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Magnesium Oxide Nano Particles Effects on Utilization of Soil Phosphorus by Maize (*Zea mays* L.) Plant

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A B S T R A C T

Magnesium (Mg) is the structural component of chlorophyll and polysaccharides and also an activator of several enzymes. Magnesium plays an important role in photosynthesis. An attempt was made in the present investigation on utilizing this property for increasing rate of photosynthesis of maize plant and subsequently higher root exudations which enhance the microbial activities in soil. Green house experiments were conducted to evaluate the effect of MgO nano particles spray on maize plant (*Zea mays* L.) in three benchmark soils of India which are phosphorus (P) deficient. Results revealed that application of MgO nano particles spray 10 mg/L (viz. 15, 28, and 35 days after sowing) enhanced the enzymatic activities like phytase and phosphatase particularly in roots of P deficient plant. With the application of both P doses (viz. 0, 13, 26, and 52 mg kg^{-1}) and MgO nano particles spray increased the different growth parameters of plants like root length, root volume, dry weight of shoot and root etc. irrespective of soils. The results can enhance our understanding on the role of MgO nano particles spray in plant root exudation and as well as the availability of soil P. These findings are of great help towards building a comprehensive understanding of the potential impact of MgO nano-particles on phosphorus nutrition of plant and soil health.

Keywords

MgO nano particle, Maize, Soils, Phosphatase, Phytase

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Introduction

Currently, nanotechnology holds a prominent position in boosting agriculture and permits broad advances in agricultural research. In industry nano-MgO has been extensively used for several decades. But its application in agricultural field is still not emphasized and practiced so as to increase the nutrient use efficiency. Before application in field characterization of nano particles is essential to understand its behaviour and reaction kinetics in soils and plants. Magnesium is the most important essential secondary nutrient, which is required to enhance the productivity of the crop. Nano-scale MgO exhibits unique optical electronic, magnetic, thermal, mechanical, and chemical properties, due to its characteristic structures. In this investigation the optical property of MgO nano particles was given importance which subsequently increased the plant root exudation and also helped to increase the energy supply and supply of C skeleton compounds to phosphorus mobilizing microorganisms. This approach would enable in breaking of existing barriers in utilization of native P and reduce dependence on imported P fertilizers. There are no studies on effect of nano-particles on root exudation but it is envisaged that higher photo energy (photosynthesis) available through root exudates to soil system would positively create favourable conditions for microbial growth and consequently enhance the native P solubilization. Maize is now widely cultivated in India, and a greater weight of maize is produced each year than any other grain. The importance of maize or corn lies in its wide variety of applications besides serving as human food and animal feed. Phosphorus (P) is one of the main plant nutrients, the lack of which limits plant growth. In most soils, the concentration of orthophosphate in solution is low and must be replenished from other pools of soil P to satisfy plant requirements (Richardson and Simpson 2011). Thus, phosphate-mobilizing microorganisms may be used to increase P availability to plants (Kalayu, 2019). The greatest challenge in Indian agriculture in the coming decades is to increase production with ecological sustainability and in order to achieve that there is a need for optimum utilization of fertilizers. To address the issues relating to increase fertilizer use efficiency, development of new agricultural technologies will be crucial in meeting the ecological needs. Many studies have reported that nano particles have favorable effects on plant growth and development. Still the knowledge available on the positive or negative effects of some nano-particles on the physiology and biochemistry of plants is meager, and does not convey any clear evidence on this issue. Against this backdrop, the present investigation was carried out to investigate the effect of MgO nano particles spray on maize plant for the utilization of soil phosphorus.

Materials and Methods

Soil sampling and analysis

Surface soil samples (0-15 cm) were collected from P deficient field from Jodhpur, Bhopal, Betul. The soils are respectively classified as Aridisol (Typic Haplustalfs), Vertisol (Typic Haplustert), and Alfisol (Typic Torripsamments). Composite surface soil samples from the above sites were processed, sieved through a 2 mm sieve and analyzed for physico-chemical properties by following the standard procedure (Page, 1982). Available phosphorous in the soil was determined by spectrophotometry (Olsen, 1954).

