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Study on the Effect of Sodium Nano Lignin Sulfonate on the Growth of Wheat Seedlings under Zinc Stress

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Keywords: Sodium nanolignosulfonate; zinc stress; wheat seedling treatment; seedling growth

Abstract: In addition to mercury, cadmium, lead, chromium and metalloid arsenic, which are biotoxic heavy metals, the environmental hazards of general heavy metals such as zinc, copper, nickel and cobalt, which are toxic to some extent, have also attracted widespread attention in recent years. Among them, the wastewater discharged from tannery, electroplating and zinc salt production has also caused harmful zinc pollution to the environment. In this study, sodium lignosulfonate with heavy metal adsorption function was used to prepare nano-sodium lignosulfonate preparations by means of nanotechnology, aiming to improve its adsorption rate of heavy metal ions and utilization rate of the preparations to a greater extent, reduce the use of adsorbent materials, reduce the operational burden and cost investment of environmental treatment, and also provide useful exploration and research for the agricultural use of the treated products.The main research works were (1) preparation of nano-sodium lignosulfonate formulations; (2) characterization of morphological structures of nano-sodium lignosulfonate formulations by laser pens, scanning electron microscopy, and optical microscopy; (3) treatment solutions of nano- and non-nano-sodium lignosulfonate formulations for Zn^{2+} adsorption were used to treat wheat seeds, and their effects on wheat germination and seedling growth under Zn ion stress were investigated .The results showed that (1) the prepared nano-adsorbents exhibited homogeneous fine granularity and uniform dispersion with particle size ranging from about 20 nm to 60 nm under room temperature neutral conditions; (2) the adsorption of the nano-formulations was higher than that of the non-nanoproducts under the same conditions. (3) The effect of nano-sodium lignin sulfonate was found to be better than that of non-nanopreparations for the alleviation of Zn stress by seedling trials in wheat, and the best effect was obtained when the addition amount was 30 ml.

1. Literature Review

1.1 Application of nanotechnology in wastewater treatment

With the development of China's economy, the use of nanotechnology environmental protection industry category to reduce the heavy pollution problems brought about by the petrochemical industry to the environment.

1.1.1 Treatment of inorganic polluted wastewater

Although the heavy metals in wastewater are harmful to human body, in another sense the loss of heavy metals is also a waste of resources. Nanopreparations can generate heavy metal ions in water bodies with extremely strong reducing power substances through photoelectric reaction [1], which adsorb the precious heavy metal ions that are oxidized very stably to the outside, and also reduce it to tiny metal crystals, which not only remove the toxicity of waste water, but also reduce the waste of resources.

1.1.2 Organic wastewater treatment

It is verified through several experiments that nanoscale titanium dioxide as a photocatalyst can catalyze the oxidation of organic pollutants in water bodies under visible light to achieve the need for rapid degradation. The method of using titanium dioxide as a catalyst has the advantage of no pollution and high degree of purification [2].

1.1.3 Nano water purifiers

New nanoscale water purification substances [3] have a water purification capacity ten to twenty times that of non-nano water purifiers, which can completely adsorb and precipitate suspended particles in the water to be treated, and can also efficiently remove many harmful impurities from it.

1.2 Overview of research on zinc ions on water environment pollution

1.2.1 Sources, distribution and behavior of zinc

Zinc is stored in large quantities in nature, reaching 70 mg/kg in the earth's crust. Zinc ores are generally polymetallic, mostly coexisting with lead, silver and cadmium, etc. China is also rich in lead and zinc ore deposits. Due to its own nature, Zn is mostly stored through compounds, most notably in the form of sulfides [4-5]. The effective state Zn in soil is influenced by various environmental factors such as soil pH, soil organic matter content, temperature and other climatic conditions [5-6].

1.2.2 Toxic effects of Zn on plants

In general, the Zn concentration will exceed the limit when it reaches about 100 mg/kg, and when the concentration exceeds 360 mg/kg, it will be toxic to most plants. Morphologically Zn in excess will lead to plant dwarfing and leaf yellowing. Liu [7] showed that when the Zn concentration of wheat seeds exceeded 10-4 mol/L, the seed germination rate and the amylase activity therein as well as the seedling growth state were significantly inhibited. Xu Qinsong[8] et al. showed that with increasing Zn stress, chlorophyll content decreased, SOD activity was reduced as well as soluble protein content significantly decreased; on the other hand, POD and CAT activities were first promoted and then inhibited [9].

