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Microbiological composition of sludge generated in water treatment plants

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ABSTRACT

Studies that focus on the microbiological composition of water treatment plants (WTP) sludge, as well as its pathogenicity, are extremely necessary, especially with regard to environmental regulations, where the microbiological characterization of WTP waste can encourage new policies related to its management. In Brazil, few studies address WTP sludge, which, in general, is classified as non-hazardous and non-inert waste, with the microbiological characterization of this material being little explored. This case study performed the microbiological characterization of sludge samples from two WTPs located in the state of São Paulo, before and after the centrifugation process. The determination of microbial density and morphology, Gram staining, and the identification of the presence of total and thermotolerant coliforms were performed with samples produced in two different years, in WTPs that used different coagulants (liquid aluminum sulfate or polyaluminum chloride and ferric chloride). Results were evaluated along with the physicochemical analysis of the composition of this waste. The presence of microalgae and protozoa in non-centrifuged WTP sludge and the presence of total and thermotolerant coliforms in WTP sludge before and after centrifugation are among the main results of this study.

Keywords: beneficial use, microbiological composition, water treatment plant sludge.

Composição microbiológica do lodo gerado em estações de tratamento de água

RESUMO

Estudos que tenham como foco a composição microbiológica do lodo de Estação de Tratamento de Água (ETA), bem como sua patogenicidade, são extremamente necessários, principalmente em relação às regulamentações ambientais, onde a caracterização microbiológica dos resíduos de ETAs podem incentivar novas políticas relacionadas ao seu gerenciamento. No Brasil, poucos estudos abordam o lodo de ETA, que, em geral, é classificado como resíduo não perigoso e não inerte, sendo pouco explorada a caracterização microbiológica desse material. Nesse sentido, este estudo teve como objetivo, realizar a caracterização microbiológica de amostras de lodo de duas ETAs localizadas no estado de São Paulo, antes e após o processo de centrifugação. A determinação da densidade e morfologia microbiana, coloração de Gram e a identificação da presença de coliformes totais e termotolerantes foram realizadas com amostras produzidas em dois anos distintos, em ETAs que utilizaram diferentes coagulantes (cloreto férrico e sulfato e alumínio). Os resultados foram avaliados juntamente com a análise físico-química da composição deste resíduo. A presença de microalgas e protozoários no lodo não centrifugado da ETA e a presença de coliformes totais e termotolerantes no lodo da ETA antes e após a centrifugação estão entre os principais resultados deste estudo.

Palavras-chave: composição microbiológica, lodo de estação de tratamento de água, uso benéfico.

1. INTRODUCTION

Access to a safe water supply is a fundamental right of citizens and essential to ensure adequate health and housing conditions, since water can directly or indirectly transmit diseases to a large number of people due to lack of hygiene, contact with polluted water, or to the presence of pathogenic organisms (Brasil, 2019; Howe *et al.*, 2017; Heller and Pádua, 2010) . In this sense, water treatment plants (WTPs) are necessary, as they guarantee the production of water in accordance with the quality standards required for human consumption in different territories (Brasil, 2021; Who, 2017).

The water treatment process in WTPs involves a combination of different technologies selected according to the characteristics of the raw water. Among the treatments used in Brazil, the most common is the conventional treatment, which includes the processes of coagulation, flocculation, decantation, filtration, and disinfection, and requires the addition of chemical products (IBGE, 2020; Howe *et al.*, 2017). This treatment produces waste, which is called WTP sludge. Its composition includes solid, organic, and inorganic substances from raw water (for example, bacteria, viruses, algae, organic particles in suspension, colloids, clay, silt, sand, iron, calcium, magnesium, and manganese), as well as aluminum hydroxides from the addition of chemical products and conditioning polymers used in the treatment (Grandin *et al.*, 1993; Marques *et al.*, 2000). In general, WTP sludge has different characteristics and properties, depending essentially on the conditions of the raw water, dosage of chemical products, and technology used in the treatment (Achon *et al.*, 2013).

In Brazil, WTP sludge is classified as solid waste and commonly classified as Class II A waste (non-inert and non-hazardous), so the release *in natura* into surface waters and soil is prohibited (Brasil, 2010; IBAMA, 2012; ABNT, 2004a). This waste must be treated and disposed of within the legal criteria established in the country, but the steps to treat this sludge usually involve high costs. Thus, in most cases, WTP sludge is released into water bodies without treatment, causing significant environmental impacts (Achon *et al.*, 2008; Brasil, 2010).

