CAPÍTULO 4

INFLUENCE OF PURIFICATION TECHNIQUES WITH HYDROCHLORIC ACID AND AUTOCLAVE ON THE CHEMICAL COMPOSITION OF SAND IN AGRICULTURAL EXPERIMENTS

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ABSTRACT: The use of sand as a substrate in nutritional studies is frequent, but there is no formal protocol for cleaning and purifying this substrate for various agronomic studies, including those related to plant nutrition. The study aimed to evaluate the changes in chemical attributes resulting from the cleaning and purification of common construction sand. The experimental study, of a quantitative nature, was carried out under greenhouse and laboratory conditions. An experiment was carried out whose treatments were levels of hydrochloric acid (HCl) concentration, immersion times in the acid solution, and purification in an autoclave. It was found that sterilization in an autoclave significantly decreased the pH of the sand and increased the phosphorus content. From a day of rest with HCl, the decrease in the pH of the sand was significant. On the other hand, the phosphorus content increased linearly with the immersion time in HCl. The use of HCl completely eliminated the levels of potassium, calcium, magnesium, and copper, and significantly decreased the contents of iron and manganese (above 70%) and zinc (above 80%). To exhaust or significantly decrease the levels of nutrients present in the sand, one should use 3% HCl, without rest, for phosphorus and one day of incubation for potassium, calcium, magnesium, and copper, iron, manganese, and zinc.

KEYWORDS: Substrate; Macronutrient; Micronutrient; Sterilization.

RESUMO: O uso da areia como substrato em estudos nutricionais é frequente, porém não há um protocolo formal de limpeza e purificação desse substrato para os diversos estudos agronômicos, incluindo aqueles ligados à nutrição de plantas. O estudo teve como objetivo avaliar as alterações de atributos químicos resultantes da limpeza e purificação da areia de construção comum. O estudo experimental, de natureza quantitativa, foi realizado em condições de casa de vegetação e laboratório. Foi realizado um experimento cujos tratamentos foram níveis de concentração de ácido clorídrico (HCl), tempos de imersão na solução ácida e purificação em autoclave. Constatou-se que a esterilização em autoclave diminuiu significativamente o pH da areia e aumentou o teor de fósforo. A partir de um dia de repouso com HCl, a diminuição no pH da areia foi significativa. Por outro lado, o teor de fósforo aumentou linearmente com o tempo de imersão em HCl. O uso de HCl eliminou totalmente os níveis de potássio, cálcio, magnésio e cobre, e diminuiu consideravelmente os teores de ferro e manganês (acima de 70%) e zinco (acima de 80%). Para exaurir ou diminuir significativamente os níveis de nutrientes presentes na areia, deve-se utilizar HCl a

3%, sem repouso, para fósforo e um dia de incubação para potássio, cálcio, magnésio e cobre, ferro, manganês e zinco.

PALAVRAS-CHAVE: Substrato; Macronutriente; Micronutriente; Esterilização.

1. INTRODUCTION

The use of sand as a substrate in agronomic trials, particularly in nutritional studies involving vegetables, cereals, legumes, or grasses, for instance, has its advantages and disadvantages compared to strictly hydroponic cultivation. Sand, a low-cost material closer to soil cultivation conditions, allows roots to develop in darkness at a consistent and suitable temperature, along with efficient drainage. On the other hand, sand requires constant monitoring of moisture levels. Trials using sand have simpler nutrient management compared to hydroponic systems, where nutritional management requires constant monitoring and greater technical knowledge. However, strictly hydroponic cultivation provides more precise control over the quantity of nutrients supplied and requires less water usage. Plant support and substrate aeration are more natural, eliminating the need for artificial aeration apparatus, making cultivation easier to manage.

When studying a specific nutrient, it’s essential to consider its absence or varying levels in the substrate. In such cases, the sand should undergo thorough cleaning through successive washings to eliminate impurities and various chemical elements. Additionally, it’s crucial to seal pots or work in protected environments to prevent air contamination (e.g., dust), especially when dealing with micronutrients. Even well-washed sand may still contain reasonable levels of iron (Fe), which can reduce the need for additional iron supplementation. Consistent attention to irrigation is also necessary. In nutrient solutions, particular care should be taken with iron (Fe) to avoid chlorosis associated with deficiency.

Several products can be used for soil remediation due to different types of contamination, such as inorganic pollutants (heavy metals) and organic pollutants (petroleum hydrocarbons and polycyclic aromatic hydrocarbons) (Anning & Akoto, 2018; Dardouri & Sghaier, 2018). In these situations, several products are recommended, depending on the specificity of each situation, including inorganic agents (water, alkaline hydroxides, salts), organic and inorganic acids, and organic and inorganic chelating agents.

Hydrochloric acid is one of the agents used for cleaning polluted soils, being effective in removing Cu and Zn. It also significantly reduces the levels of Al, Fe, Mg, Mn, and dissolved organic matter, which often may not be plausible for soil decontamination, including due to the generation of wastewater (Yoo et al., 2018; Liu et al., 2021). However, its use for cleaning sand for agronomic experimentation is interesting due to the low concentration and volumes used and efficiency in reducing or eliminating chemical elements.