1.2.3 Accumulation and distribution of zinc in plants

Experiments by L.Y. Bai showed that with increasing doses of exogenous Zn, the amount of Zn absorbed by wheat plants increased, along with the amount of Zn in wheat seeds, and the accumulation size of Zn in various plant parts was: stem and leaves > seeds > root system > glumes [10]. Other studies found that along with the increase in the concentration of exogenous Zn incorporation reagent, there was a significant accumulation of Zn ions in the roots and stems and leaves of wheat to the extent of root>stem>seed [11]. It was found that the accumulated Zn in the root system mainly accumulates in the cell wall, and when the amount of Zn input into the cell is excessive, the plant presses the excess Zn in the cytoplasm into the vesicles, while when the Zn involved in metabolism is insufficient, it sends Zn from the vesicles into the cytoplasm until the Zn in the vesicles is completely consumed [5-12].

1.3 Treatment of heavy metal wastewater by lignin and its sulfonates

1.3.1 Introduction to lignin

There have been many studies on the application of lignin, and from the end of the nineteenth century to the present, as many as two hundred lignin products have been developed internationally [13-14], mainly for dye dispersants, concrete admixtures, water treatment agents, animal feed additives, drilling fluid diluents, tertiary oil recovery aids, and various adhesives and complexing agents [14-22]. Three basic structures, including guaiac-based structure, lilac-based structure and p-hydroxyphenyl structure, are considered to be the main ones [23-25], as shown in Figure 1.

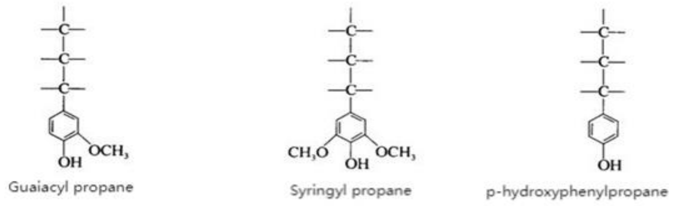

Figure 1: The three basic structures of lignin

1.3.2 Application of lignin-based in heavy metal wastewater treatment

Experiments showed that the adsorption amounts of alkaline lignin for zinc, lead, copper and cadmium ions were 7.5 mg/g, 9 mg/g, 137.14 mg/g and 87 mg/g, respectively [26-27]; the adsorption amounts of hydrolyzed lignin and lignin made from paper black liquor were 17.97 mg/g and 1.72 mg/g for lead and cadmium ions, respectively [28-29] ; lignin modified by alkaline glycerol showed adsorption of 7.5 mg/g and 9 mg/g for lead and cadmium ions, respectively; lignin extracted from straw was effective in adsorbing chromium ions in water [30]; lignin modified by methane-based thioethering was effective in adsorbing many harmful heavy metal ions from nitrates [31].

2. Preparation of nanoformulations

2.1 Main experimental apparatus and reagents

2.1.1 Main experimental apparatus

Electronic balance: FA2104B, measuring range 0~210g, d=0. 1mg, Shanghai Pingxuan Scientific Instruments Co.

Magnetic stirrer: 81-1, power 25W, 0~2000rpm, Jiangsu JintanHuangyu Scientific Instruments Co.

Ultrasonic cleaning machine: FRQ-1030XH, Hangzhou Farrant Ultrasonic Technology Co.

Field emission scanning electron microscope: SU8010, Hitachi.

ICP Inductively Coupled Plasma Emission Spectrometer: SPECTRO BLUE, SPECTRO, Germany.

Scanning electron microscope: ZEISS EVO18, Carl Zeiss, Germany.

Transmission electron microscope: HT7700, Hitachi.
UV spectrophotometer: Changzhou Fipu Experimental Instrument Factory.
Centrifuge: Liaoning Fuyi Machinery Co.

2.1.2 Experimental materials and reagents

Sodium lignin sulfonate: Hubei Xing Yinhe Chemical Co.
Zinc oxide: Tianjin Bodi Chemical Co.
Sodium tripolyphosphate (STTP): Weifang Chenyang Chemical Co.
Sodium hydroxide: Guangzhou Dixindo Scientific Instruments Co.
Hydrochloric acid: Jinzhou Chemical Hydrochloric Acid Factory.
Wheat: Purchased from Dalian Pulandian Seed Company.

