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Major controlling factors and predictions for cadmium transfer from the soil into spinach plants

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ABSTRACT

Predicting the mobility, bioavailability and transfer of cadmium (Cd) in the soil–plant system is of great importance with regards to food safety and environmental management. In this study, the transfer characteristics of Cd (exogenous salts) from a wide range of Chinese soils to spinach (*Spinacia oleracea* L.) were investigated. The major controlling factors and prediction equations for Cd transfer in the soil–plant system were also investigated. The results showed that plant Cd concentration was positively correlated with soil Cd concentration. The maximum transfer factor (ratio of the Cd concentration in the plant to that in the soil) was found in acid soils. The extended Freundlich-type function was able to describe the Cd transfer from soil to spinach plants. Combining soil total Cd, pH and organic carbon (OC) content in the prediction equation greatly improved the correlation performance compared with predictions based on total Cd only. A slight protection effect of OC on Cd uptake was observed at low soil Cd concentrations. The results are a useful tool that can be used to predict Cd transfer from soil to plant.

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1. Introduction

Trace element accumulation in agricultural soils is of considerable concern due to the food safety implications and potential health risks, as well as the detrimental effects on soil ecosystems (McLaughlin and Singh, 1999). Of all the potentially toxic elements in soils, either endogenous or exogenous, cadmium (Cd) is one of the most dangerous of the trace elements because of its high solubility and toxicity (Barcelo and Poschenrieder, 1992; Das et al., 1997). Soil-to-plant transfer of Cd is the major exposure pathway for humans with regards to soil contamination. Excessive dietary intake of Cd accumulates in the body over a lifetime and can lead to kidney malfunction and disease (McLaughlin et al., 1999).

The main soil factors that influence Cd uptake by plants have been identified (Alloway, 1995; Adams et al., 2004; Yanai et al., 2003). For example, Cd uptake increases with decreasing soil pH (Lagerwerff, 1971; Miller et al., 1976), whereas it decreases with increasing soil organic carbon (OC) content (Eriksson, 1988).

Root and leafy vegetables have a greater soil Cd uptake efficacy than fruit and seed vegetables (Lund et al., 1981; McLaughlin et al., 1999; Alexander et al., 2006). Spinach is classified as a plant with high capacity for Cd uptake according to Kuboi et al. (1986) and

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some other studies (Bingham et al., 1975; Bradford et al., 1975; Davis and Carlton-Smith, 1980). Previous investigations have focused on the soil-to-plant transfer characteristics of Cd, but the relationship between Cd uptake by spinach and soil factors has not been investigated. Furthermore, the soil types and sampling areas used in most studies have been generally limited to small variation ranges, so the identified influential factors and prediction relationships may lack broader representativeness. In this study, the transfer characteristics of Cd (exogenous Cd salts) from a wide range of Chinese soils to the edible parts of spinach (*Spinacia oleracea* L.) were investigated, the major controlling factors were identified and prediction equations were developed.

2. Materials and methods

2.1. Soil samples and experimental design

A set of fifteen soils covering a wide range of soil properties was collected from typical locations in China, which were representative of the major soil types and the distributions of soil pH and organic matter content of agricultural soils in China. Selected physical and chemical characteristics of the soils are shown in Table 1. Soil pH and electrical conductivity (EC) were measured in deionized water (soil: solution ratio, 1:5); cation exchange capacity (CEC) was determined using the unbuffered silver–thiourea method; OC was measured by dry combustion and clay content was determined using the standard pipet method. The carbonate (as CaCO₃) content of the soils was determined by treating each soil with HCI and measuring the liberated CO₂. The background Cd in the soils was determined using an aqua regia (a 1:3 fresh mixture of concentrated HNO₃ and HCI) digestion.

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Table 1Selected properties of the soil samples.