Sand, due to its relatively large particles, has a low specific surface area with little capacity for water and nutrient retention (Brady & Weil, 2013). The presence of contaminants such as silty-clayey material, iron and/or manganese hydroxides, and associated nutrients in sand (Ferreira & Daitx, 2003), even in small quantities, is the main disadvantage compared to hydroponics for nutritional studies, particularly with micronutrients. However, sand allows the added nutrient solution to have a lower pH without significantly affecting the plant's root system. The same can be said for potentially toxic elements, requiring higher doses compared to pure nutrient solution to affect the plant. This fact is linked to the lower diffusion of nutrients in the medium and their adsorption to sand particles (Hewitt, 1966).

Sand purification for agronomic experimentation can be carried out for the total or partial elimination of nutrients, or alternatively, with the concurrent use of autoclaving, which eliminates substrate biota through high-pressure steam. It is essential for studies requiring different and controlled levels of specific nutrients in the substrate or for studies involving microorganisms, such as symbiotic nitrogen (N) fixation.

Moist heat sterilization in an autoclave is the most commonly used method in laboratories. It offers an excellent option in terms of both time and cost, with good performance in sterilizing bacteria and fungi (Querejeta, 2023; Li et al., 2023). Research on nutrient solutions is longstanding, as are studies that combine nutrient solution with sand or similar solid substances to soil (McCall, 1916), aiming to eliminate undesirable variables for a particular objective, such as other elements besides the one under study or microorganisms.

Knowledge of acid use for sand cleaning, especially for industrial purposes in glass and mirror manufacturing, dates back to early literature, such as Curtin and Parker (1940) and Earle (1934). Sand purification with acidic solutions for agronomic purposes is based on separating adsorbed elements in substrate oxides through leaching. In this process of sand washing, nutrients dissolve in the aqueous and/or acidic solution of the leaching agent, specifically in the case of sand (Heck, 2007).

Typically, the literature mentions the use of washed sand with or without hydrochloric acid. However, it often lacks specific details regarding concentration and cleaning methodology. Given this lack of specificity, our study aimed to evaluate the chemical attribute changes resulting from cleaning common construction sand. We employed hydrochloric acid for this purpose, with or without autoclave sterilization, focusing on its application in agronomic research, particularly related to soil fertility and plant nutrition.

2. METHODOLOGY

For references on methods of studying plant nutrition and fertility in sand and nutrient solution, one can refer to fundamental works in the field, such as Hewitt (1966), Silva (2009), and Maathuis (2013). These books provide support for the methodology employed in this study.

Plots of 400 g of commercial sand sourced from a single supplier were utilized to conduct two experiments following a completely randomized design. These experiments were structured in a 2x3x2 factorial scheme with two supplementary treatments, each replicated four times. Additionally, a third experiment was carried out to validate the findings from the initial two experiments. This validation study involved four distinct samples of commercial sand and was organized in a completely randomized design with a 4x3 factorial arrangement, replicated four times.

To maintain a uniform washing methodology, each experimental unit consisted of 400 g of sand, placed in a plastic tray with a width of 360 cm and a length of 44 cm. The washing process involved tap water (two liters, manually agitated for 30 seconds), followed by draining the water after settling, repeated five times. Next, the same procedure was performed with distilled water, added in sufficient volume for the sand to be covered by a three-centimeter column above the surface. For acid treatments, after the described procedure, the sand was washed with hydrochloric acid (31.5% HCl solution by weight of commercial product), diluted according to the treatment, leaving the solution three centimeters above the sand. Then, a uniform mixture between the sand and the acid solution was made, followed by a resting period according to the treatment. Finally, the acid solution was drained, and the sand was washed again with distilled water as mentioned earlier. In treatments involving sterilization, moist heat was applied in an autoclave at 122°C and 1.7 kgf/cm² pressure for 60 minutes. This procedure was carried out following the final wash.

The analyses of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sum of bases (SB), base saturation (BS%), cation exchange capacity (CEC) - potential and effective, pH, and organic matter (OM) were performed according to Teixeira et al. (2017). Statistical analyses were performed using the Sisvar program (Ferreira, 2014), with means compared using the t-test at a 1% probability level.

2.1. EXPERIMENT 1

The treatments, distributed in a factorial scheme, consisted of autoclaving (with and without), three concentrations of HCl (3%, 5%, and 10%), and two resting times (24 and 168 hours, equivalent to 1 and 7 days).