Test NPs

The MgO NPs were purchased from Sigma-Aldrich Company, St. Louis, MO, USA.

Dynamic Light Scattering (DLS) Analysis

Particle size analyzer (Malvern, USA) was used for size measurement and confirmation of nano particles size distribution.

Green house experiment

Green house experiments were conducted with three different soils of India. Four levels of P (viz. 0, 13, 26, and 52 ppm as reagent grade KH_2PO_4 were applied in solution form in soil and the treated soil was then mixed thoroughly and flooded with distilled water for three days prior to transplanting of maize crop. Basal application of NK at the rate of 100-60 mg kg^{-1} soil was made through AR-grade urea and KCl, respectively. Each treatment was replicated for thrice. A pot (height 13.5 cm and inside diameter 11 cm) culture experiment was conducted in a green house. Temperature, light and humidity were not regulated. Temperature varied between 20 and 35 ^{o}C, photo flux density at leaf level about 400µ mol cm^{-2}, humidity varied from 70-80%. Spray of Mg nano particle (<50 nm), 10ppm (viz. 15, 28, and 35 days after sowing) were applied on maize plant. The soils were watered to field capacity with deionized water (gravimetric field capacity water content was 27% for Vertisol, 10% for the Alfisol, and 16% for the Aridisols) and allowed to incubate for 15 days in the green house before sowing. The pots were watered daily to field capacity with deionized water re-randomized every second day. The soil was sown with maize (*Zea mays*) plant. Ten germinated seeds were sown at 1cm depth into each pot. Plants were thinned to 5 plants per pot at the 2-leaf stage, leaving 5 uniform plants. Crop was harvested after 45 days of sowing. The plant shoot and root sample for each pot were combined, washed thoroughly with distilled water, and weight after drying at 70 ^{O}C for 2 days. All dried plant sample were digested with mixture of nitric acid- perchloric acid (9:4). Phosphorous concentration in the digested plant sample was determined with an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

Seeds

Seeds maize (*Zea mays L.)* plant was purchased from National Seed Corporation, India. The average germination rates of both the plants were greater than 90% as shown by a preliminary study. Seeds were kept in a dry place in the dark under room temperature before use.

Root studies

The root samples collected after harvesting were initially washed with distilled water to make them free from any soil particles and following observations were made:

Root length

Root length was computed using the modified version of Newman (1966) formula proposed by Tennant (1975) as:

Root Length: 11/14* number of intersections (N) *grid unit.

Root volume

Root volume was determined by displacement method (Mishra and Ahmed, 1987).

Enzyme assay

Phytase activity

The enzyme activity was determined by following Heinonen and Lahti (1981) method.

Phosphatase activity

The enzyme activity was determined following the method described by Parfsh (1974).

Statistical analysis

Each treatment was conducted with three replicates, and the results were presented as mean ± SD (standard deviation).

Results and Discussion

Physico chemical properties of experimental soils

The experimental soils varied in pH from 5.65 to 8.98, EC from 0.24 to 0.40 dS m^{-1}, organic carbon content from 2.2 to 6.6 g kg^{-1}, cation exchange capacity from 7 to 45 cmol (p+) kg^{-1}, clay content from 7.87 to 55.5% and

$CaCO_3$ content from trace to 2.7%. The available P content varied from 4.58 to 6.12 mg kg^{-1} (Table 1).

TEM study of nano-MgO particles

The TEM image of the magnesium oxide NPs revealed at their spherical, truncated and uneven nature with an average size of approximately 50 ± 20 nm (Figure 1).

The TEM micrographs indicated that the copper oxide nano particles were mono dispersed with a narrow size distribution and near spherical morphology. Analysis of particles in TEM monograph indicated hexagonal particles with the average size of 50 nm.

DLS study of nano-MgO particles

The size of nano MgO particles were measured by dynamic light scattering (DLS) technique using particle size analyzer (Figure 2) and it was found that the MgO particle recorded a size of <50 nm.