Soil no	Location ^a	рН	OC (%)	CEC (cmol ⁺ /kg)	EC (µS/cm)	Background Cd (mg/kg)	Clay (< 2 µm, %)	CaCO3 (%)	CD-Al (g/kg)	CD-Fe (g/kg)	CD-Mn (g/kg)
S1	Haikou, Hainan	4.93	1.5	8.7	110.8	0.092	66.1	0	9.478	83.920	0.574
S2	Qiyang, Hunan	5.31	0.9	7.5	74.1	0.260	46.1	0	3.293	26.154	0.422
S3	Hailun, Heilongjiang	6.56	3.0	33.6	153.0	0.142	40.4	0	1.244	6.559	0.396
S4	Hangzhou, Zhejiang	6.80	2.5	12.8	203.3	0.042	38.9	0	0.631	8.413	0.153
S5	Chongqing	7.12	1.0	22.3	71.0	0.072	27.3	0	0.370	7.727	0.315
S6	Guangzhou, Guangdong	7.27	1.5	8.3	136.7	0.172	25.3	0.2	1.488	11.411	0.048
S7	Lingshan, Beijing	7.48	4.3	22.7	92.5	0.149	19.9	4.3	0.706	6.950	0.276
S8	Hulunbeier, Inner Mongolia	7.66	2.7	22.7	888.0	0.057	37.1	0.3	0.956	5.259	0.322
S9	Gongzhuling, Jilin	7.82	2.2	28.8	146.9	0.137	44.6	0.3	1.067	6.932	0.366
S10	Shijiazhuang, Hebei	8.19	1.0	11.7	302.0	0.268	21.4	3.8	0.579	7.544	0.261
S11	Yangling, Shaanxi	8.83	0.6	8.5	83.2	0.174	27.5	8.9	0.461	7.193	0.350
S12	Langfang, Hebei	8.84	0.6	6.4	5.7	0.219	10.1	2.4	0.273	3.729	0.112
S13	Zhengzhou, Henan	8.86	1.6	8.5	108.7	0.125	16.3	0.2	0.361	4.289	0.166
S14	Zhangye, Gansu	8.86	1.0	8.1	151.8	0.120	19.6	7.8	0.451	8.356	0.331
S15	Dezhou, Shandong	8.90	0.7	8.3	111.8	0.152	17.6	6.2	0.369	4.965	0.219

^a Soil sample locations are listed in the order of increasing pH.

Soil active Al, Fe and Mn were determined using a dithionate-citrate extraction: a 0.8 g soil sample (< 0.5 mm) was mixed with 0.8 g sodium dithionite and 40 ml of 22 percent sodium citrate solution and shaken for 16 h. Then five drops of Superfloc were added and the extract was again shaken, centrifuged and filtered, after which the extract was diluted and left loosely stoppered for two days before analysis. All the metals were determined using inductively coupled plasma–mass spectrometry (ICP–MS, Agilent 7500a, Agilent Technologies Co. Ltd., USA).

All soils were bulked samples from the top 20 cm of the soil profile. They were then air-dried at 25 °C and sieved to <2 mm prior to analysis. Exogenous Cd (as Cd (NO₃)₂·4H₂O) at concentrations of 0, 1, and 2 mg/kg were added and thoroughly mixed with 1 kg air-dried soil. Each concentration treatment was repeated four times. After three months' homogenization, spinach seeds purchased from Beijing Jiahe Seeds Co. Ltd, China, with radicles less than 3 mm were sown in each pot. The pots were placed on randomized racks inside a greenhouse at a temperature of 25 ± 3 °C during the daytime and 20 ± 3 °C at night with a natural light photoperiod. Soil moisture was maintained at 60 percent to 70 percent of field capacity using deionized water during the entire plant growing period.

2.2. Sampling and analysis

The edible parts of the spinach plants were harvested at six weeks after sprouting. The samples were cleaned with deionized water before being oven-dried at 105 °C for 30 min, followed by 70 °C for 48 h. The dry weights of the edible parts of the spinach biomass were measured before being ground. Subsamples of 0.5 g were then weighed and digested in 6 ml concentrated HNO₃ (70 percent w/v) and 3 ml H₂O₂ using a CEM Mars × microwave oven (CEM Mars) at a pressure of 31. MPa. The cadmium concentration in the digestion solution was determined by inductively coupled plasma–mass spectrometry (ICP–MS, Agilent 7500a, Agilent Technologies Co. Ltd., USA).