Two additional treatments were also evaluated: no washing and washing with running water + deionized water (five times), as follows: T1) no washing; T2) washing with running water + deionized water (five times); T3) HCl 3%, without autoclaving + 1 day rest (24 hours); T4) HCl 5%, without autoclaving + 1 day rest (24 hours); T5) HCl 10%, without autoclaving + 1 day rest (24 hours); T6) HCl 3%, without autoclaving + 7 day rest (168 hours); T7) HCl 5%, without autoclaving + 7 day rest (168 hours); T8) HCl 10%, without autoclaving + 7 day rest (168 hours); T9) HCl 3%, with autoclave + 1 day rest (24 hours); T10) HCl 5%, with autoclave + 1 day rest (24 hours); T11) HCl 10%, with autoclave + 1 day rest (24 hours); T12) HCl 3%, with autoclave + 7 day rest (168 hours); T13) HCl 5%, with autoclave + 7 day rest (168 hours); T14) HCl 10%, with autoclave + 7 day rest (168 hours). Treatments T3 to T14 include washings with water before and after mixing the sand with the acid solution.

2.2. EXPERIMENT 2

In this experiment, only one concentration of HCl, presence or absence of autoclaving, and six resting times (0, 24, 48, 72, 96, and 168 hours, equivalent to 0, 1, 2, 3, 4, and 7 days) were used in a factorial scheme. As in experiment 1, two additional treatments were evaluated: no washing and washing with running water + deionized water (five times), as follows: T1) no washing; T2) washing with running water + deionized water five times; T3) HCl 3%, without autoclaving, without rest (0 hours); T4) HCl 3%, without autoclaving + 1-day rest (24 hours); T5) HCl 3%, without autoclaving + 2-day rest (48 hours); T6) HCl 3%, without autoclaving + 3-day rest (72 hours); T7) HCl 3%, without autoclaving + 4-day rest (96 hours); T8) HCl 3%, without autoclaving + 7-day rest (168 hours); T9) HCl 3%, with autoclave, without rest (0 hours); T10) HCl 3%, with autoclave + 1-day rest (24 hours); T11) HCl 3%, with autoclave + 2-day rest (48 hours); T12) HCl 3%, with autoclave + 3-day rest (72 hours); T13) HCl 3%, with autoclave + 4-day rest (96 hours); T14) HCl 3%, with autoclave + 7-day rest (168 hours). Treatments T3 to T14 include washings with water before and after mixing the sand with the acid solution.

2.3. EXPERIMENT 3

The treatments, distributed in a factorial scheme, consisted of four samples of commercial sands with plots of 400 g and three washing levels (no washing and washing with HCl followed by a 24 or 168-hour rest). For each tested sand, the following treatments were used: T1) no washing; T2) HCl 3% + 1-day rest (24 hours); T3) HCl 3% + 7-day rest (168 hours). Treatments T2 to T3 include washings with water before and after mixing the sand with the acid solution.

To maintain a uniform washing methodology, each experimental unit consisted of 400 g of sand, placed in a plastic tray with a width of 360 cm and a length of 44 cm. The washing process involved tap water (two liters, manually agitated for 30 seconds), followed by draining the water after settling, repeated five times. Next, the same procedure was performed with distilled water, added in sufficient volume for the sand to be covered by a three-centimeter column above the surface. For acid treatments, after the described procedure, the sand was washed with hydrochloric acid (31.5% HCl solution by weight of commercial product), diluted according to the treatment, leaving the solution three centimeters above the sand. Then, a uniform mixture between the sand and the acid solution was made, followed by a resting period according to the treatment. Finally, the acid solution was drained, and the sand was washed again with distilled water as mentioned earlier. For autoclaving treatments (122 °C and 1.7 kgf cm^{-2} pressure for 60 minutes), this was done after the final wash.

The analyses of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sum of bases (SB), base saturation (BS%), cation exchange capacity (CEC) - potential and effective, pH, and organic matter (OM) were performed according to Teixeira et al. (2017). Statistical analyses were performed using the Sisvar program (Ferreira, 2014), with means compared using the t-test at a 1% probability level.

3. RESULTS AND DISCUSSION

When cleaning sand for agronomic experimentation, a simple and efficient methodology is sought for the extraction of most nutrients, without altering the pH value, resulting in a suitable substrate.

The first step of sand cleaning involves the physical separation of coarser particles, including stones, various aggregates, organic material etc., through sieving. Subsequently, cleaning is done with tap water and, in sequence, with distilled water. At this stage, the aim is to remove silty and clayey particles and organic material, where nutrients are adsorbed. This water-only washing allows for many experiments, especially those related to N doses and other works that do not require very low or zero nutrient levels, mainly macronutrients.

In soil analyses, extracting the relevant nutrient fraction for plant nutrition studies relies on establishing a balance between the soil's solid phase and the liquid phase of the extracting solution (COTTENIE, 1980), whose composition and methodology vary depending on the nutrient and laboratory/region. Common extractors include salts (KCl, $CaCl_2$, $NaHCO_3$, $NH_4CH_3CO_2$ etc.), acids (HCl, H_2SO_4, NH_4F, etc.), chelating agents (DTPA, EDTA, and others), and water/hot water (HAVLIN et al., 2005). In the case of cleaning pre-washed sand with tap and distilled water, the use of HCl solution allowed a significant decrease or complete elimination of nutrients, as will be seen later. Analysis showed a null result for unwashed or water-washed sand, which is why N levels are not indicated in the tables. Regarding the analysis of nitrogen (N), the result was undetectable for both unwashed and water-washed sand, hence there is no indication of the presence of this element in the tables.

