) by dipping wheat seeds in it for ten minutes followed by five washings with de-ionised water.

Cd Treatment

Cadmium chloride ($CdCl_2.H_2O$) salt of high purity (98%) was purchased from Merck chemicals, Germany and used to prepare desired Cd concentrations. Two levels of Cd 5 and 20 mg L^{-1} were used in the experiment along with control (without Cd).

Experimental Conditions

Two separate experiments were conducted to achieve above objectives. About 200 g sand of 2 mm size particle was filled in thermophore plates having dimensions 4:4:2 cm length, width and depth, respectively. Two filter paper sheets (Whatman#40) were wrapped in each thermophore plate in order to retain moisture for longer period of time. Sand was moistened with 50 mL after every two days whereas filter paper with 3 mL daily by each Cd level. Control plants treated with distilled water with 50 and 3 mL in sand and filter paper media, respectively. Surface disinfected ten seeds were placed in each thermophore plate at uniform depth in soil or distance in filter paper medium. Each experiment was arranged in CRD with three replicates and run under axenic conditions with 14/10 h of light and dark cycles. After 7 days trial was harvested and data regarding seed germination, radicle and plumule length was recorded.

Determination of Plant Parameters

Final germination of seeds was obtained by counting number of seeds germinated on 7th day of planting. Radicle and plumule length was measured with the help of Delta T-scan. Germination and vigor indices were estimated using following formula reported by (Hu and Cai, 2001; Ruan *et al*., 2002).

Germination index (GI) $=\Sigma$ (Gt/Tt)

Where Gt: Number of the germinated seeds in the t day.

Tt: Time corresponding to Gt in days

Vigor index (VI) = GI × seedling height

Tolerance indices (T.I) were determined with the formula given by Iqbal and Rahmati (1992).

$$Tolerance\ indices = \frac{\text{Mean radicle length in Cd solution}}{\text{Mean radicle length in distilled water}} \times 100$$

Growth inhibition (I) was estimated according to the formula given below:

$$Growth\ Inhibition = \frac{\text{Mean RL or PL in control} - \text{Mean RL or PL length in Cd solution}}{\text{Mean RL or PL in control}} \times 100$$

Where RL = Radicle length; PL = Plumule length

Cd Concentration in Radicle and Plumule

Cd concentration was determined from wheat tissue after drying all the samples in an oven at 70ºC for 24 h. Each plant sample was ground, weighed and digested with nitric (HNO_3) and perchloric acid ($HClO_4$) in 3:1, then heated on hot plate at 350ºC till dense white fumes appeared. Cool the flasks, filtered and stored in plastic bottles for further determination on atomic absorption spectrophotometer (PerkinElmer, 100 Analyst, Waltham, USA) (Yang *et al*., 2009). Bioaccumulation factor (BAF) was determined according to the formula:

$$\text{BAF} = (\text{Cd conc. in wheat tissue})/(\text{Total Cd conc. in soil or solution})$$

Statistical Analysis

Data was analyzed by calculating the standard error of means, ANOVA was performed and all the means were compared with LSD value to determine the significance at $p<0.05$ (Steel *et al*., 1997). Pearson correlation was calculated by using STATISTIX software v8.1.

Results

Final Germination

Germination of wheat seeds was relatively better in soil than filter paper medium (Table 1). Seed germination gradually decreased on successive application of Cd. It was also observed that the wheat seed germination was inhibited more stringently in filter paper compared to soil medium. Maximum inhibition was found at 20 mg L^{-1} which was significant ($p<0.05$) compared to control. The results further revealed that the seed germination of wheat varied in these different growth media on successive exposure to Cd contamination (Fig. 3). Seeds germinated on soil medium after second day of Cd exposure while it took four days to germinate on filter paper. Significant ($p<0.05$) differences in seed germination were observed on both growth media under Cd stress. Cd applied at 5 mg L^{-1} stimulate seed germination on both media although soil medium showed significant ($p<0.05$) difference.