After harvesting the spinach, soil samples from the pots were air-dried and thoroughly mixed. Subsamples weighing 10 g were collected using a chessboard method and sieved to < 0.26 mm. Subsamples of 5.0 g were weighed into 50 ml digestion tubes and mixed end over end for 2 h with 25 ml 0.05 M Na₂-EDTA. The suspensions were then centrifuged at 4000 rpm for 0.5 h and filtered through 0.2 μ m pore-size cellulose acetate filters before analysis. Another 0.5 g subsample was weighed and added to digestion tubes containing 9 ml HNO₃ and 3 ml HF. The cadmium concentrations in both the extraction solution and the digestion solution were measured using inductively coupled plasma–mass spectrometry (ICP–MS, Agilent 7500a, Agilent Technologies Co. Ltd., USA).

For analytical quality control, standard certified reference plant and soil materials were used for determination of physic-chemical properties of soils, and for determination of total metal concentrations in soils and plants.

2.3. Data analysis

The ratio of the Cd concentration in the plant to that in the soil was defined as the transfer factor (*TF*, also called the uptake factor, accumulation factor or concentration factor in the literature). Currently, two methods are used to calculate the Cd transfer factor. The first is based on total Cd in the soil, which can be formulated as Alexander et al. (2006):

where C_{plant} is the Cd concentration in the plant and C_{soil} is Cd concentration in the soil. Taking the source of Cd into account, the second method is as follows:

 $TF_{added} = (C_{plant} - C_{controlplant}) / (C_{soil} - C_{controlsoil})$

where C_{plant} is Cd concentration in the test plant, $C_{\text{control plant}}$ is the Cd concentration in the control plant, C_{soil} is the Cd concentration in the test soil and $C_{\text{control soil}}$ is the Cd concentration in the control soil.

SPSS 17.0 for Windows[®] (SPSS Inc, Chicago, IL, USA) was used for statistical analysis of the significant differences and Origin 8.0 (OriginLab Co., Northampton, MA, USA) was used for the regression analysis.

3. Results

3.1. Relationship between Cd concentrations in the plants and in the soils

A significant and positive correlation (r^2 =0.534, p < 0.001) was obtained between the Cd concentrations in the plants and in the soils (Fig. 1). The soil Cd concentration in the fifteen selected soils varied between 0.04 and 1.49 mg/kg (including the control), which corresponded to a Cd concentration in the plants of between 0.02 and 6.17 mg/kg (including the control). The log-transformation of the data, as recommended by Webster (2001), provided a much better (r^2 =0.878, p < 0.001) correlation due to assurance of variance homogeneity.

Different views exist regarding the relationship between the plant Cd concentration and the soil Cd concentration. McBride (1995) proposed three possible trends in metal solubility (or plant availability) as a function of the metal concentration in soils: (i) the linear model (constant partitioning model), (ii) the plateau model (saturation model) and (iii) the Langmuir sorption model. Other scientists (Kuboi et al., 1986; Castilho and Chardon, 1995; Fytianos et al., 2001) contend that there is no correlation between plant Cd and soil Cd concentration. However, it is generally accepted that plant Cd concentration is considered to be related to Cd^{2+} activity in the soil solution. The distribution of Cd between the soil solid phase and the soil solution can be described by a Freundlich sorption function (given that the solubility of Cd at low soil Cd concentration is sorption-controlled rather than precipitation-controlled). Though diffusion limitation has been demonstrated for Cd supply from the soil solution to the plant root (Degryse et al., 2006) and the correlation between the metal concentration in the plant roots and above-ground biomass depends on many factors affecting translocation (including species and cultivar of plant, environmental conditions and competing

(2)

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7.0 Normal 6.0 y = 1.537x - 0.153R Sq = 0.534 5.0 Plant Cd (mg/kg) 4.0 3.0 2.0 1.0 0.0 0.0 2.0 0.5 1.5 1.0 Soil Cd (mg/kg) 1.0 Log-transformed 0.5 y = 1.339 x + 0.044R Sq = 0.878 0.0 log [plant Cd] -0.5 -1.0 -1.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 log [soil Cd]

Fig. 1. Relationship between the Cd concentrations in the plants and in the soil in normal scales and in log–log scales with upper and lower confidence (95 percent) interval curves of the regression slope.

ions) (McBride, 1995), a Freundlich-type function relationship (discussed in detail below) could still be expected between plant Cd concentration and soil Cd concentration, as was the case (log [plant Cd] vs. log [soil Cd]) in this study.