The search for economically viable and environmentally advantageous alternatives for the final disposal of WTP sludge and its use is still a global challenge. Disposal in landfills, use in composting, manufacture of materials in civil construction, and composition of some types of pavement are among the alternative methods (Richter, 2001; Di Bernardo *et al.*, 2012, Da Silva *et al.*, 2017; Alves and Marques, 2021).

WTP sludge is a heterogeneous by-product and its composition varies according to the raw water quality, the applied water treatment and the chemicals used (Bernegossi *et al.*, 2022).

With regard to raw water, it can be influenced by increased urbanization, untreated wastewater discharges (eg domestic and industrial), soil pollution, deforestation, agriculture, among other changes in land use (Boretti and Rosa, 2019; Bernegossi *et al.*, 2022). All over the world, the surface raw water purification processes use chemical coagulants to improve solid-liquid separation, where Al and Fe salts are added mainly as coagulants during the treatment process to remove colloidal and suspended impurities. In this way, these impurities together with the coagulant products also constitute the waste generated in the WTPs (Ahmad *et al.*, 2017; Nayeri and Mousavi, 2022).

To enable the safe handling and use of WTP sludge, assessing the presence of microorganisms in its composition and the possible associated risks is essential (Acquolini, 2017; Zhao *et al.*, 2019; Kristanti *et al.*, 2022). Water treatment plants are an unexpected source of biodiversity in terms of environmental microorganisms and their interactions at the community level are still poorly understood, where the heterogeneous and seasonal composition of water treatment plant (WTP) sludge must be specifically assessed for each water treatment plant (Bruno *et al.*, 2017; Rodgher *et al.*, 2023).

In this sense, studies that focus on the microbiological composition of WTP sludge, as well as its pathogenicity, are extremely necessary, especially related to environmental regulations, since the microbiological characterization of waste from WTPs can encourage new policies related to their management, where the absence of WTS-specific guidelines currently allows reuse of the WTS without any restrictions (Ahmad *et al.*, 2017; Giglio and Sabogal-Paz, 2018; Bernegossi *et al.*, 2022). Several studies address the microbiological diversity present in WTP sludge, showing the importance of microbiological characterization, especially related to close contact with workers, which can represent a risk of infection (Makovcova *et al.*, 2015; Giglio and Sabogal-Paz , 2018; Xu *et al.*, 2018; Ullmann *et al.*, 2019; Ranković *et al.*, 2020;Wang *et al.*, 2021; Bernegossi *et al.*, 2022).

Thus, this study presents the microbiological composition of sludge produced in two WTPs in the state of São Paulo, before and after the centrifugation process, showing the human and environmental risks of the handling, recycling, and final disposal of the sludge, depending on of its physicochemical and biological compositions and the need for evolution of Brazilian regulations to enable the beneficial use of this waste.

2. METHODOLOGY

2.1. Contextualization

In this study, the sludge produced in two WTPs located in the state of São Paulo, Brazil (WTP 1 and WTP 2), which perform surface water capture and make the water potable to meet the Brazilian quality standard (Brasil, 2021), was evaluated using treatments with the stages of coagulation, flocculation, decantation, filtration, and disinfection (pre- and post-treatment), fluoridation, and pH correction. These stages produce about 20 m^3/s of potable water, which is used for public supply. During the study period, aluminum coagulants: liquid aluminum sulfate ($Al_2(SO_4)$) or polyaluminium chloride ($Al_2(OH)_5Cl$) were used in WTP 1 and ferric chloride ($FeCl_3$) in WTP 2. In these WTPs, the sludge produced in the decantation stage was centrifuged with the help of Polyacrylamide polymers (anionic for WTP1 and cationic for WTP2) and, after adjusting the moisture content, it is sent for final disposal in licensed landfills.