3.1 ORGANIC MATTER

In soils, autoclaving can alter the structure of organic matter, with greater dissolution in the aqueous phase. High temperatures break the chemical bonds of organic compounds, resulting in a reduction in the amount of OM (Berns et al., 2008). However, in the present study, the organic matter (OM) content in the sand was naturally low, less than 1 g kg^{-1}. Washing with water or supplementing with acid, at any concentration, with or without sterilization, did not practically change the OM content (Tables 1 and 3).

3.2 pH

In experiments 1 and 2, washing the sand with water did not alter the pH (Tables 1 and 3). Washing with the acidic solution, at any of the concentrations tested (Table 1), equally decreased the pH compared to the treatment without acid addition. Therefore, increasing the HCl concentration (between 3% and 10%) did not significantly modify the sand pH. However, there was a significant decrease in pH when the sand was incubated for seven days (experiment 1, Table 1). In experiment 2, this trend was observed starting from one day of incubation with the acid (Table 3). Since there is an increase in the activity of H^{+} ions by adding HCl to the washing water, the pH tends to decrease, but this decrease tends to be small if the sand is not left incubated with the HCl solution.

There was an interactive effect between resting with HCl and autoclave purification on the sand pH in experiments 1 and 2 (Tables 2 and 4), as described below.

Table 1: Parameters in the analysis of the fertility of the sand used for purification and washing test (experiment 1)

Treatment	P	K	Ca	Mg	Cu	Fe	Mn	Zn	pH_{water}	SB	CEC	$CEC_{ef.}$	BS	OM
	mg dm^{-3}									cmol dm^{-3}			%	g kg^{-1}
T1	2,7 a	11,7 a	80 a	12 a	0,35 a	70 a	10,9 a	1,8 a	7,1 a*	0,53 a	1,66 a	0,53 a	31,8 a	0,9 abcde
T2	1,9 b	5,9 b	40 b	12 a	0,20 b	84 a	10,7 a	1,4 b	7,0 a	0,32 b	1,44 b	0,32 b	22,4 b	1,0 abcd
T3	1,1 hij	2,0 cd	0 c	0 b	0,00 c	18 b	3,4 bcd	0,3 cd	6,0 b	0,01 cd	1,10 c	0,01 cd	0,4 c	1,0 abc
T4	0,9 j	2,0 cd	0 c	0 b	0,03 c	20 b	4,0 b	0,5 c	5,8 b	0,01 cd	1,05 c	0,01 cd	0,3 c	0,8 cdef
T5	1,0 ij	1,0 d	0 c	0 b	0,00 c	16 b	3,1 bcd	0,3 d	5,9 b	0,00 d	1,10 c	0,03 c	0,1 c	0,8 def
T6	2,0 b	0,0 d	0 c	0 b	0,00 c	14 b	1,2 g	0,2 d	5,4 c	0,00 d	1,10 c	0,00 d	0,0 c	0,8 bcdef
T7	1,4 efg	0,0 d	0 c	0 b	0,03 c	14 b	2,0 efg	0,2 d	5,4 c	0,00 d	1,07 c	0,00 d	0,0 c	0,7 f
T8	1,7 bcd	0,0 d	0 c	0 b	0,00 c	12 b	1,2 g	0,2 d	5,1 c	0,00 d	1,10 c	0,00 d	0,0 c	0,7 ef
T9	1,3 fgh	0,0 d	0 c	0 b	0,03 c	17 b	3,8 bc	0,4 cd	5,4 c	0,00 d	1,02 c	0,00 d	0,0 c	0,9 abcde
T10	1,3 fgh	1,0 d	0 c	0 b	0,00 c	19 b	2,9 cde	0,4 cd	5,2 c	0,00 d	1,10 c	0,00 cd	0,1 c	0,8 cdef
T11	1,2 ghi	3,9 bc	0 c	0 b	0,00 c	16 b	2,8 def	0,2c d	5,2 c	0,01 c	1,08 c	0,01 cd	0,6 c	0,7 def
T12	1,6 cde	0,0 d	0 c	0 b	0,03 c	12 b	1,4 g	0,2 d	5,1 c	0,00 d	1,07 c	0,03 cd	0,0 c	0,8 cdef
T13	1,4 efg	0,0 d	0 c	0 b	0,00 c	15 b	1,8 fg	0,2 d	5,1 c	0,00 d	1,13 c	0,00 d	0,0 c	1,1 a
T14	1,6 def	0,0 d	0 c	0 b	0,00 c	11 b	1,1 g	0,2 d	5,1c	0,00 d	1,07 c	0,00 d	0,0 c	0,7ef

*Means followed by the same letter, vertically, do not differ statistically from each other, by the t-test at the 1% probability level.

