Assess the influence of using treated wastewater by nano hydroxyapatite and its modification on some soil and faba bean plant properties

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Abstract: The effect of using wastewater (WW) and treated wastewater (TWW) in irrigation on some faba bean plant and soil properties was studied under pots experiment. In this study, using nano hydroxyapatite (nHP) powders, which prepared by microwave heating based wet chemical method and its modification with humic acid (nHP-HA complex) as a sorbents for wastewater treatment (WWT). The nHP and nHP-HA complex were characterized by different techniques. The treatment process was carried out by the fixed bed column technique with a flow rate (80 ml/h) to treat 11iter. Heavy metals concentrations, total organic carbon (TOC), major cations and anions were determined before and after treatment. Moreover, some factors were used to evaluate the quality of WW for irrigation purposes. The results showed that TWW by nHP-HA was more suitable for irrigation than treated with nHP. This referred to a significant reduction of TDS, inorganic and organic pollutants that caused improved in TWW chemical properties. Pots experiment was conducted to study the effect of irrigation with WW and TWW by nHP and nHP-HA complex on some soil and Faba bean plant properties. Plant and soil analysis showed significant changes in plant and soil properties due to irrigation with TWW than WW. Furthermore, plant height, dry and fresh weight, number of leaves and photosynthetic pigments were significantly affected by irrigation with WW and TWW and some soil properties such as EC were also affected.

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Key words: Wastewater, nano hydroxyapatite, Humic acid, soil, Faba bean plant.

1. Introduction

Increasing global population causes that the hole between the sources and demand for water is reaching to disturbing levels in semi-arid and arid countries (Lucia *et al.*, 2018). Therefore, many studies are working on new ways of conserving water and finding non-conventional water resources such as wastewater and drainage water for irrigation and other purposes nowadays (Jiménez, 2006).

The municipal wastewater considers an important alternative source of water for irrigation. Conversely, it can also cause real risks to public health, especially once used non -treated for crop irrigation (Meneses *et al.*, 2010). Besides, it is causing risks to plant health, soil and groundwater pollution. Therefore, we must reduce the risks of WW reuse in agriculture, in particular those to public health through properly planned and managed wastewater irrigation practices (Zavadil, 2009).

Hence, to applying the wastewater for irrigation as another source of water, it has to gain some certain criteria of the qualification parameters such as a sodium adsorption ratio (SAR), salinity (EC), soluble sodium percentage (SSP) and residual sodium carbonate (RSC), inorganic and organic pollutants (Jaishankar *et al.*, 2014) have to consider before applying of WW in agricultural. Moreover, these measures run out regular to observing and certify the functionality of the system. A variety of physical and chemical parameters should be monitored at regular intervals to verify the performance of wastewater treatment system (WHO, 2006) and the respective recommended levels of trace elements in the irrigation water was reported by USEPA (2012).

Several techniques could be used to eliminate wastewater contaminates, while adsorption technology is considered as the best technique to eliminate pollutants from effluents among these techniques due to its efficiency, simplicity and economical to operate for eliminating pollutants (El-sayed *et al.*, 2018).

Many natural and artificial materials have been used as sorbents for removing pollutants from WW, but nano structure materials consider novel materials to eliminate WW pollutants. Nano structure sorbents have exhibited much higher efficiency and faster rates in water treatment due to their large surface area and high reactive capacities. Nano hydroxyapatite is a natural mineral and an example of ideal sorbent material because of its high sorption capacity for organic and inorganic pollutants (Mobasherpour *et al.*, 2011; Lyczko *et al.*, 2014), accessibility and high stability (Krestou *et al.*, 2004).

Humic acid can adsorb onto hydroxyapatite surfaces, modifying the physicochemical properties and enhancing the adsorption capacities of the nHP. Where, HA is one of the most important component of soil organic matter, and generally hold both hydrophobic and hydrophilic moieties as well as many functional groups (-COOH, -C=O, and -OH) bonded to the aliphatic or aromatic carbons in the macromolecules. Thus, HA can react with inorganic and organic pollutes wherever the hydrophobic fraction of HA interacts with organic contaminates by hydrophobic-hydrophobic interaction mechanism while carboxylic and hydroxyl group interact with heavy metals by electrostatic and complexation mechanisms (Peng *et al.*, 2012; Zhang *et al.*, 2013).