Effect of MgO nano-particles spray on dry matter yield of maize plant

A perusal of the data (Table 2-7) revealed that the biomass yield of maize plant harvested after 45 days of sowing in different soils like Vertisol, Alfisol, and Aridisol showed distinctly different response to P nutrition to maize plant treated with and without MgO nano particles spray. The shoot and root dry matter yield of maize plant treated with MgO NP spray recorded 0.67 and 0.66 times more yield respectively than the plant without any MgO NP spray in Vertisol (Table 2 and 3) of Bhopal region. Similarly in case of root length and root volume of treated plant showed 0.64 and 0.57 times more growth than the untreated plant. Experimental results also revealed that with the increasing rate of application of different doses of P (0, 13, 26 and 52 mg kg^{-1}), enhanced the dry matter yield of shoot and root of maize plant. The highest dry matter yields of shoot and root (2.95 g and 0.29 g) was recorded at the dose of 52 mg P kg^{-1} in Vertisol. The increase in growth and yield parameters of maize plant was observed and the difference could be attributed to additive effect of the 10 ppm MgO NP spray. Root length and root volume of treated plant showed 990 cm and 28 cc in comparison to control plant 387 cm and 7 cc respectively.

The stunted growth and low yield was observed for control plant and the reason could be due to reduction in soil nutrient supply to the crop without any MgO NP spray.

The highest P content in shoot and root i.e. 0.15 % and 0.11% respectively was recorded in case of higher dose of P application (52 mg kg^{-1}). For a better understanding of the effect of MgO NP spray on maize plant in different soils, however, studies on multiple soils like Vertisol, Alfisol, and Aridisol were conducted. In case of Alfisol, the MgO NP treated shoot and root dry matter yield were also shown an increasing trend i.e. 0.55 and 0.57 times more than untreated plant (Table 4 and 5). The root length and root volume recorded 0.54 and 0.51 times more than the plant without MgO NP spray.

The increase in biomass production of maize plant could be as a result of good P supply facilitated by application of MgO NP spray that resulted in good crop growth and increase yield. The beneficial effects were attributed to the improvement in soil nutrition. Another possible reason could be that the improved soil P status with the MgO NP spray encouraged root growth and nutrient use. Thus growth and yield attributes increased. Amongst the soils compared, the Aridisol recorded lowest dry matter yield of shoot and root (Table 6 and 7).

Table.1 Physico- chemical properties of the experimental soils

Sl. No.	Soil sub group and Site	pH (1:2.5 H_2O)	EC (dS m^{-1})	Organic Carbon (%)	CEC cmol (p+)kg^{-1}	Clay	$CaCO_3$	Available P (mg/kg)
						--------%-----------		
1	Bhopal Typic Ustrochrept	7.85	0.40	0.66	45.0	55.50	2.7	5.85
2	Betul Typic Haplustalf	5.65	0.35	0.40	8.0	24.9	Tr.	4.58
3	Jodhpur Typic Torripsamments	8.98	0.24	0.22	7.0	7.87	1.80	6.12

Table.2 Physiological parameters of maize plant grown in Vertisol with MgO nano particle spray

Sl. No.	P levels (mg/kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (%)	Root P content (%)
1	0	0.52 ± 0.02	0.09 ± 0.007	387 ±1.50	7 ± 0.20	30 ± 0.55	12.5 ± 0.30	0.02 ± 0.001	**0.01 ± 0.001**
2	13	1.15 ± 0.01	0.15 ± 0.008	560 ± 2.10	13 ± 0.65	22 ± 0.31	8.20 ± 0.25	0.08 ± 0.002	**0.06 ± .004**
3	26	2.90 ± 0.07	0.26 ± 0.01	940 ± 3.20	25 ± 0.70	12 ± 0.25	4.30 ± 0.15	0.11 ± 0.011	**0.09 ± 0.005**
4	**52**	**2.95 ± 0.06**	**0.29 ± 0.01**	**990 ± 5.0**	**28 ± 0.68**	**5.5 ± 0.20**	**3.10 ± 0.25**	**0.15 ± 0.03**	**0.11 ± 0.02**