3.2. Effects of different soil types on Cd transfer in the soil-spinach system

As shown in Fig. 2, TF_{total} after Cd addition increased substantially in the different soils compared with the control, due to the higher bioavailability of added Cd. Generally, the bioavailability of added metal salts to soils is higher than the metals in soils and from other sources, such as wastes. Because other sources are heterogeneous, the approach of added metal salts to soils for risk assessment is often used in the procedures to set soil quality standards (Smolders et al., 2009). There was a non-significant difference in the TF_{total} after the addition of 1 mg/kg and 2 mg/kg Cd. In the control soil, the two maximums for TF_{total} (1.89 and 1.79) were observed in S1 and S2 (pH 5.31 and 4.93). This result confirms that Cd under acidic conditions is more highly bioavailable and leads to increased absorption by the plant (Lagerwerff, 1971; Miller et al., 1976; Hinesly et al., 1984; Castilho and Chardon, 1995; Adams et al., 2004). TF_{total} in S15 (pH 8.90) was lower than



Fig. 2. Effects of different soil types on the transfer factor based on total Cd in soils (TF_{total}) and added Cd to soils (TF_{added}) .

that in S1 and S2, but significantly higher than in the other twelve soils, which may be ascribed to the low OC (0.7 percent) and clay content ($< 2 \mu m$, 17.6 percent) in S15. Increased Cd solubility, due to complexation with dissolved organic matter (DOM), could be seen at elevated pH values, which was expected in soils with low OC and clay contents (Castilho and Chardon, 1995). The values of *TF*_{total} in S3–S10, S12 and S13 were intermediate, ranging from 0.35 to 0.53. The two minimums for TF_{total} (0.25 and 0.22) were found in S11 and S14, which coincided with the highest CaCO₃ content of 7.8 percent and 8.9 percent, respectively. Under Cd addition at 1 mg/kg, the two TF_{total} maximums were still observed in S1 and S2. Insignificant differences for TF_{total} were observed in S5, S8, S10, S12, S13, with pH values of 7.12, 7.66, 8.19, 8.84 and 8.86, respectively. Noticeably, no significant difference was found between S5 and S13, which had a difference in pH of 1.74. This was probably due to the difference in CEC between the two soils (22.3 and 8.5). Cations adsorbed on soil colloids could exchange for Cd in the soil, thus decreasing the bioavailability of Cd and reducing its transfer from the soil to the plants. It can therefore be concluded that the compensation role of CEC in S5 offset its low pH effect compared with S13 (Haghiri, 1974; Miller et al., 1976). Under Cd addition at 2 mg/kg, S1 and S2 still had the highest TF_{total} values among the fifteen soils. The values of TF_{total} in S3, S4, S5, S8, S10, S12, S13 and S15 were intermediate (1.03 to 1.54), with S6, S7, S9, S11 and S14 recording low values for TF_{total} (0.70–0.80).

For TF_{added} , there was no significant difference between Cd addition at 1 mg/kg and 2 mg/kg in S1 and S2 and these soils recorded the highest values out of all the soils. Nevertheless, the elimination of the control Cd concentration in the calculation formula resulted in increased Cd transfer factors for each soil compared with TF_{total} .

3.3. Major factors affecting plant uptake of Cd in different soil types

In most cases, the inclusion of soil factors improves the correlation performance between Cd concentration in the plant and in the soil compared to those based on total Cd concentration only. In this study, r^2 between log [soil Cd] and log [plant Cd] was 0.878 (p < 0.001). When soil Cd and pH were combined, the controlled variance was improved to $r^2=0.926$ (p < 0.001) and when OC was introduced into the equation, the regression coefficient rose to $r^2=0.944$ (p < 0.001). Other soil factors, such as CEC, clay, Fe and Mn contents failed to significantly improve the correlation performance and were therefore excluded. The simple relationships between plant Cd and pH, and plant Cd and OC are shown in Fig. 3

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Fig. 3. The simple relationships between the Cd concentration in plants (plant Cd) and soil pH, and organic matter in soils (OC).