Accordingly, the objective of this study is to evaluate the quality of WW and TWW and investigate the effect of using WW and TWW in irrigation on some soil and Faba bean plant properties.

2. Materials and Methods Materials

Collecting wastewater samples

The municipal WW samples were collected in plastic bottles from Sohag (Eldare area), Egypt. Complete chemical analysis of wastewater: TOC, heavy metals, EC, pH, major cations, major anions and microbial existence community before and after treatment step were determined as described by Fishman and Friedman (1989). The microbial species including fungi and bacteria were isolated following single-spore isolation techniques as described by Moubasher (1993) and Bauer *et al.* (1969). The isolated species were purified and morphologically identified following different identification keys.

Nano hydroxyapatite

The nHP powders were synthesized as described later in the method section from high purity Calcium Chloride 2-hydrate CaCl₂.2H₂O (99%) and orthophosphoric acid H₃PO₄ (85%).

2.1.3. Humic acid

Humic acid in its potassium salt was obtained from Sigma-Aldrich Co., Germany.

Soil Samples

Soil samples were taken from the surface layer (0-30 cm) for pot experiment of an experimental farm, at the Shandwell Agricultural Research station, Sohag Governorate, Egypt.

Seeds

Faba bean (Balady) seeds (Giza 843) were used in the experiment. These seeds were obtained from the Ministry of Agriculture, Egypt.

Methods

The nHP Synthesis

The nHP powder was produced according to the wet chemical method with the use of precipitation from the aqueous medium through a titration process by reacting calcium chloride 2-hydrate CaCl₂.2H₂O (99%) and orthophosphoric acid H₃PO₄ (85%), as the main reactant materials in accordance to the molar

ratio of Ca/P = 1.67 as described elsewhere (Puvvada *et al.*, 2010; El-Sayed *et al.*, 2018).

Preparation of nHP- HA complex

The nHP-HA were carried out by mixing 25 gm of nHP and 0.25 g/L of HA solution (at pH 6.0 and ionic strength, 0.001 mol dm⁻³ KCl and the total volume was 1 liter) in an orbital shaker at 5000 rpm for 1 day (El-Sayed *et al.*, 2018). The nHP and nHP-HA were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and surface area (S_{BET}).

Wastewater treatment

The wastewater treatment by using a fixed bed column model through the adsorption process was carried out with nHP and nHP-HA complex (Karunarathne and Amarasinghe, 2013). Column studies are carried out in the column within 3 cm diameter and 30 cm length with the rate flow 80ml/h. Adsorbents are loaded on the column over glass wool layer. The purpose of the glass wool was to support the adsorbents. The adsorbents and wastewater solution are added from the top of the column. The rate of adsorption process was detected every day. TWW was collected and stored for complete analysis. The statistical analysis system (f test) has been used to analysis the results and determine the least significant difference (LSD 0.05).

Pots experiment

A pot experiment was done to explore the impact of WW and TWW on some soil and plant properties. An experiment was done during the winter seasons of 2016 and 2017 at the experimental station of Agriculture Research Centre, Sohag Governorate, Egypt, to investigate the effect of using WW and TWW in irrigation on the soil's chemical and physical properties and the vegetative growth of Faba bean plant after 60 days. Pots are loaded with 10 Kg of soil sample. Then, the seeds were cultivated in pots and were irrigated during planting with three types of wastewater: WW and TWW by nHP, and nHP-HA complex from stock TWW solutions. Throughout the vegetative growth stage (growing stage was 60 days), three random plants were taken after the experiment end from each pot per treatment for determination of plant height (cm), number of leaves per plant, fresh and dry weight per plant (g), leaf area per plant (cm^2) according to Ghoneim and El-Araby (2003). At harvest, the weight of 100 seeds and seeds yield were calculated. The data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) and means were separated using LSD 0.05.

Soil analysis

Some soil properties such as: mechanical analysis, bulk density, pH value, calcium carbonate content, organic matter (OM), EC, soluble cations and

anions were estimated before and after the experiment (Carter and Gregorich 2008).