2.2 Preparation and characterization of sodium lignosulfonate nanoparticles

2.2.1 Preparation of sodium lignosulfonatenanopreparations

After adjusting the pH of a certain volume of saturated solution to neutral with alkaline solution, the absorbance of the solution at different pH values (wavelength=420nm) was measured after adding buffer solution to fix the volume. According to the experiment, the absorbance measured at room temperature was A=0.302, and the absorbance was calculated to be 3.02L/g-cm.

To the flask containing 4g of sodium lignosulfonate, deionized water was added to make it fully dissolved. The product was filtered and cleaned and dried in acetone, and then fixed in a volumetric flask with a volume of 500ml and stirred for a period of time at room temperature, and the pH was adjusted to 4.5. After adding an appropriate amount of sodium tripolyphosphate (STPP) to the third group and stirring to fully dissolve, the optimum acidity was adjusted. Finally, the solution was put into the ultrasonicator and ultrasonically shaken for a period of time to obtain the desired nanoformulations.

2.2.2 Characterization of nano-sodium lignosulfonate formulations

(1) Tindal phenomenon detection

The prepared nano-sodium lignosulfonate formulations were left to stand and observed by the naked eye for the appearance of emulsion color, and then the nano formulations were irradiated with a laser pointer to observe whether the Tindal phenomenon was produced.

(2) Scanning electron microscope (SEM) detection.

The prepared sodium lignosulfonate solution was added dropwise to the aluminum sheet, dried at room temperature, sprayed with gold and observed under SEM.

(3) Transmission electron microscope detection

The prepared sodium lignosulfonatenanopreparations were diluted to five times, carefully dipped in a small copper mesh, dried to dry state at room temperature, and then put under transmission electron microscope for characterization.

2.3 Effect of sodium lignosulfonate on the growth of wheat seedlings

2.3.1 Treatment and preparation of materials

The researchers selected evenly sized wheat seeds and washed them with distilled water.The seeds were disinfected with 75% alcohol, soaked for 30s, and finally washed with distilled water for 1 to 3 times.

Three groups of 10 ml, 20 ml and 30 ml of nano and non-nano preparations of 4 mg/ml were used to adsorb 20 ml of Zn^{2+} reaction solution with a concentration of 50 mg/ml; then distilled water as well as 360 ppm of Zn^{2+} standard solution were used as controls to soak the seeds for 1 d, respectively.

2.4 Experimental method

After the seeds were treated, the seeds were rinsed with distilled water 3 to 4 times, and the residual water on the surface of the seeds was absorbed cleanly using filter paper, and two sheets of cut circular filter paper were put on the Petri dishes, and 30 seeds were put on each group of filter paper, and the filter paper was always kept moist, and the Petri dishes were put into the incubator with constant light and temperature, and three parallel experimental groups were set up for each experimental condition.

2.4.1 Index measurement and data processing

The germination rate of each group of seedlings was calculated and counted at the 7th d separately, and the average germination rate of the three parallel groups was calculated. Then the root length and seedling length of seedlings were measured at 14d, and the average growing root length and seedling length were calculated, and their dry weight and fresh weight were measured, and finally summarized for significance analysis.

(1) Measurement of seedling morphological indicators in wheat

The germination potential of seedlings was observed at 7 d, the number of germination was recorded, and the average germination rate of the three parallel groups was calculated. The root length and seedling length of wheat and mung bean seedlings in each group were measured at 14 d using vernier calipers. And the dry weight was measured separately after killing at 105°C for 15 min and drying at a constant temperature of 75°C until constant weight.

(2) Determination of chlorophyll content in wheat seedlings.

0.2g of fresh wheat leaves were weighed separately, cut with scissors and then ground with quartz sand, and then 10mL of 80% acetone was added for chlorophyll extraction. And after centrifuging the extract for five minutes, the supernatant was taken and its OD values were measured at 663 nm and 645 nm, respectively, and the measured OD values were substituted into the following equation to calculate the content of chlorophyll (mg/g Fw):

3. Analysis and discussion of the results

3.1 Morphological characterization of sodium lignosulfonatenanopreparations

3.1.1 Direct observation

It can be used as a direct judgment of the success of the nanoformulations by directly observing whether there is a milky white light or not. Further by irradiation of the laser pointer, the observation of whether it can produce Tyndall phenomenon was used as the initial detection standard, and the results are shown in Figure 2.