Table 1: Effect of growth media on the performance of wheat under Cd stress (column shows similar letter don't differ significantly at $p<0.05$). Data presented as means ± SD. (average of three replicates)

Growth Medium	Cd levels (mg L^{-1})	Final germination (%)	Germination index	Vigor index	Radicle length (cm)	Plumule length (cm)	Root/shoot ratio
Filter paper	0	73.33±5.77[ab]	3.60±0.71[a]	37.05±6.76[a]	5.40±0.53[a]	4.67±0.42[a]	1.16±0.10[a]
	5	76.67±5.77[a]	4.21±0.42[a]	36.15±5.55[a]	4.76±0.25[a]	4.00±0.20[a]	1.19±0.01[a]
	20	63.33±5.77 [b]	4.43±0.58[a]	28.87±2.69[a]	3.40±0.53[b]	3.16±0.35[b]	1.08±0.17[a]
Soil	0	76.67±5.77[a]	7.74±0.11[a]	101.47±4.03[a]	6.33±0.58[a]	6.76±0.25[a]	0.99±0.12[a]
	5	73.33±5.77 [a]	7.34±0.30[a]	88.50±1.76[b]	5.86±0.23[a]	6.20±0.20[b]	0.95±0.02[a]
	20	73.33±5.77 [a]	6.85±0.24 [b]	77.14±5.12[c]	5.60±0.53[a]	5.67±0.35[b]	0.94±0.04[a]

Means sharing same letter differ non-significantly ($P>0.05$)

Table 2: Correlation between Cd and various plant parameters; Asterisks show significant differences at 5% probability

	Filter paper medium	Soil medium
Cd vs final germination	-0.55ns	-0.27ns
Cd vs germination index	0.57ns	-0.88*
Cd vs vigor index	-0.53ns	-0.95*
Cd vs plumule length	-0.91*	-0.89*
Cd vs radicle Length	-0.89*	-0.61ns
Cd vs seedling growth	-0.92*	-0.84*

*Significant at $P<0.05$ and ns, non-significant

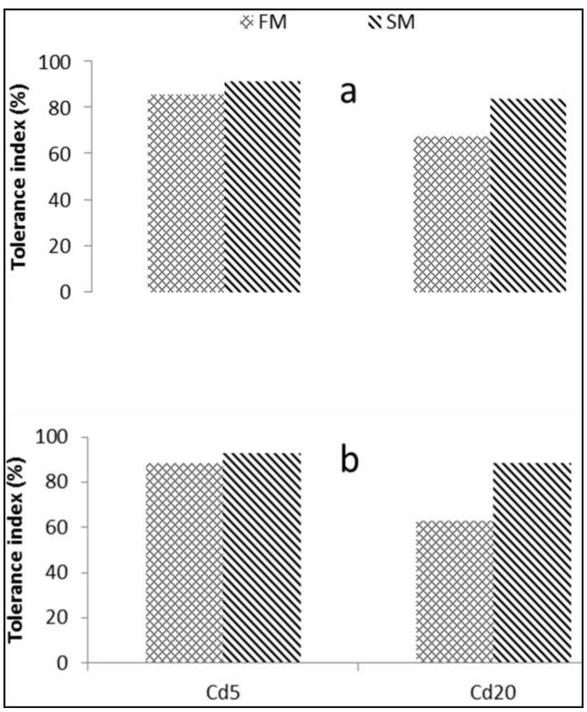

Fig. 1: Tolerance indices of wheat seedlings (a:Plumule length and b:Radicle length) grown on different growth media. FM: Filter paper medium, SM: Soil medium