3. Results and discussion Characterization of nHP and nHP-HA complex

The XRD patterns of the prepared nHP and nHP-HA complex are presented in fig. 1, the prepared nHP were in good agreement with the reference pattern of the pure nHP (Seaf Elnasr *et al.*, 2017) while slightly change in nHP-HA complex XRD chart at 30 and 70 in due to HA adsorption (Chilom and Rice, 2005). The average particle size (D) of nHP and nHP-HA complex were estimated using Debye Scherer equation (Seaf Elnasr *et al.*, 2017).

 $D = \frac{K \lambda}{\beta \cos \theta}$

(20°)

Where λ, β , and θ is X-ray wavelength light, the complete width of the half maximum of diffraction peak, and the diffraction angle respectively. K is a shape factor and usually taken as 0.89. It was found that the particle sizes of nHP and nHP-HA complex were on the nanoscale about 35 and 40 nm, respectively.

The morphology and particle size of the nHP and nHP-HA complex were tested by TEM. The nHP and nHP-HA complex particles were a short rod-like crystal structure. The crystallite size of nHP and nHP-HA complex particles obtained from the Debye-Scherrer equation and those obtained from TEM images were on the nanoscale. Moreover, the TEM images also showed HA adsorption on the surface of the nHP and slightly increasing in nHP-HA complex particle size.

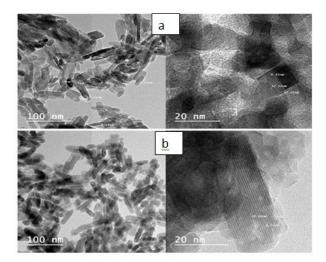


Fig. 1 The XRD patterns and TEM images of (a) nHP and (b) nHP-HA complex.

The surface area of nHP and nHP-HA complex were 60.618 and 78.165 m²/g, respectively, where humic acid enhanced the surface area of nHP (El-Sayed *et al.*, 2017). The average pore diameter of nHP and nHP-HA complex were determined to be 15.164 and 14.454 nm respectively, suggesting that nHP and nHP-HA complex correspond to a mesoporous material. The modification process of nHP with HA was highly significant due to t-test statistical results on surface area and average pore diameter. The values of probability (p) at t_{0.05} were 0.0074 and 0.01 for surface area and the average pore diameter respectively.

Wastewater treatment

The wastewater treatment process was done by adsorption onto two sorbents "nHP and nHP-HA complex " by using a fixed bed column model with rate flow 80 ml/h (El-Sayed *et al.*, 2018). The results in table 1 evident that nHP-HA complex was much a

better than nHP in WWT from heavy metals and organic contaminants. The f-test results showed that the treatment process by the nHP and nHP-HA complex had a significant effect on all heavy metals concentration and TOC of wastewater except on Cr^{6+} . Furthermore, treatment by nHP-HA complex had a significant effect on Se⁴⁺, B³⁺, Co²⁺, Mn²⁺, Cu²⁺ and Fe²⁺ than nHP due to LSD results (C.f. table 1). This refers to highly active site contents, function groups and BET surface area of nHP-HA complex rather than nHP.

The results in table 2 showed that nHP-HA complex enhanced the chemical analysis (EC, pH, TDC, major cations, and major anions) of wastewater rather than of nHP. Additionally, f-test results showed that the treatment process by the nHP and nHP-HA complex had a significant effect on the chemical analysis of wastewater except had an insignificant

effect on Ca^{+2} and SAR. This insignificant effect refers to the composition of nHP with calcium. Likewise, the WWT by nHP-HA complex had a significant effect on some chemical analysis than nHP due to LSD values (El-Sayed *et al.*, 2018).

Microbial test

Pathogen analysis was determined for wastewater before treatment. The results showed that

there were one type of fungi species under genus of aspergillas sp and two bacteria types of Bacilli species (gram -ve and gram +ve). Therefore, drops of chloride were added to the WW before treatment. Pathogen examination was repeated after treatment and the results indicated that TWW came to be free from both fungi and bacteria species.