3.4. Prediction equations for Cd transfer from the soil to spinach plants

The Freundlich-type function is often used to describe Cd transfer from the soil to plants (Efroymson et al., 2001; Krauss et al., 2002).

$$C_{\text{plant}} = 10^{a} C^{b}_{\text{soil}} \text{ or } \log[C_{\text{plant}}] = a + b \log[C_{\text{soil}}]$$
(3)

The Freundlich-type function can be extended using soil factors like pH, OC, CEC, etc. It has been well established that Cd uptake by plants is dependent on soil total Cd, soil pH and OC content (McBride et al., 1997; Sauvé, 1999; Sauvé et al., 2000). In the present study, the relationship between plant Cd concentration and soil total Cd concentration was established using the extended Freundlich-type function and included pH and OC.

$$\log[\text{plant Cd}] = a + b\log[\text{soil Cd}] + cpH + d\log[\text{OC}]$$
(4)

The optimum prediction equation was acquired by minimizing the residual sum of squares between the predicted *TF* and the measured *TF* (Fig. 4). In this study, the predicted *TF* was calculated as the ratio of predicted plant Cd concentration from Eq. (4) to soil Cd concentration and the measured *TF* was expressed as the quotient of the measured plant Cd concentration and the soil Cd concentration. *TF*_{total} and *TF*_{added} were analyzed separately and



Fig. 4. Relationship between the measured log [plant Cd] and the predicted log [plant Cd] for different Cd sources (control, total and added Cd only) with upper and lower confidence (95 percent) interval curves of the regression slope.

took into account the origin of the Cd. *TF* values at 1 mg/kg and 2 mg/kg were combined as there was a negligible difference between the two (as discussed above).

Table 2 shows the prediction equations for the different Cd sources (control, total and added). The equation and all parameters for the control soil were significant at p < 0.05, while significance

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 Table 2

 Prediction equations for the different Cd sources.

Cadmium sources	Prediction equations	r ²	р
Control Cd	log [plant Cd]=1.21+0.89log [soil Cd]-0.20pH-0.58log [OC]	0.711	< 0.05
Total Cd	log [plant Cd]=1.29+1.01log [soil Cd]-0.15pH-0.47log [OC]	0.721	< 0.001
Added Cd	log [plant Cd]=1.26+0.98log [soil Cd]-0.14pH-0.48log [OC]	0.754	< 0.001



Fig. 5. Relationship between the measured log [plant Cd] and the predicted log [plant Cd] for the combined observations (control, total and added Cd together) with upper and lower confidence (95 percent) interval curves of the regression slope.

was observed at p < 0.001 for the total Cd and the added Cd equations. All equations displayed the same trend, in that plant Cd concentration was positively related to soil Cd and negatively correlated with soil pH and OC. The correlation between plant Cd and soil added Cd was higher than that for soil total Cd. The concept of extrogenity was introduced and applied to the calculation of Cd transfer from soil to plant in order to exclude the effect of soil background Cd (control Cd). The higher correlation between plant Cd and soil added Cd indicated a better representation of the Cd transfer characteristics.

Incorporating the control Cd with total Cd (45 observations) considerably improved the equation predictability from r^2 =0.721 to r^2 =0.944, as indicated in Eq. (5) and Fig. 5.

log[plant Cd] = 1.29 + 1.01 log[soil Cd] - 0.15 pH - 0.47 log[OC] (5)

For the sake of consistency and comprehension, several published data sets are listed for comparison in Table 3.

Similar prediction approaches have been applied by other researchers, with the same positive and negative relationship for fitted terms as was found in this study. All the parameters in Eq. (5) were significant at p < 0.001. All coefficients of log [soil Cd] and log [OC] were significant at p < 0.001 in Eqs. (6)–(8), but the coefficient of pH in Eq. (6) and the coefficient of intercept and pH in Eq. (8) were not significant. The weak significance (p=0.154) for pH in Eq. (6) may be ascribed to the strong effect of soil OC on controlling Cd uptake by the crops when pH and OC were simultaneously accounted for. The non-significance (p=0.340) of pH in Eq. (8) can be attributed to the narrow pH range in the soils. Both the pH range and OC interval were wide enough to guarantee the significance of the coefficients for pH and OC in this study. Notwithstanding the results, a slight protection effect of OC on Cd uptake, with r^2 changing from 0.926 to 0.945, was observed at low soil Cd concentrations.