Note: T1) No Washing; T2) Washing with running water + deionized water (5 times); T3) 3% HCl, without autoclaving + 1 day rest; T4) 5% HCl, without autoclaving + 1 day rest; T5) 10% HCl, without autoclaving + 1 day rest; T6) 3% HCl, without autoclaving + 7 days rest; T7) 5% HCl, without autoclaving + 7 days rest; T8) 10% HCl, without autoclaving + 7 days rest; T9) 3% HCl, with autoclave + 1 day rest; T10) 5% HCl, with autoclave + 1 day rest; T11) 10% HCl, with autoclave + 1 day rest; T12) 3% HCl, with autoclave + 7 days rest; T13) 5% HCl, with autoclave + 7 days rest; T14) 10% HCl, with autoclave + 7 days rest. Washings with water are included in treatments T3 to T14, both before and after mixing the sand with the acid solution.

Source: The author

Table 2: Summary of analysis of variance for pH and P, considering treatments involving the use of HCl, autoclave sterilization, and resting periods of one or seven days (T3 to T14) - experiment 1

Autoclave (F1) Residual (F2)	**D.F.**	**Medium squares**	
		pH_{water}	**P**
HCl (F3)	1	2,15**	0,05^{ns}
F1xF2	1	1,61**	2,47**
F1xF3	2	0,09*	0,21**
F2xF3	1	0,70**	0,58**
F1xF2xF3	2	0,01^{ns}	0,09^{ns}
Treatments	2	0,03^{ns}	0,129*
Residual	2	0,03^{ns}	0,01^{ns}
Autoclave (F1)	11	0,44**	0,36**
Rest (F2)	36	0,02	0,03
CV%		2,88	12,22
Overall average		5,40	1,37

** Significant at the 1% probability level by the t-test.

* Significant at the 5% probability level by the t-test.

Source: The author

3.3 AUTOCLAVING

In experiment 1, the use of autoclave in the one-day rest treatment, at various HCl concentrations, had a significant effect on reducing the pH more than treatments without autoclaving, compared to the treatment with water-only washing. However, with seven days of rest, without autoclaving, the pH was similar to treatments with one or seven days of rest with sterilization (Table 1). On average, there was a 17% decrease in pH when using HCl, at any concentration, for cleaning non-autoclaved sand with one day of rest. With autoclaving, under the same conditions, the decrease was 26%. With seven days of rest, there was no difference in pH with or without autoclaving, with an average decrease of 26% (Table 1).

In experiment 2, it is observed again that the presence of autoclaving had an additive effect on the decrease in sand pH, with no difference between sands with and without autoclaving only after four days of incubation (Table 3). The decrease in pH signifies greater availability of H+ ions, whose solubility is directly proportional to the increase in temperature and pressure of the medium, conditions achieved with the use of autoclaving after sand washing (YANG, 2012), as also observed in other studies with soil (RAZAVI and LAKZIAN, 2007), where autoclave sterilization significantly decreased soil pH. There was no effect of autoclaving on the other parameters evaluated, except for the concentration of P, as will be seen later.

3.4 PHOSPHORUS

In experiments 1 and 2, P levels decreased by about 30% and 40%, respectively, simply by washing the sand with water. However, the different concentrations of acidic solutions in experiment 1 did not practically influence the P content after washing, as the significant difference that occurred at the intermediate dose involved very close and low values (Table 1). On the other hand, the P concentration was affected by the resting time with the acid solution and substrate sterilization (Tables 2 and 4). These significant changes occurred even considering the low initial levels of the element in the sand.

In experiment 1, at any HCl concentration, with or without autoclaving, the seven-day resting period generally showed higher P levels compared to just one day of incubation (Table 1). In experiment 2, the longer the immersion time in the acid solution, the greater the tendency for P adsorption in the sand, increasing its concentration in the subsequent chemical analysis, even after thorough washing with water (Table 3, Figure 1).

Table 3: Fertility of sand washed with 3% HCl (1), with or without autoclaving, at different resting periods (experiment 2)