2.2. Sample collection and preparation

Two sludge samples were collected, before and after the centrifugation process in WTP 1 and 2 (Fiore *et al.*, 2020). The first sampling took place in October 2018 and the second in August 2019. Considering data from the historical series of rainfall in the city of São Paulo, collected at the National Institute at the meteorological stations close to the WTP studied (83856 and 83781), the samples were collected in periods of greater rainfall in 2018 and in the dry period of 2019. Considering these data, this study was carried out with sludge samples that represent the rainy and dry periods, respectively.

For the microbiological characterization of the sludge and identification of filamentous organisms, the collections were performed according to the Brazilian guidelines for sample collection and preservation (CETESB and ANA, 2011). On these same dates, centrifuged samples were also collected for the physicochemical characterization of the sludge, according to Method 1060 described in Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 1998).

2.3. Identification of filamentous organisms

The identification of filamentous organisms was the identification of phytoplankton and protozooplankton communities, which was observed by an optical microscopy (Zeiss Microscope, Germany) with 400× magnification, in quintuplicate. Samples were analyzed within a maximum period of three hours, in order to ensure the vitality of the entire microbial community (CETESB and ANA, 2011). Specialized bibliographies for the identification of phytoplankton (Komárek and Foot 1983; Round *et al.*, 1990; Hoek *et al.*, 1995; Bicudo and Menezes, 2006) and protozooplankton organisms (Pennak, 1953; Lee *et al.*, 1985; Foissner and Berger, 1996; Ruppter *et al.*, 2005) were used.

2.4. Microbiological characterization

For the microbiological characterization of sludge, microbial density and morphology (Hermoso *et al.*, 2006), Gram staining, and colorimetric alterations presumptive of the presence of total and thermotolerant coliforms were analyzed, besides the counting of colonies of enterobacteria suggestive of *Escherichia coli* (Morris *et al.*, 1987; Meherdad *et al.*, 2014; BD, 2014). To identify the presence of coliforms and *E. coli*, sludge samples were filtered by sterilized overlapping meshes with a minimum diameter of 0.7 mm to remove suspended solids with larger dimensions. For the centrifuged samples, preliminary procedures of resuspension, concentration, and filtration were performed. The centrifuged sludge samples were resuspended in sterile saline solution before filtration. The counting of colonies suggestive of *E. coli* was performed on diluted sludge samples (10^{-1}–10^{-6}) in sterile saline solution (0.9% NaCl).

To determine the main microbial morphology of sludge samples, microscopic analyses were performed, in quintuplicate, at 1,000× magnification/oil immersion (Zeiss Microscope, Germany). A total of 10 fields were observed in each slide. To identify the presence of total coliforms and *E. coli*, chromogenic (ONPG)/fluorogenic (MUG) (Colilert®) substrates were used. Moreover, 0.1-ml aliquots of the suspensions were plated on MacConkey agar to quantify colonies suggestive of *E. coli.* After incubation under aerobic conditions for 24 hours, at a temperature of 37ºC, tests were read. These tests were performed in duplicate.

2.5. Physicochemical characterization

In Fiore *et al.* (2020), the analytical methods used to characterize the organic and inorganic parameters of the sludge samples and the physical-chemical data for the gross mass, leachate and solubilized extract are presented. The analyses were carried out by different laboratories accredited by the National Institute of Metrology, Standardization and Industrial Quality, in accordance with standards 10004, 1005 and 10006 of the Brazilian Association of Technical Standards (ABNT, 2004a; 2004b; 2004c). Average data on the sludge composition for each WTP were compared with the Brazilian reference values that classify solid waste in terms of risks to the environment and human health NBR 10.004 (ABNT, 2004a) and with studies with sludge containing aluminum and iron species.

3. RESULTS AND DISCUSSION

Tables 1 and 2 present the results of the characterization of algal and protozoan communities, respectively, in the non-centrifuged (NC) WTP sludge sample. For WTP Sludge 1 (NC/2018), the qualitative analysis showed five taxa for microalgae distributed in the classes Chlorophyceae (four) and Bacillariophyceae (one). For WTP Sludge 2 (NC/2019), the qualitative analysis showed three taxa of microalgae distributed in the classes Chlorophyceae (two) and Bacillariophyceae (one). Regarding protozoa, in WTP Sludge 1, we found taxa distributed in the phyla Alveolata and Amoebozoa, four taxa in October 2018 and two taxa in August 2019. In October 2018, the microscopic analyses of WTP Sludge 2 (before and after the centrifugation process) did not show representatives of the communities. In August 2019, we found two taxa of microalgae of the class Chlorophyceae and one taxon of the phylum Alveolata, protozoa group, in WTP Sludge 2.