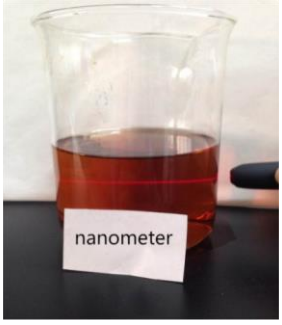

Figure 2: Butadar phenomenon of sodium lignosulfonate nanoparticles

3.1.2 Scanning electron microscope characterization

By the photos of sodium lignosulfonate under 400x optical microscope in Figure 3, and the photos under field emission electron scanning microscope in Figure 4, it shows that the original sodium lignosulfonate itself has uneven particle distribution and large particle size.

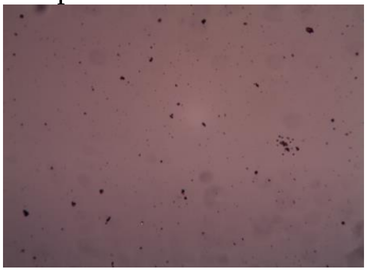

Figure 3: 400x optical microscope image of sodium lignosulfonate

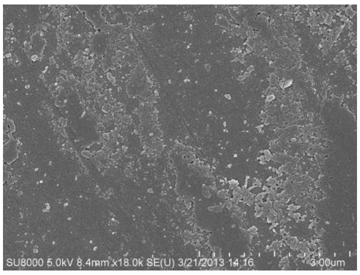

Figure 4: Image of sodium lignosulfonate under electron scanning microscope

Figure 5 and Figure 6 show the images of the nano-sodium lignosulfonate preparation under field emission electron scanning microscopy at 45kx and 200kx, respectively. The photographs show that the prepared sodium lignosulfonate reagents are regularly and uniformly distributed, with particle sizes in the range of 20 nm to 60 nm, and exhibit a distinct spherical shape.

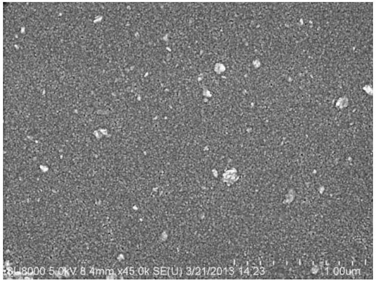

Figure 5: Image of sodium lignosulfonate nanoparticles under 45k magnification electron scanning microscope

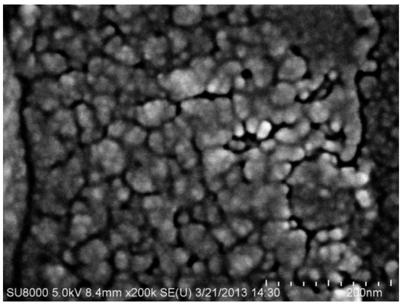

Figure 6: Image of sodium lignosulfonate nanoparticles under electron scanning microscope at 200k magnification

4. Effect of sodium lignosulfonate and its nanoformulations on morphological indicators of wheat seedlings

Figure 7 shows the growth of wheat seedlings under zinc stress with sodium lignosulfonate, from left to right: distilled water, nano-sodium lignosulfonate solution containing 30 ml of Zn^{2+} solution in 100 mg of Zn^{2+} solution, and photos of wheat seedlings under zinc ion stress with 30 ml of original sodium lignosulfonate in Zn^{2+} solution and 360 ppm Zn^{2+} solution dipped in seeds.