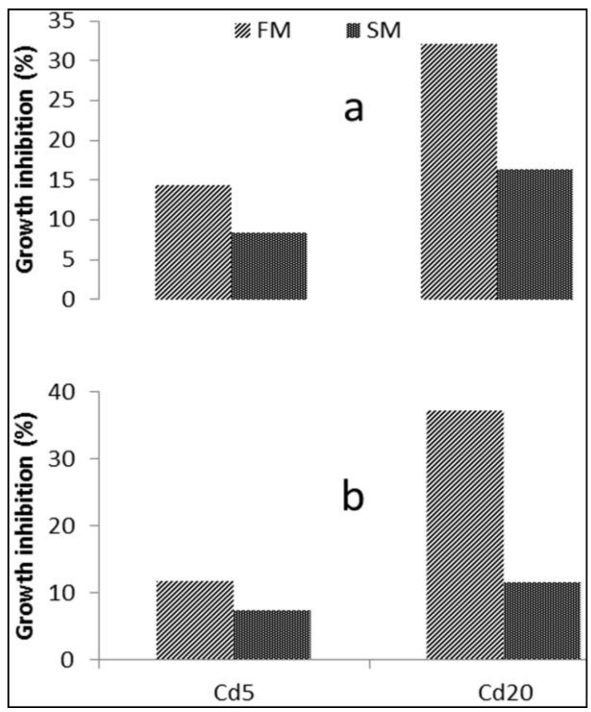

Fig. 2: Tolerance indices of wheat seedlings (a: Plumule length and b: Radicle length) grown on different growth media. FM: Filter paper medium, SM: Soil medium

Germination and Vigor Indices

Germination and vigor indices were statistically non-significant ($p>0.05$) in filter paper test, whereas significant ($p<0.05$) in soil medium (Table 1). Germination index increased while vigor index was decreased by each increment of Cd from 0 to 20 mg L^{-1} in filter paper medium. On the other hand both germination and vigor indices were decreased in soil medium. Maximum inhibition in germination and vigor index, 11 and 24%, respectively was observed at 20 mg Cd L^{-1} in case of soil medium. Vigor index was decreased 28% in filter paper and 24% in soil medium at 20 mg Cd L^{-1} compared to treatment which received no Cd.

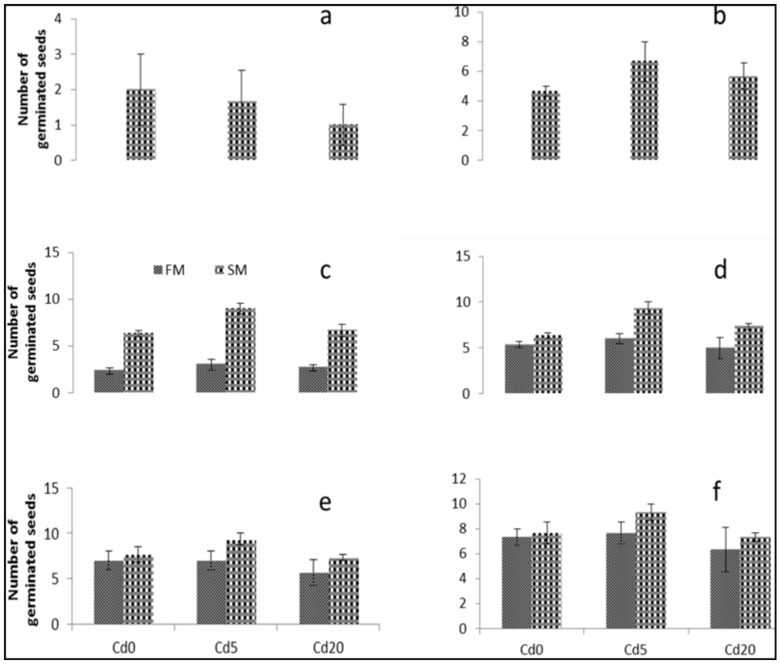

Fig. 3: Comparison of growth media (FM: Filter paper medium, SM: Soil medium) on the ability of seed germination under Cd stress. Lowercase letters indicate number of seed germinated in a respective day; a: 2nd; b: 3rd; c: 4th; d: 5th; e: 6th; f: 7th