Table 1. Representatives of the microalgae group found in WTP sludges samples (non centrifuged samples).

WTP	Month	Division	Class	Genus
1	October 2018	Chlorophyta	Chlorophyceae	*Pediastrum* sp.
				Dictyosphaerium sp.
				Desmodesmus sp.
				Scenedesmus sp.
		Heterokonthophyta	Bacillariophyceae	*Aulacoseira* sp.
	August 2019	Chlorophyta	Chlorophyceae	*Pediastrum* sp.
				Binuclearia sp.
		Charophyta	Zygnematophyceae	*Staurastrum* sp.
2	August 2019	Chlorophyta	Chlorophyceae	*Pediastrum* sp.
				Microspora sp.

Table 2. Representatives of the protozoa group found in WTP sludges samples (non centrifuged samples).

WTP	Month	Phylum	Subphylum	Genus
1	October 2018	Alveolata	Dinoflagellata	*Glenodinium* sp.
				Ceratium sp.
			Ciliophora	*Euplotes* sp.
		Amoebozoa		*Arcella* sp.
	August 2019	Alveolata	Dinoflagellata	*Ceratium* sp.
		Amoebozoa		*Arcella* sp.
2	August 2019	Alveolata	Dinoflagellata	*Ceratium* sp.

The phytoplankton and zooplankton communities tend to respond to the limnological and climatic variables, such as pluviosity, winds, water temperature, nutrients concentration, water transparency, among others (Tundisi and Tundisi 2008; Esteves, 2011). In present study, the seasonal response of microalgae and protozoan groups could be related to changes in composition of WTP sludge. Low temperature in the dry season (August 2018) became an important factor reducing primary production and planktonic diversity (Zhao *et al.* 2019) which will lead to a change in the community structure (Pulsifer and Laws, 2021).

In the months analyzed, we found no microorganism of the microalgae and protozoan communities in the centrifuged WTP samples. According to Reali (1999), the centrifuge dewatering process has a principle of phase separation very similar to what occurs with gravity-settled particles, but with a force intensity hundreds or thousands of times greater than that of gravity. In this way, during centrifugation, microalgae and protozoa can be carried away with the water, not adhering to the flakes. Another possible cause may also be associated with the fact that centrifuged samples become dry and microorganisms may lose viability more quickly, making it impossible to identify them.

It should be noted that in WTP 1 and 2 studied, the sludge from the filter washing decanters is destined for reserve tanks. In these tanks the WTP sludge arrive with about 2 to 5% solids content. In these tanks, polymers are incorporated that will contribute to the sequential dewatering process, which is carried out using centrifuges. After dewatering, the solids content of the WTP sludge is raised to about 18 to 22%. At WTP 1, the WTP sludge still undergoes new drying processes inside the unit, before being sent to landfills. In WTP 2, after centrifugation, the WTP sludge are sent for final disposal.

Table 3 presents the results of the morphological characterization and Gram staining, as well as the quantification of total and thermotolerant coliforms (*E. coli*). In the 2018 WTP Sludge 1, microorganisms existed in all analyzed fields. In most of them, we found Gram-positive bacilli in both the non-centrifuged and centrifuged samples. In the 2019 sample, the density of microorganisms was low in both samples. By the Colilert™ test, we found total coliforms and *E. coli* in both 2018 samples, which was confirmed by the presence of colonies suggestive of *E. coli*. In the 2019 sample, we also found total coliforms and *E. coli*, but the counting of enterobacteria showed low density and no colonies suggestive of *E. coli* in both samples.

Table 3 shows that WTP Sludge 1 presented a high number of microorganisms in the period analyzed, higher than that of WTP Sludge 2. In the 2018 and 2019 WTP Sludge 2, the presence of microorganisms was lower in comparison with WTP Sludge 1. By the Colilert™ test, we found total coliforms and *E. coli* in the centrifuged sample and only total coliforms in the 2018 non-centrifuged sample. In the 2019 sample, we found total coliforms and *E. coli* in the non-centrifuged and centrifuged samples. The number of enterobacteria remained the same in the non-centrifuged sample, the density of enterobacteria was low, and the 2018 sample presented only one colony suggestive of *E. coli*. In the 2019 sample, the density of enterobacterial colonies was low and we observed a growth of colonies suggestive of *E. coli* only in the non-centrifuged sample.