Each value is the Mean ± SD of three replicates

Table.3 Physiological parameters of maize plant grown in Vertisol soil without MgO nano particle spray

Sl.No.	P levels (mg/kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (%)	Root P content (%)
1	0	0.35 ± 0.03	0.06 ± 0.006	250 ±1.10	4 ± 0.10	20 ± 0.40	8.50 ± 0.30	0.008 ± 0.001	**0.005 ± 0.001**
2	13	0.90 ± 0.01	0.09 ± 0.002	400 ± 2.50	8 ± 0.55	15 ± 0.21	4.20 ± 0.20	0.04 ± 0.002	**0.02 ± .003**
3	26	1.85 ± 0.06	0.19 ± 0.01	750 ± 5.00	16 ± 0.60	7 ± 0.22	2.10 ± 0.05	0.08 ± 0.004	**0.05 ± 0.004**
4	**52**	**1.99 ± 0.07**	**0.21± 0.01**	**780 ± 5.30**	**18 ± 0.65**	**5 ± 0.20**	**1.30 ± 0.06**	**0.12 ± 0.04**	**0.08 ± 0.01**

Each value is the Mean ± SD of three replicates

Table.4 Physiological parameters of maize plant grown in Alfisol with MgO nano particle spray

Sl.No.	P levels (mg/ kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (mg/kg)	Root P content (mg/kg)
1	0	0.36 ± 0.03	0.07 ± 0.005	220 ± 1.50	5.80 ± 0.20	35 ± 0.40	16 ± 0.50	150 ± 2.0	**85 ± 1.0**
2	13	0.65 ± 0.04	0.11 ± 0.004	415 ± 3.50	9.51 ± 0.55	25 ± 0.11	12 ± 0.30	570 ± 3.0	**300 ± 3.0**
3	26	1.25 ± 0.06	0.19 ± 0.01	780 ± 5.00	19.50 ± 0.65	16 ± 0.10	6.80 ± 0.05	750 ± 6.5	**560 ± 3.0**
4	**52**	**1.95 ± 0.08**	**0.23 ± 0.02**	**858 ± 5.50**	**22.30 ± 0.70**	**8 ± 0.05**	**4.30 ± 0.06**	**990 ± 7.2**	**780 ± 4.0**

Each value is the Mean ± SD of three replicates

Table.5 Physiological parameters of maize plant grown in Alfisol without MgO nano particle spray

Sl.No.	P levels (mg/kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (%)	Root P content (%)
1	0	0.20 ± 0.01	0.04 ± 0.004	120 ± 1.00	3.0 ± 0.15	22.1 ± 0.20	9.2 ± 0.30	60 ± 1.0	**40 ± 1.0**
2	13	0.45 ± 0.02	0.07 ± 0.003	310 ± 2.20	5.1 ± 0.20	18 ± 0.11	5.1 ± 0.20	220 ± 2.0	**135 ± 0.15**
3	26	0.85 ± 0.04	0.10 ± 0.01	585 ± 5.00	9.2 ± 0.35	9.2 ± 0.18	2.5 ± 0.008	450 ± 3.5	**310 ± 2.0**
4	**52**	**0.98 ± 0.06**	**0.15 ± 0.01**	**710 ± 5.30**	**12.5 ± 0.42**	**6.5 ± 0.08**	**1.6 ± 0.006**	**770 ± 5.5**	**500 ± 3.5**

Each value is the Mean ± SD of three replicates

Table.6 Physiological parameters of maize plant grown in Aridisol with MgO nano particle spray