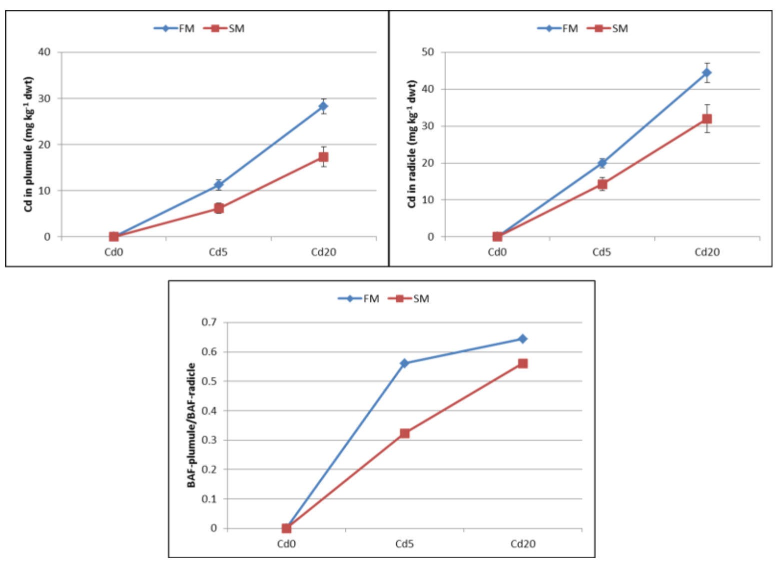

Fig. 4: Cd concentration in plumule and radicle of wheat on different growth media. Bioaccumulation factor (BAF) shows ratio between (BAF-plumule/BAF-radicle) on different growth media. (FM: Filter paper medium and SM: Soil medium). Data presented as means ± SE

Seedling Growth

Radicle and plumule length was rigorously reduced in filter paper medium under Cd stress (Table 1). They were inhibited by 37 and 32%, respectively at 20 mg Cd L^{-1} compared to treatment received no Cd. In contrast, Cd reduced radicle length in soil medium but the difference was statistically non-significant ($p>0.05$) in comparison to treatment received no Cd. Plumule length also decreased in soil medium, which received Cd and it was significantly ($p<0.05$) less compared to un-amended soil. Maximum inhibition 12 and 16% was observed in radicle and plumule length, respectively at 20 mg Cd L^{-1} compared to the treatment, which received no Cd. Root shoot ratio was found statistically non-significant ($p>0.05$), while it showed decreasing trend as the Cd level increased in soil. Moreover, it was less affected in filter paper, while greatly reduced in soil medium.

Cd Concentration in Plumule and Radicle

Fig. 4 shows the effect of growth media on Cd accumulation in plumule and radicle of wheat. Radicle was act as the major sink for Cd whereas plumule accumulated Cd but in very less quantity. Cd accumulated in radicle was almost 2 times greater than Cd accumulated in plumule. Growth media showed prominent difference in the accumulation of Cd in radicle and plumule; wheat grown on filter paper medium accumulated significantly ($p<0.05$) much higher Cd compared to soil medium. Cd concentration in wheat tissue (radicle and plumule) depends upon the Cd concentration applied in growth media. Moreover, it was observed that Cd concentration in wheat tissue increased with increasing concentration in the growing media. Bioaccumulation factor (BAF) in wheat tissue was determined to investigate extent of Cd accumulation in wheat tissue. Bioaccumulation factor was depended on bioavailable fraction of Cd to the growing plant (Fig. 4). Filter paper medium showed higher BAF-plumule to BAF-radicle ratio implied higher amount of Cd available to accumulate in wheat tissue in filter paper medium compared to soil medium. Highest BAF ratio (BAF-plumule/BAF-radicle) 0.64 was observed in filter paper medium.

Tolerance Indices

Tolerance indices on the basis of plumule and radicle growth clearly elaborated the response of growth media in mitigating Cd stress (Fig. 1). Soil medium comparatively engrossed stress more competently than filter paper medium. Increased Cd concentration in any medium severely inhibited plant growth thus reduced tolerance. Almost soil endures equal stress at 20 mg L^{-1} as compared to 5 mg L^{-1} in filter paper medium. Tolerance indices were 68 and 63% on the basis of plumule and radicle growth respectively, at 20 mg Cd L^{-1} in filter paper medium while at the same Cd concentration 84 and 88% tolerance was observed in soil medium.

Growth Inhibition

Radicle length was tremendously affected by the Cd compared to plumule length in filter paper medium whereas small inhibition of growth was recorded in soil medium (Fig. 2). Increased Cd concentration was severely inhibited plumule and radicle growth at 20 mg Cd L^{-1} in filter paper medium. Overall results indicated that radicle length was inhibited at a greater rate than plumule length.

Pearson Correlation

Strength of relationship between Cd and various indices of wheat was observed on both growth media and noted that each medium performed differently under Cd stress (Table 2). Relationship changed from positive to negative as it was observed in case of germination index which was positive in filter paper while strongly inhibited on soil medium. Except germination and vigor index all other parameters were strongly affected in filter paper compared to soil medium. Seedling growth, plumule and radicle length was reduced on filter paper whereas germination and vigor index, seedling growth and plumule length was significantly reduced on soil medium.