4. Discussion

Generally, plants readily take up Cd in the soil solution in either free ionic or complexed forms. Soil pH is the primary factor influencing the phytoavailability of Cd. The direct effect of pH is its influence on Cd speciation. Cd^{2+} is generally the predominant soluble Cd species below pH 9.0 and can comprise 40 percent to 90 percent of the soil solution Cd (Hirsch and Banin, 1990; De Villarroel et al., 1993; Holm et al., 1995). The ultimate effect of pH is its influence on the solution activity of Cd and the distribution of Cd between the soil phase and the solution phase (Christensen, 1984; Eriksson, 1990; Naidu et al., 1994). The increased sorption of Cd at elevated pH values would reduce the solution concentration and thus decrease Cd phytoavailability.

Other soil factors, such as soil organic matter (OM), texture, salinity, redox potential and nutrient status, might also affect Cd uptake. Soil OM is the major contributor to the pH-dependent negative charge in soils that gives rise to the soils' ability to retain cationic metals. It must be stressed, however, that OM appears to have quite different roles in controlling Cd uptake by plants, depending upon whether it is soluble (fulvic acid) or insoluble (humic acid).

Predicting the mobility and bioavailability of Cd in soil has been a major area of research recently in both the agricultural and the environmental fields. Both mechanistic and empirical models have been adopted to predict plant concentration from the soil or from the soil solution concentration. Mechanistic models are based on plant and soil parameters that are difficult to determine, e.g., diffusion coefficients of ions in the soil solution, root geometry and size and kinetic parameters for the uptake of ions by the roots (Rengel, 1993; Barber, 1995). For this reason, far more studies seek to use empirical models (Dudka et al., 1996; Mench et al., 1997) using expressions that are as simple as possible. Different authors have adopted distinct prediction models (equations) based on different hypotheses (Browne et al., 1984; McBride, 2002; Krauss et al., 2002; Wang et al., 2004). An advantage of the Freudlich-type function as applied to the soil to plant transfer relationship is its simplicity and applicability.

This study identified the major controlling factors, added them into the empirical prediction equations and improved the reliability of the equation. However, extrapolation of the present experiment results and its broader application to other plants and field conditions, particularly in the agricultural landscape, still needs further investigation.

5. Conclusions

Plant Cd concentration was positively correlated with soil Cd concentration and the log-transformed data improved the correlation. The maximum TF_{total} and TF_{added} values for the fifteen soil types occurred in acid soils. TF_{added} was higher than TF_{total} for each soil type. The extended Freundlich-type function described well the Cd transfer from soil to spinach plant. Combining soil total Cd, pH and OC in the prediction equation greatly improved the correlation performance compared with one based on total Cd only, which will be a useful tool that can be used to predict Cd

Table 3				
Comparison of prediction	equations	for the	different	sources

Equation no.	Prediction equations	Conditions
(6)	log [plant Cd] = 5.83+2.09log [soil Cd] - 0.19pH-2.09log [OC]	With and without sewage sludge, Swiss chard, pH 6.5–7.9, OC 13–27% $$
(7)	n=6, $r=0.984$ (km et al., 1988) log [plant Cd]=3.36+1.27log [soil Cd]-0.24pH-1.27log [OC]	Long-term sewage sludge, lettuce, pH 5.0–5.4, 6.2–6.9, OC 1.25–2.07%
(8)	$n=40$, $r^2=0.924$ (Brown et al., 1998) log [plant Cd]=1.24+0.77log [soil Cd]=0.091pH=0.77log [OC] $n=24$, $r^2=0.523$ (Ge et al., 2000)	Inactive railway yards, vegetation, pH 6.39–8.55, OC 1.39–15.0%

transfer from the soil to the plant. A slight protection effect of OC on Cd uptake was observed at low soil Cd concentrations.

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