Treatment		P	K	Ca	Mg	Cu	Fe	Mn	Zn	pH_{water}	SB	CEC	CECef	BS	OM
		mg dm^{-3}									cmol dm^{-3}			%	g kg^{-1}
	No washing	4,4 a*	11,7 a*	106,7 a**	12,0 a*	0,3 a*	60,9 b*	9,9a*	1,6 a	6,5 a**	0,7 a*	2,1 aa*	0,7 a*	32,3 a*	0,5 ab*
	Water washing (2)	1,8 bc	3,9 b	60,0 b	0,0 b	0,2 b	47,6 d	7,8b	1,1 b	6,6 a	0,3 b	1,4b	0,3 bc	22,9 b	0,4 bc
No autoclaving	0 day	1,0 d	3,9 b	26,7 c	0,0 b	0,1 cd	54,9 c	7,5b	0,7 c	6,6 a	0,1 c	1,1 c	0,1 bc	12,5c	0,4 c
	1 day	1,1 cd	1,3 c	13,3 de	0,0 b	0,0 e	20,6 e	2,2c	0,3 d	5,0 c	0,1 c	1,2 bc	0,2 bc	10,2 cd	0,4 bc
	2 days	1,1 cd	0,0 c	6,7 ef	0,0 b	0,0 e	17,6 ef	1,7cd	0,3 d	5,0 c	0,0 d	1,2 bc	0,1 c	2,5 e	0,5 bc
	3 days	1,3 cd	1,3 c	0,0 f	0,0 b	0,0 e	14,8 f	1,2de	0,2 d	5,4 b	0,0 d	1,1 c	0,1 c	0,2 e	0,4 bc
	4 days	1,5 cd	0,0 c	0,0 f	0,0 b	0,0 e	16,0 ef	0,9de	0,2 d	4,8 cd	0,0d	1,0c	0,1 c	0,0 e	0,6 a
	7 days	1,8 bc	0,0 c	6,7 ef	4,0 b	0,0 e	14,3 f	0,8e	0,2 d	4,8 cd	0,1 cd	1,2 bc	0,2bc	4,6 de	0,4 bc
With autoclaving	0 day	1,3 cd	3,9 b	20,0 cd	4,0 b	0,1 c	69,9 a	8,2b	0,8 c	5,7 b	0,1 c	1,1 bc	0,4 b	11,9 cd	0,5 abc
	1 day	1,1 cd	0,0 c	6,7 ef	0,0 b	0,0e	20,7 e	2,0c	0,3 d	4,5 d	0,0 d	1,1 c	0,1 c	0,0 e	0,5 abc
	2 days	1,4 cd	0,0 c	0,0 f	0,0 b	0,0 e	17,8 ef	1,4cde	0,2 d	4,7 cd	0,0 d	1,1 c	0,1 c	0,0 e	0,5 abc
	3 days	1,6 cd	0,0 c	0,0 f	0,0b	0,0e	17,4 ef	1,2de	0,2 d	4,9 c	0,0 d	1,1 c	0,1c	0,0 e	0,4 bc
	4 days	1,8 bc	0,0 c	0,0 f	0,0 b	0,0 de	15,2 ef	1,1de	0,2 d	4,8 cd	0,0 d	1,1 c	0,1 c	0,0 e	0,4 bc
	7 days	2,4 b	1,3 c	0,0 f	0,0 b	0,0 e	15,2 ef	0,7e	0,2 d	4,7 cd	0,0 d	1,1 c	0,1 c	0,2 e	0,5 abc

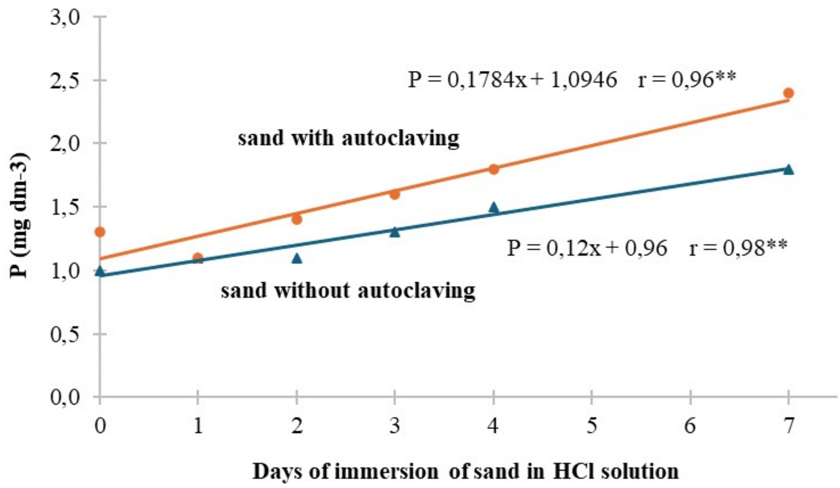
Means followed by the same letter, vertically, do not differ statistically from each other, by the t-test at the 1% probability level. ** Means followed by the same letter, vertically, do not differ statistically between them, by the t-test at the 5% probability level. ns Not significant ($p>0.05$). (1) In treatments with resting periods from zero to seven days, with or without autoclaving, washings with water before and after mixing the sand with the acid solution are included. (2) Washing with running water + deionized water (5 times)

Source: The author

Many physical and chemical properties of the soil influence the adsorption and solubility reactions of P in the substrate, affecting its concentration in the solution. In the sand, P exists in inorganic and organic forms, although the latter has an insignificant presence due to the small amount of organic matter. In the inorganic form, P is associated with amorphous and crystalline sesquioxides, mainly of Fe, Al, and Mn, which impose sorption processes and Ca oxides, where sorption and precipitation reactions predominate. pH is the main factor controlling the forms of inorganic P, and Al, Fe, Mn, and Ca present in the substrate determine the quantities of these forms of P. For example, sorption by Fe and Al oxides increases with declining pH (Havlin et al., 2005; Sharpley, 1999). Thus, the subsequent analysis of the sand, with a decrease in pH, presented proportionally higher P values.