Sl. No.	P levels (mg/kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (%)	Root P content (%)
1	0	0.23 ± 0.01	0.05 ± 0.003	150 ± 1.00	4.0 ± 0.10	39 ± 0.30	22.5 ± 0.25	90 ± 1.5	**60 ± 1.0**
2	13	0.50 ± 0.02	0.09 ± 0.004	365 ± 2.50	7.5 ± 0.15	30 ± 0.21	15.2 ± 0.15	350 ± 2.5	**255 ± 1.5**
3	26	0.96 ± 0.03	0.15 ± 0.008	680 ± 4.00	14.2 ± 0.40	20 ± 0.15	9.3 ± 0.008	430 ± 4.0	**385 ± 2.5**
4	**52**	**1.15 ± 0.05**	**0.19 ± 0.01**	**715 ± 5.50**	**16.5 ± 0.45**	**11 ± 0.08**	**5.2 ± 0.006**	**650 ± 6.2**	**470 ± 3.5**

Each value is the Mean ± SD of three replicates

Table.7 Physiological parameters of maize plant grown in Aridisol without MgO nano particle spray

Sl.No.	P levels (mg/kg)	Dry shoot wt (g)	Dry root wt (g)	Root length (cm)	Root Volume (cc)	Phytase activity (Nitrite µmol/hr) (Root)	Acid Phosphatase activity µmol $h^{-1}g^{-1}$ (Root)	Shoot P content (%)	Root P content (%)
1	0	0.15 ± 0.01	0.03 ± 0.003	78 ± 1.50	2.1 ± 0.10	25.5 ± 0.30	12.1 ± 0.25	50 ± 1.1	**30 ± 1.0**
2	13	0.25 ± 0.02	0.06 ± 0.005	250 ± 2.00	3.5 ± 0.10	20.3 ± 0.21	6.2 ± 0.10	120 ± 2.3	**90 ± 1.8**
3	26	0.55 ± 0.03	0.12 ± 0.008	458 ± 3.50	6.9 ± 0.20	11.2 ± 0.15	2.88 ± 0.008	260 ± 3.5	**220 ± 2.6**
4	**52**	**0.68 ± 0.04**	**0.17 ± 0.02**	**620 ± 4.50**	**7.3 ± 0.35**	**8.20 ± 0.08**	**1.75 ± 0.006**	**390 ± 5.2**	**285 ± 3.7**

Each value is the Mean ± SD of three replicates

Fig.1 Tranmission-electron micograph of the MgO nanoparticle (< 50 nm)

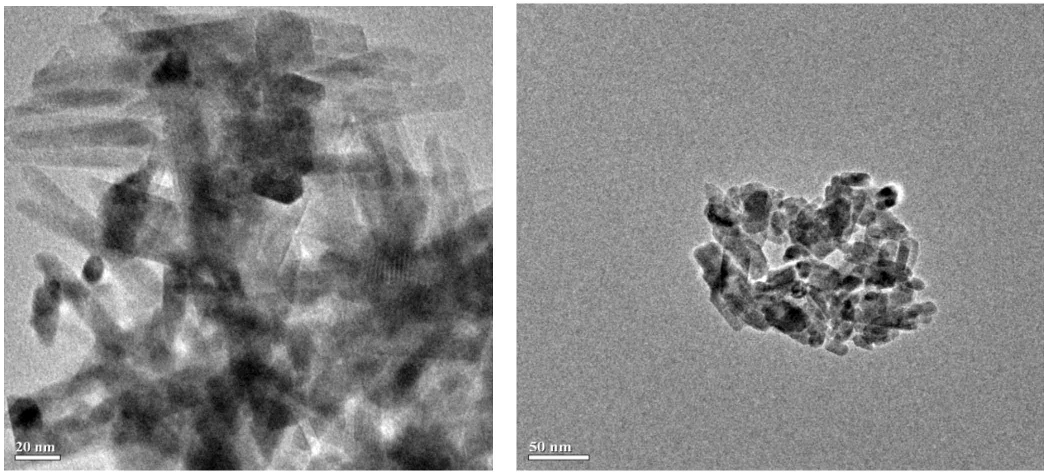
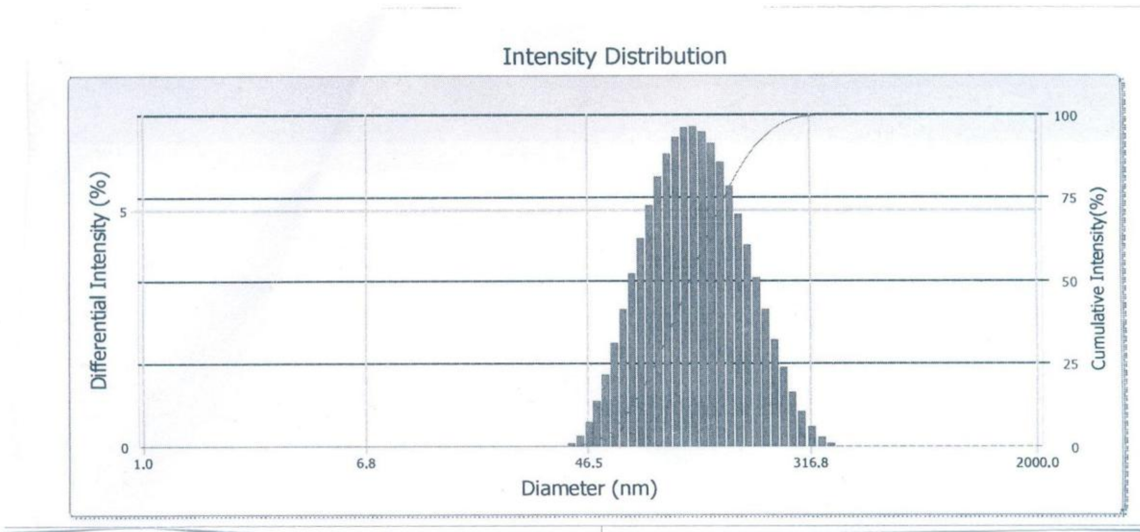