Discussion

In the current study Cd toxicity has been assessed by means of seed germination, radicle and plumule length of wheat seedlings as previously reported (Baker and Walker, 1989; An, 2004). Soil medium provided some relief to plants grown under Cd stress therefore awe some increase in growth parameters has been observed as compared to filter paper medium. The results of this study demonstrated very clear effects of both growth media. Germinating ability of wheat seeds significantly decreased at higher Cd concentration in filter paper medium which might be due to direct contact of Cd to seed. Although low Cd concentration increased the germinating ability while it strongly inhibited at higher concentration which implied that wheat seed tolerate Cd up to a certain limit than repressed on further addition of Cd. On the other hand soil assuages the effect of Cd which led to decrease in final germination non-significantly. Similar findings have been reported by An (2004) stating that Cd concentration does not show any inhibition on wheat seed germination except at 640 mg Cd kg^{-1} soil. Germination index increased whereas vigor index was decreased non-significantly upon exogenous application of Cd on filter paper medium. But in contrast to it both indices significantly decreased in soil medium which meant Cd induced germination at the cost of vigor loss. It also indicated that to assess Cd toxicity in soil; these two indices especially vigor index tells the real story. These results are in line with our previous findings which showed an increase in germination index at 5 while significant decrease at 20 mg Cd L^{-1} (Ahmad *et al.*, 2012).

Radicle and plumule growth showed great reduction on exposure to 20 mg Cd L^{-1} in filter paper medium while same concentration inhibited plumule growth in soil medium. Addition of Cd to the growing medium hampered shoot and root length, dry weight and area of maize leaves (Aycicek *et al.*, 2008; Shafiq *et al.*, 2008; Jun-yu *et al.*, 2008; Perveen *et al.*, 2011). Plumule growth was strongly inhibited on both media, which indicated its higher vulnerability to Cd stress while radicle growth was reduced but non-significantly to the treatment which received no Cd. Similarly, Aycicek *et al.*

(2008) reported variable effects of Cd on root whereas shoot length was very sensitive to Cd stress (AI-Rumaih *et al*., 2001; Ci *et al*., 2010; Ahmad *et al.*, 2012). Tolerance index was decreased as the concentration of Cd increased in growth medium. Similar results have been reported by Shafiq *et al*. (2008). Moreover, tolerance indices on the basis of plumule and root length elaborated the ability of soil to slack Cd stress on wheat thus tolerance indices were increased in soil medium. Similarly, growth inhibition results indicated significance of soil over filter paper medium.

Cd accumulated in radicle at greater rate than plumule, it might be due to direct contact of root tissues to applied Cd that bind and immobilized Cd and restricted its transport to shoot. Cd immobilization by roots reported in other plants garlic (Jiang *et al.*, 2001); cucumber, sorghum (An *et al.*, 2004); vegetables (Yang *et al.*, 2009). Wheat has many thin roots this property might be helpful for higher Cd accumulation in wheat radicle. It was reported that plants have numerous thin roots able to accumulate higher amount of metals (Das *et al.*, 1997). Moreover, An *et al.* (2004) also reported higher bioaccumulation factor (BAF-shoot/BAF-root) in cucumber and concluded root as major sink of the metals. Cd is highly mobile element in soil therefore its accumulation and translocation in food chain is very high. In current study, Cd accumulated in wheat tissue in higher amount when it grown on filter paper medium compared to soil medium. It was possible reason that bioavailable fraction of Cd was much higher to growing plant in filter paper than soil medium. Being a negatively charged body soil has ability to reduce toxic effects of Cd thus it is considered as less sensitive to Cd stress than filter paper medium.

In conclusion wheat seedlings were very sensitive and their growth reduced on exposure to Cd. Seed vigor index and plumule length was extremely sensitive and therefore it can be applied to assess Cd toxicity under both growth media. Cd accumulated in wheat tissue was the function of available concentration of Cd in growth media. Further, Soil medium was less sensitive in inducing Cd toxicity to wheat plant compared to filter paper medium.

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