When autoclaving the sand in experiment 1, there was a significant decrease in pH (Table 1). As observed, this pH variation led to an increase in the content of P. Similarly, in experiment 2, autoclaving resulted in a significant increase in P concentration, also related to the pH change (Table 3; Figure 1). The proportion of P forms may also undergo alteration with the use of autoclave. For instance, Anderson and Magdoff (2005) found significantly higher concentrations of soluble phosphorus in autoclaved soils compared to non-sterilized soils, with 78% more orthophosphate monoesters, 60% more orthophosphate diesters, and 54% more soluble inorganic phosphorus.

Figure 1: Phosphorus content in sand after washing with 3% HCl at different immersion periods, including washings with water before and after mixing the sand with the acid solution (experiment 2)



Source: The author

Table 4: Summary of analysis of variance for pH and P, considering treatments involving the use of HCl and resting periods of zero, one, two, three, four, and seven days (T3 to T14) - experiment 2

Source of Variation	**DF**	**Medium Squares**	
		pH_{water}	**P**
Autoclave (F1)	1	1,15**	0,84**
Rest (F2)	5	1,71**	0,88**
F1xF2	5	0,16*	0,07*
Treatments	11	0,96**	0,50**
Residue	24	0,05	0,03
CV%		4,59	10,34
Overall average		5,08	1,44

Source: The author

3.5 Ca, Mg, K, SUM OF BASES (SB), AND BASE SATURATION (BS%)

Simple washing with water reduced the presence of calcium and potassium in the sand by 50%. However, it did not decrease the magnesium content (Table 1). In experiment 2, under the same conditions, the decrease in Ca and K was 56% and 33%, respectively. In relation to Mg, its elimination occurred with simple washing with water (Table 3). The concentration of 3% HCl and one day of rest, regardless of autoclaving, was sufficient to completely remove residues of Ca and Mg from the sand and practically all potassium (experiment 1, Table 1). Experiment 2 corroborates the data regarding Ca and K, although there were low levels of Ca with zero and one day of rest (Table 3). Anyway, one day of rest, at the lowest HCl concentration, was sufficient for the almost complete removal of Ca and total removal of Mg and K.

As SB represents the combined amount of Ca, Mg, and K, washing with water alone represented a decrease of about 40% in this parameter in experiments 1 and 2. With washing with HCl, the decrease reached 100% in experiments 1 and 2, the latter starting from two days of rest. In practice, the difference in the SB can be considered negligible, at any rest period, with the acidic solution in experiment 2. The same applies to BS%, where there was a decrease of about 30% with washing with water in experiments 1 and 2, reaching levels close to or equal to zero with the use of HCl, at any of the concentrations tested in experiment 1 (Tables 1 and 3).

3.6 POTENTIAL AND EFFECTIVE CEC

Sands naturally have a low cation exchange capacity (CEC), which represents the total negative charges that retain cations such as Ca, Mg, K, in addition to H and Al ions. In these cases, leaching is a predominant factor in the decrease of bases, as occurred with Ca and K only with washing with water. Potential CEC represents the total of basic cations (Ca, Mg, K, Na) and acid cations (H and Al) at pH 7.0. Effective CEC reflects the retention of cations near natural pH, considering the sum of Ca, Mg, K, and Al (HAVLIN et al., 2005). Just as with base saturation and sum of bases, washing with water in experiment 1 decreased total and effective CEC by about 15% and 40%, respectively. The use of hydrochloric acid, regardless of other variables, caused a sharp drop in potential CEC, about 60%, leaving only H+ ions and traces of K. Effective CEC followed the remaining levels of basic cations, which were nil or close to zero with HCl from the lowest concentration (Table 1). In experiment 2, the decrease in potential and effective CEC occurred similarly to that described for experiment 1 (Table 3).

3.7 COPPER

Initial Cu levels in the sands were very low. Washing with water alone significantly reduced the Cu content, about 35% in experiments 1 and 2, but the hydrochloric acid treatment, at any concentration, was effective in eliminating any residue of the element, with no influence from autoclaving or rest after one day.

3.8 IRON

Unlike Cu, washing with water alone did not reduce the element content in experiment 1 (Table 1), but there was a 22% reduction in experiment 2 (Table 3). The use of hydrochloric acid, at any concentration, with one or seven days of rest, reduced the Fe content in the sand by about 80% (Table 1). The data from experiment 2 corroborate the result since the use of acid from one day of rest also reduced the Fe concentration by 80%. Acids such as hydrochloric, fluorosilicic, or sulfuric acid can eliminate part of the Fe contained in the sand, but not its entirety. In this case, other methods can be used, such as the combined use of hydrofluoric acid, but there is the inconvenience of silica solubilization (Hewitt, 1966).