The sludge analyzed in this study came from WTP decanters and is essentially constituted of dirt from raw water, coagulants, and polymers used in the dewatering stage. Thus, the origin of these microorganisms may be related to the composition of captured water, operational parameters, and environmental conditions (Schuroff *et al.*, 2014; Souza and Ferreira, 2021; Roque *et al.*, 2021; Bernegossi *et al.*, 2022).

In Brazil, WTP sludges are solid waste and can be characterized in terms of risks to the environment and human health with the use of NBR 10.004 (ABNT, 2004a), which establishes pathogenicity as one of the factors that make waste hazardous. Considering our findings and, especially, that the referred standard only excludes from the pathogenicity classification sludge produced in sewage treatment plants, WTP Sludges 1 and 2 should be classified as hazardous waste, contrary to what is usually performed in Brazil, including the reference to the dangerousness of WTP sludges in the IBAMA Normative Instruction (IBAMA, 2012). However, considering that this is an exploratory study, the need for further research aimed specifically at microbiological characterizations of WTP sludge is suggested.

Table 3. Presence of microorganisms in WTS SLUDGE 1 and 2 before and after centrifugation, collected in 2018 and 2019. (+) refers to presence and (-) to absence.

WTP	Microbiological indicator	2018		2019	
		Non-centrifuged	Centrifuged	Non-centrifuged	Centrifuged
1	Presence of microorganisms in the fields analyzed	100%	100%	100%	96%
	Morphology and Gram staining	Low density in fields with a predominance of Gram-negative bacilli (90%), 8% Gram-positive cocci, and 2% Gram-positive bacilli.	Gram-negative bacilli in 98% of the fields and one spiryl in 2% of the fields.	Low density in 90% of the fields, with a predominance of Gram-negative bacilli, Gram-positive cocci, Gram-positive bacilli, and yeast.	Sparse distribution, with a predominance of Gram-negative bacilli, Gram-positive cocci, Gram-positive bacilli, and yeast.
	Total coliforms	+	+	+	+
	Thermotolerant coliforms (*E. coli*)	+	+	+	+
	Number of Enterobacteriaceae and colonies suggestive of *E. coli* (CFU/ml)	4000 and 200	3200 and 500	Low density and 0	
2	Presence of microorganisms	60%	80%	20%	80%
	Morphology and Gram staining	Sparse presence of Gram-negative bacilli, yeast, and Gram-positive cocci.		Low presence of Gram-negative bacilli.	Sparse presence of Gram-positive cocci, Gram-negative bacilli, and Gram-negative cocci
	Total coliforms	+	+	+	+
	Thermotolerant coliforms (*E. coli*)	-	+	+	+
	Number of Enterobacteriaceae and colonies suggestive of *E. coli* (CFU/ml)	0	Low density and 1	Low density and 600	Low density and 0

Solid waste characterization is an essential activity to assess the existence of chemical and/or biological compounds, which pose risks to the environment and human health, and determines all activities for safe waste management. The identification of pathogenic organisms in the samples studied should guide the reuse of WTP sludges in their various applications, especially when the inhalation of particles containing pathogens is possible, which would represent a risk for individuals who work directly with sludge, such as workers, transporters, and sludge spreaders (Moreira *et al.*, 2009; Alves and Marques, 2021). However, the exception of the Brazilian classification standard for sludge from sewage treatment plants should also be applied to WTP sludge, aiming to expand the potential for beneficial use of this material. Moreover, establishing specific methods for the identification of microorganisms in WTP sludge is necessary, as it already exists for sludge from sewage treatment plants in São Paulo. Table 4 presents the results of the leaching and solubilization analyses in the sludge samples from WTPs 1 and 2**.**

Table 4. Results of the leaching and solubilization analyses of sludge from WTPs 1 and 2.