	Cd ²⁺	Fe ²⁺	Zn ²⁺	Cu ²⁺	Mn ²⁺	Co ²⁺	Cr ⁶⁺	Pb ²⁺	B ³⁺	Se ⁴⁺	TOC
Samples	ppm	ppm	%								
Non - TWW	0.019	0.965	0.109	0.075	0.082	0.179	0.049	0.054	0.132	4.030	0.40
TWW by nHP	0.00	0.352	0.000	0.049	0.019	0.047	0.000	0.000	0.053	0.294	0.01
TWW by											
nHP-HA complex	0.00	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.022	0.00
LSD _{0.05}	0.0014	0.0377	0.0057	0.0064	0.0058	0.0118	0.1133	0.0028	0.0192	0.2297	0.0288

Table 1: Heavy metals concentrations and TOC of non -TWW and TWW.

Tuble 2. Major cations and amons concentrations of non-1 vv vv and 1 vv vv.												
Samples	ոս	EC	EC TDS		Na ⁺	\mathbf{K}^{+}	Ca ²⁺	Mg ²⁺	Cľ	SO_4^{2-}	NO ₃	HCO ₃ ⁻
Samples pH		ds/m	mg/l	SAR	meq/l	meq/l	meq/l	meq/L	meq/l	meq/l	meq/l	meq/l
Non -TWW	8.58	0.95	0.61*10 ³	2.48	4.08	0.40	1.61	3.80	4.60	1.77	0.49	2.48
TWW by nHP	8.01	0.81	0.52*10 ³	2.26	3.36	0.32	1.23	3.20	4.11	1.70	0.30	2.00
TWW by nHP-	7.62	0.74	$0.48*10^{3}$	1.09	2.80	0.27	1.00	3.00	3.80	1.57	0.10	1.60
HA complex	7.62	0.74	0.48*10	1.98	2.60	0.27	1.00	5.00	5.60	1.57	0.10	1.00
LSD _{0.05}	0.2088	0.1073	16.9687	3.22	0.2336	0.0860	1.675	0.2007	0.3324	0.1696	0.0702	0.1945

Evaluation of WW and TWW quality for irrigation

Some parameters such as SAR, EC, SSP and RSC presented in table 3 had been used to evaluate the suitability and the possibility of TWW for irrigation purposes.

Sodium adsorption ratio (SAR)

The sodium concentration of WW is causing a serious problem when using WW in irrigation because of its impact on soil permeability and water infiltration at high sodium concentration. The SAR is an important parameter to assess the sodium effect which calculated by the following formula (El-Sayed *et al.*, 2017)

$$SAR = \frac{Na^{+}}{\sqrt{(Ca^{2+} + Mg^{2+})/2}}$$

Where Na^+ , Mg^{2+} and Ca^{2+} are in meq/l.

The SAR results in table 3 indicated that the WW and TWW from the study area were excellent water class (S1) in water classification according to the SAR and may be used in irrigation for nearly all soils, with little hazard of the development of harmful levels of exchangeable sodium (US Salinity Laboratory, 1954). Salinity (EC)

Wastewater salinity is one of the most brutal environmental factors and has a dual effect on plant and soil properties lead to decreasing the crop productivity as well as increasing soil salinity. The EC results of wastewater, WW and TWW by nHP and nHP-HA complex are presented in table 3. The WW and TWW by nHP samples lie in the class of C3 in the table of the irrigation water classification according to salinity indicating high salinity water, which can be used for soil with restricted drainage and plants with good salt tolerance should be selected. While TWW by nHP-HA complex EC value lies in the class of C2 according to salinity indicating medium salinity water, that may be used after adding the amount of leaching and some plants can be grown without special practices for salinity control (US Salinity Laboratory, 1954).

The Soluble Sodium Percentage (SSP)

The SSP was estimated by equation (Alobaidy *et al.*, 2010):

$$SSP = \frac{(Na^{+}) \times 100}{Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}}$$

Where all the ions are expressed in meq/L.

The SSP also used to evaluate the sodium hazard of irrigation water like SAR, but it expresses the

percentage of sodium out of the total cations and not as SAR correlating the sodium with the Ca^{2+} and Mg^{2+} only. The calculated SSP values of WW and TWW by nHP (c.f. table 3) indicated that this water is permissible class in table of classification of irrigation water based on SSP while the calculated SSP value in case of TWW by nHP-HA complex indicated that this water is good class for irrigation in the same classification (Todd, 1980).