Fig.2 Intensity distribution of MgO nano particles through Photon Collision Spectroscopy.(Dynamic light scattering techniques)



Although the similar trend was observed in relation to MgO NP spray to maize plant, growth rate and P content were recorded the lowest in comparison to other two soils.

Effect of MgO nano-particles spray on enzymatic activity of maize plant

Spray of Mg nano particle (<50 nm), 10 mg kg^{-1} (viz. 15, 28, and 35 days after sowing) were applied on maize plant and experimental results depicted that the activities of two enzymes viz. phytase and acid phosphatase were positively affected by the spray in irrespective of soils (Table 2-7). In Vertisol, the application of MgO NP spray increased the phytase enzyme activity 0.57 times more than the non treated plant. Similarly in case of acid phosphatase enzyme, activity was enhanced by 0.66 times. The activities of the two enzymes in Alfisol and Aridisol followed the similar trend. But amongst the soils, the enzymes activities were recorded lowest in Aridisol.

In this investigation MgO NPs were taken based on its major role in plant photosynthesis and to utilize the impact of size effects on its optical properties. The hypothesis of the present experiment is that MgO nano-particles improved light absorbance and promoted the photosynthetic activity of plant and thus accelerated plant growth. The increase of carbohydrates may stimulate the growth and activity of microorganisms, which in turn increased the synthesis of enzyme. Uptake and distribution of MgO nano particles by the plant cells can be exploited for efficient and increased solar energy trapping that might improve the photosynthetic efficiency of plants. The small-sized nano-particles will have higher degree of freedom for movement, and hence, they would be more efficiently absorbed by the plant. Nano-particles when sprayed normally penetrate through the stomata due to its high surface area and kinetic energy. Nano-particles of specific size are capable of penetrating and migrating to different regions of plant cells (Corredor *et al.*, 2009). In this present investigation it was observed that application of MgO NP governs a prominent role to enhance the availability of soil P.

Overall experimental results depicted that 10 mg/L MgO NP spray on maize leave caused better utilization of applied and native soil P through root enzymes in irrespective of soils. Enhanced phosphatic enzyme activities and increased growth and yield parameters of maize plant which may be attributed to the fact that MgO NP aggravated the photosynthetic and specific metabolic activity of the plant grown in different soils.

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