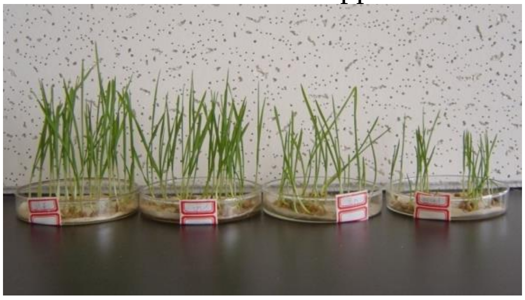

Figure 7: Effects of Zn stress on wheat seedling growth

4.1 Effects on root length, seedling height and germination rate of wheat seedlings at seedling stage

Table 1: Effect of different adsorbent addition on wheat seedlings

	Root length (cm)	Seedling height (cm)	Germination rate (%)
10mloriginal	5.69 ±0.23	5.80 ±0.21	81.5
10mlnano formulation	6.95 ±0.15	6.55 ±0.24	89.5
20mloriginal	6.25 ±0.40	6.53 ±0.33	88.5
20mlnano formulation	7.11 ±0.20	7.75 ±0.40	91.3
30mloriginal	6.67 ±0.10	6.85 ±0.10	90.5
30mlnano formulation	7.75 ±0.32	7.94 ±0.15	92.5
Distilled water	7.95 ±0.20	7.97 ±0.23	92.5
360ppmZn^{2+}	3.34 ±0.17	4.32 ±0.30	65.6

As can be seen from Table 1, after 30 ml of sodium lignosulfonatenanopreparation adsorption, the seedling growth of wheat was optimal, and the increase in root length as well as seedling height and germination rate were better than the original agent group, and when the increase was 30 ml of nanopreparation, the difference between root length and seedling height and the original agent reached and its significant level ($P<0.05$), and the effect of the nanopreparation group was better than the original agent group at the same dose.

The principle of action may be due to the best absorption effect for Zn2+ in water with the addition of 30 ml of nano-adsorbent, and the reagents obtained after nanotechnology treatment, whose particles are smaller and more conducive to adsorption, so they are all in better growth state than the non-nano reagent treatment.

4.2 Effect on fresh and dry weight of wheat seedlings

The effects of zinc stress on fresh weight and dry weight of mung bean seedlings under different amounts of adsorbent addition are shown in Figs. 8 to 9.

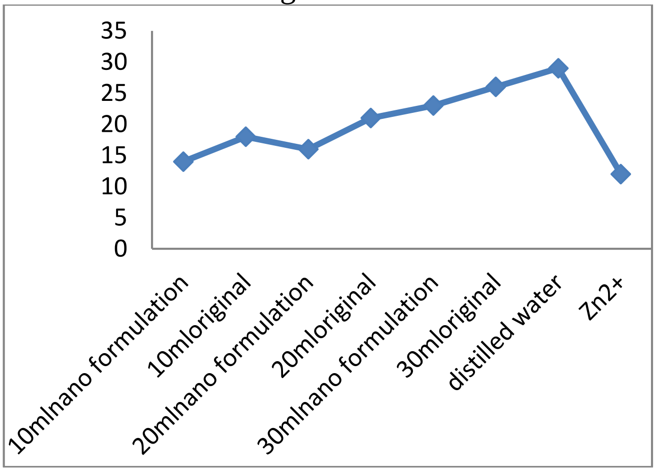

Figure 8: Effect of zinc stress on fresh weight of wheat seedlings at seedling stage

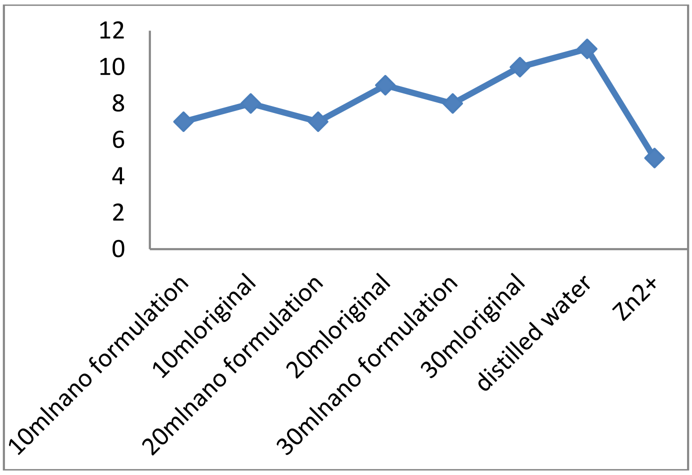

Figure 9: Effect of zinc stress on dry weight of wheat seedlings at seedling stage

From Figs. 8 and 9, it can be concluded that the trend of plant weight increase is similar to the trend of plant height increase. With the increase of sodium lignosulfonate treatment, the fresh weight of treated wheat increased, and the effect of nano group was better than the original agent group at the same dose. When the increased amount was 30 ml of nano formulation, the dry weight and fresh weight reached the maximum, and the measured results of fresh weight and dry weight were 90.3% and 95.8% of the distilled water of the control group, and 39% and 46.3% of the weight gain than the zinc ion group, which effectively alleviated the effect of zinc stress on wheat.