The residual sodium carbonate (RSC)

The RSC was calculated by the following equations (Ghoneim and EL-Araby, 2003):

$$RSC = (CO_3^{2-} + HCO_3^{-}) - (Ca^{2+} + Mg^{2+})$$

The high RSC means that high pH value of water. The results showed (c.f. table 3) that all samples have RSC less than zero and are more suitable for irrigation purposes according to the USSL (US Salinity Laboratory, 1954).

Thus, non TWW and TWW by nHP are permissible for irrigation while TWW by nHP-HA complex is good class for irrigation.

Sample	SAR	EC (ds/m)	SSP	RSC (meq/l)
WW	2.48	0.95	41.4	< 0
TWW by nHP	2.26	0.81	41.3	< 0
TWW by nHP-HA complex	1.98	0.74	39.6	< 0

Table 3: The SAR, EC, SSP and RSC values of WW and TWW.

The effect of using WW and TWW in irrigation

In order to study the effect of WW and TWW on some plant and soil properties, Faba bean seeds were sown in pots and were irrigated by different irrigation sources of WW and TWW by the nHP and nHP-HA complex. Pots experiment was completely random and in three replicated.

Effect of irrigation with WW and TWW on some properties of soil samples

The texture of a soil under investigation was Clay loam (38.9% clay, 35.8% silt, and 25.3% sand) and CaCO₃ content was 3.36%. Data in table 4 represented some properties of the soil before and after irrigation by WW and TWW. The results indicated that slightly change in OM contents, Bulk density, and SP of soil under different irrigation sources with little increasing after using non TWW than TWW. Although, these results refer to the EC values and OM contents of WW and TWW as described before in table 2 are not high enough to change these soil properties.

However, some chemical properties of soil such as values of pH, EC (ds/m), cations (meq/L): Ca²⁺,

 Mg^{2+} , Na⁺, K⁺ and anions (meq/L): CO_3^{2-} , HCO_3^{-} , Cl⁻, SO_4^{2-} of the soil before and after irrigation with WW and TWW. The results explained that chemical properties of the soil samples under investigation affected by irrigation with WW and TWW.

The soil EC had significantly increased by using WW as compared to using TWW in irrigation of soil under investigation. This is may be referred to the alkaline nature of WW (Alghobar et al., 2014). The values of EC, major cations and major anions increased after irrigation with WW while the EC value decreased after irrigation with TWW and become much better after irrigation with TWW by nHP-HA complex (Zein et al., 2003). Soil pH and EC consider important factors that affect nutrient availability for plant roots. Likewise, the results showed that major cations and anions of the soil decreased after irrigation by TWW by nHP-HA complex than nHP. Consequently, some soil properties became much better after irrigation with TWW by nHP-HA complex than nHP. On the other hand, the irrigation with WW led to increase the salinity and major cations and anions of soil.

Soil Somula	pH SP		OM%	Bulk density		Cations me/L				Anions me/L			
Soil Sample	1:2.5	mL/100g	UM %	g/cm ³	ds/m	Ca ²⁺	Mg ²⁺	Na^+	K ⁺	CO_{3}^{2}	HCO ₃	Cl	SO_4^{2-}
Blank before planting	7.90	56	0.62	1.39	0.9	2.7	2.9	3.1	1	-	5.2	3	1.21
Control	7.90	56	0.56	1.39	0.80	2.4	2.5	2.7	0.7	-	4.2	3.4	0.85
WW	8.00	57	0.7	1.38	1.1	3	3.3	3.6	0.8	-	5.3	4.5	1.3
TWW by nHP	7.90	55	0.52	1.40	0.9	2.7	2.9	3.0	0.9	-	4.1	3.9	1
TWW by nHP-HA complex	7.90	55	0.53	1.40	0.85	2.5	2.55	2.8	0.75	-	3.9	3.3	0.8

Table 4: Some properties of soil samples under study

Control = normal water

Effect of irrigation with WW and TWW on Faba bean properties (vegetative growth characters)

Some Faba bean plant properties were represented in table 5 after irrigation with WW and

TWW. Plant height, plant fresh and dry weight, number of leaves per plant, photosynthetic pigments (Chlorophyll a, Chlorophyll b and Carotenoids) were significantly affected, while No. of branches, leaf width, root zone and leaf area were insignificantly affected after irrigation with WW and TWW due to LSD values.