Sample analysis (mg/l)	Parameter	WTP SLUDGE 1		WTP SLUDGE 2		Reference value (NBR 10,004/2004)
		2018	2019	2018	2019	
Solubilized	Barium	0.03	--	7.59	--	0.7
	Cadmium	--	--	--	--	0.005
	Lead	--	--	--	--	0.001
	Fluoride	--	--	1.1		1.5
	Aluminum	0.08	--	--	0.49	0.2
	Chloride	53.8	52.5	21.9	20.9	250
	Total iron	1.73	4.92	0.53	18.21	0.3
	Total manganese	10.75	1.94	0.94	0.22	0.10
	Sodium	3.18	9.99	1.96	19.38	200
Leached	Barium	--	0.36	7.72	0.47	70
	Cadmium	--	--	0.02	--	0.5
	Lead	--	--	0.16	--	1.0
	Fluoride	--	0.4	--	0.21	150

(--) refers to concentrations below the detection limits.

Despite the lack of physicochemical characteristics that infer dangerousness to WTP sludge (flammability, corrosivity, reactivity, and toxicity), the analyses of the solubilized sludge samples from WTP s 1 and 2 showed high concentrations of iron and manganese, higher than the reference limits for inert residues. Thus, following the specifications of NBR 10.004 (ABNT, 2004a), sludge from WTPs 1 and 2 could be classified as Class II A waste, that is, non-hazardous and non-inert. The concentration of barium above the reference limit found in the solubilized 2018 WTP Sludge 2 also characterizes this waste as non-hazardous and non-inert, even if not recurrent in other previous samplings.

Some studies report that products containing these elements in their composition are added during the water treatment process, also considering their relationship with the composition of solids, since this is one of the main elements of soil formation (Roque *et al.*, 2021; Ahmad *et al.*, 2017; Ackah *et al.*, 2018; Carneiro *et al.*, 2013; Petris *et al.*, 2019, Alves and Marques, 2021), which would justify the high concentration in the sludge samples analyzed.

The accumulation and release of manganese can represent an expensive and difficult problem to solve for WTPs. According to Moruzzi and Reali (2012), the presence of manganese in supply waters is associated with the presence of iron, in the soil or mineralized, usually in the form of manganese dioxide. Its soluble form usually appears in groundwater in the form of manganese bicarbonate and its insoluble form appears mineralized as insoluble carbonates, of which rhodochrosite ($MnCO_3$) and pyrolusite (MnO_2) are the most abundant.

Iron is an essential element for cellular metabolism. Xu *et al.* (2020) showed that the growth rate of bacteria such as *E. coli* can increase at high concentrations of iron. Thus, controlling the level of iron during the water treatment process is essential to reduce the concentration of potential pathogens, due to its harmful effects on the ecosystem and humans. In this study, we could not assess the correlation between a higher concentration of iron and presence of *E. coli*.

Manganese is harder to be removed from water, and the damage caused by its presence of manganese in water for human consumption is similar to that caused by the presence of iron, but on a larger scale. On the other hand, its purple color gives aesthetic limitations to the use of this water (Moruzzi and Reali, 2012). Grassi *et al.* (2020) showed that the presence of iron in the chemical composition of WTP sludge gives it a catalytic potential for the degradation of organic contaminants in wastewater.

4. CONCLUSION

According to the results, it was possible to identify algae, protozoa and bacteria in the WTP sludges studied, with greater diversity in the sludge produced at the station that used an aluminum-based coagulant. It was also possible to infer that the centrifugation process influences the increase in the concentration of microorganisms present in the WTP sludges.

In this exploratory research, cross-sectional sampling of the microbiological community was carried out, but due to its findings, it is recommended that further studies be carried out with representative samplings of the heterogeneity and seasonality of these residues. It is noteworthy that the disinfection of water in the WTPs studied occurs exclusively after the separation of the solid waste from the captured water; therefore, studies correlating the microbial community present in the raw water and in the generated sludge can contribute to the determination of the predictability of pathogenicity in WTP sludges.

Another important detail observed is that the studied WTP sludges should be classified as Class 1 waste according to the ABNT (2004a) standard, as they contain pathogens, contrary to the provisions of the current normative instruction in the country (IBAMA, 2012). These results point to the need for establishing safe procedures for the management of these residues and to reassess the Brazilian classification regulations regarding environmental risk and human health, since this excludes the risk associated with the pathogenicity of sludge from sewage treatment plants, but not for WTP sludges.

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