4.3 Effect on chlorophyll content of wheat seedling growth

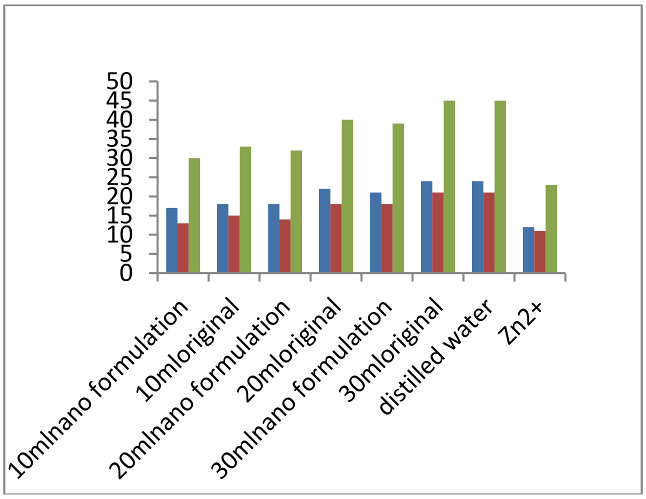

Figure 10: Effect of Zn stress on chlorophyll in wheat seedlings

According to Figure 10, it can be seen that the effect of the reaction solution of Zn^{2+} with the same adsorption content of sodium lignosulfonate original agent and nanoformulation on the chlorophyll content of wheat seeds after immersion: it can be concluded from the graph that the reaction solution after adsorption of nanoformulation has less effect on chlorophyll content of wheat than the reaction solution after adsorption of original agent. And the reaction solution with 30 ml of nanopreparation added, the chlorophyll a content was 96% of the control group of distilled water imbibed seeds, the chlorophyll b content was 99.7%, and the total chlorophyll was 97.8% of the control group, which were increased by 40%, 44.7% and 42.2% than the zinc ion group. It can be seen that the adsorption of this group is more complete and has little effect on the environment and the plant itself, which is a green organic adsorbent.

5. Conclusion

Sodium lignosulfonatenanopreparations were prepared using sodium lignosulfonate as the object of study. The prepared sodium lignosulfonatenanopreparations were characterized, and the size of the prepared nanopreparations in terms of granularity was determined by optical microscopy and electron scanning microscopy. Then the nanopreparations were used as adsorbent materials for heavy metal effluent containing Zn^{2+}, and the adsorbed reaction solution was used as seeding dip for seedling experiments on wheat to measure its physiological and biochemical indexes. The experimental results were as follows.

(1) The conditions for the preparation of sodium lignosulfonatenanopreparations were: pH neutral (7 to 8); room temperature (20 to 25 ℃); sodiumlignosulfonate concentration: 2.4 mg/ml, dispersant (STPP) concentration: 0.8 mg/ml, and the mass ratio of both was 1:3; the optimal magnetic reaction stirring and ultrasonic shaking times were derived from the experiments: 20 min and 30 min, respectively. By SEM characterization, the prepared nanoparticles showed a uniform distribution of regular spherical shape with particle size ranging from 20 nm to 60 nm.

(2) When the same content of Zn^{2+} solution was added to a volume of 30 ml of sodium nanolignosulfonate with a concentration of 4 mg/ml, the effect of Zn stress on wheat seedlings was low because the adsorption effect on Zn ions was best at this time, and the content of Zn ions in the solution was low.

In summary, the use of sodium lignosulfonatenanopreparation as adsorbent not only has a good adsorption effect on zinc ions, the adsorbed mixture solution has low zinc stress effect on the plant itself, but also sodium lignosulfonate itself has the effect of promoting plant growth, which is a more effective plant growth regulator. Therefore, sodium lignosulfonatenanopreparation, is a green, environmentally compatible, widely available, organic adsorbent.

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