It is obvious that using TWW by nHP-HA complex in irrigation provided the highest values of all studied characters and more closer to using normal water followed by using TWW by nHP then non - TWW. The significant reduction in some plant vegetative growth characters under irrigation with non TWW refers to EC values of non -TWW and excessive concentration of major ions such as: Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and HCO₃⁻. Furthermore, plant nutrient availability affected by soil and irrigation water pH (Al-Tahir and Al-Abdulsalam, 1997). From table 2, the WW pH was 8.58 while pH value was 8.01

and 7.62 of TWW by nHP and nHP-HA complex, respectively. Therefore, the nutrients availability and plant growth were affected under irrigation with non - TWW than TWW by nHP and nHP-HA complex. Moreover, the toxicity effect of heavy metals also had a significant effect on plant properties, where the heavy metals concentrations were highly reduced after the treatment process especially with nHP-HA complex. Furthermore, decreasing in photosynthetic pigments refers to WW contaminants.

Data in table 6 explained that seeds yield and weight of 100 seeds were affected by irrigation source due to LSD value and significant change in some plant properties such as plant height, fresh and dry weight and photosynthetic pigments.

Consequently, the treatment of wastewater by adsorption onto nHP-HA complex in the fixed bed column model is very economical, simple and safe method to treatment WW for irrigation purpose.

Table 5. Means of	vegetative growth	characters of Faha l	hean nlant after 60 d	days (Means of two seasons).
Table 5. Means of	vegetative growth	Characters of Faba	Jean plant after 00 t	lays (micans of two seasons).

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Water Source	Plant height (m)	Dry weight (g)	Fresh weight (g)	No. of leaves	No. of Branches	Leaf width (m)	Root zone (m)	Average leaf area of pot (m ²)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)
Control	0.64	49.2	142.4	6	4.2	0.052	0.054	0.06	22.1	39.00	8.82
WW	0.42	24.4	74.8	4	3	0.03	0.043	0.04	10.1	19.2	2.98
TWW by nHP	0.48	32.5	101.5	5	3.3	0.04	0.045	0.05	17.1	29.3	4.83
TWW by nHP-HA complex	0.54	47.4	131.4	6	4.1	0.051	0.053	0.06	21.9	38.31	8.678
LSD _{0.05}	0.011	0.100	0.54	0.01	ns	ns	ns	ns	0.251	0.378	1.62

Control = normal water

Table 6: Faba bean	yield under different water irrigate sources

Water Source	Seeds yield Per plant (g)	The weight of 100 seeds (g)	Seeds yield (kg/fed)
Control	49.7	43.6	405.2
WW	36.88	34.7	310.22
TWW by nHP	42.78	38.90	358.93
TWW by nHP-HA complex	47.6	40.8	395.2
LSD _{0.05}	0.100	0.556	0.528

4. Conclusion

In this paper, nHP and nHP-HA complex have been used as sorbents in WWT. The nHP was modified by HA, which improve nHP surface properties due to HA structure and its functional groups (hydroxyl and carboxyl groups). Moreover, the results indicated that nHP-HA complex. The results explained that WWT by nHP-HA complex was better than nHP and had more significant effect on heavy metals, TOC and major cations and anions.

Some parameters such as: SAR, EC, SSP, and RSC were used to assess the suitability of water for irrigation purposes. Also, the results showed that TWW by nHP-HA complex is good for irrigation. While non -TWW and TWW by nHP are permissible for irrigation, but may be causes problems for soil and plant on long term. Furthermore, the result showed that using TWW by nHP-HA complex in irrigation has better on some soil and plant properties. Hence, WWT by nHP-HA complex considers safe, simple and economical method for WWT and to reuse it again in agriculture with significant effect on plant and soil properties.

Conflict of interest:

No potential conflict of interest was reported by the author

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