Table 5: Fertility of three commercial sands washed with 3% HCl, without autoclaving, at two resting periods (experiment 3)

Treatment	P	K	Ca	Mg	Cu	Fe	Mn	Zn	pH_{water}	H+Al	SB	CEC	CECef	BS	OM
	mg dm^{-3}									cmol dm^{-3}				%	g kg^{-1}
Sand A															
no washing	2,3 a*	2,6 a	40,0 a	0,0 a	0,3 a	36,2 a	1,8 a	1,7 a	5,6 a	1,1 a	0,2 a	1,3 a	0,2 a	16,6a	0,7 a
1 day	0,6 c	0,0 a	0,0 b	0,0 a	0,0 b	3,8 b	0,1 b	0,1 b	5,4 a	0,9 b	0,0 b	0,9 b	0,0 b	0,0b	0,5b
7 days	1,1 b	1,3 a	0,0 b	0,0 a	0,0 b	1,9 c	0,0 b	0,1 b	5,2 a	0,9 b	0,0 b	0,9 b	0,0 b	0,2b	0,4 b
Sand B															
no washing	1,8 b	11,7 a	60,0 a	12,0 a	0,3 a	122,4 a	12,7 a	2,1 a	6,2 a	0,9 b	0,4 a	1,4 a	0,4 a	31,1 a	0,6 a
1 day	1,3 c	2,6 b	0,0 b	0,0 b	0,0 b	24,7 b	2,8 b	0,3 b	4,7 b	1,1 a	0,0 b	1,1 b	0,1 b	0,4 b	0,8 a
7 days	2,7 a	0,0 b	0,0 b	0,0 b	0,0 b	18,2 b	0,9 b	0,2 b	4,7 b	1,1a	0,0 b	1,1 b	0,1 b	0,0 b	0,7a
Sand C															
no washing	3,8 a	14,3 a	113,3 a	12,0 a	0,4 a	57,5 a	9,6 a	1,5 a	6,6 a	0,9 a	0,7 a	1,6 a	0,7 a	43,5 a	0,8 a
1 day	1,1c	1,3 b	0,0 b	0,0 b	0,0 b	17,9 b	1,6 b	0,2 b	4,8 b	1,1 a	0,0 b	1,1 b	0,1 b	0,2 b	0,5 b
7 days	2,0b	0,0 b	0,0 b	0,0 b	0,0 b	19,7 b	0,7 b	0,2 b	4,5 b	1,1 a	0,0 b	1,1 b	0,1 b	0,0 b	0,6 b
Sand B															
no washing	2,4 a	6,5 a	60,0 a	12,0 a	0,2 a	91,3 a	11,4 a	1,0 a	6,4 a	0,9 c	0,4 a	1,3 a	0,4 a	31,2 a	0,6 a
1 day	1,2 b	0,0 b	20,0 b	0,0 b	0,0 b	30,8 b	2,5 b	0,3 b	4,4 b	1,2 b	0,1 b	1,3 b	0,3 a	7,0 b	0,7 a
7 days	2,5 a	0,0 b	0,0 b	0,0 b	0,0 b	32,1 b	1,6 b	0,4 b	4,0 c	1,5 a	0,0 b	1,5 b	0,4 a	0,0 b	0,6 a

Source: The author

3.9 MANGANESE

Washing with water alone did not decrease the Mn content in experiment 1. And just as with Fe in experiment 2, there was a significant 21% reduction. In experiment 1, there was a significant effect with the use of HCl at any of the concentrations tested, with the effect being greater and significant with seven days of rest – an 87% decrease compared to exclusive washing with water and about 70% with one day of rest. Considering the concentration variation over seven days (experiment 2), the trend was a constant decrease, but with one day of rest, the decrease in Mn content was already approximately 80% (Table 3).

3.10 ZINC

Washing with water decreased the Zn content in the sand by around 25%. The use of HCl at any concentration in experiment 1, however, allowed an 80% reduction with one and seven days of rest, respectively (Table 1). Similar values were obtained in the second experiment, with an 81% reduction for one day of rest (Table 3). But washing with acid solution, without rest, already allowed a significant reduction since the concentration of the element in the sand was naturally quite low.

3.11 VALIDATION OF RESULTS

In order to corroborate the results concerning experiments 1 and 2, the washing of three commercial sands was carried out, in a simpler design, with one concentration of HCl and two rest periods, in addition to a treatment with a larger amount of sand (Experiment 3). The evaluated parameters, discussed earlier, showed results similar to those of the preceding experiments (Table 5).

4. CONCLUSION

To deplete or significantly reduce nutrient levels present in the sand, 3% HCl should be used with one day of incubation for P, K, Ca, Mg, Cu, Fe, Mn, and Zn.

Autoclave sterilization significantly decreased the pH of the sand and increased the phosphorus (P) content. From one day of rest with 3% HCl, the decrease in the sand's pH was significant. On the other hand, the P content increased linearly with the immersion time in HCl.

For the levels found in the sands studied in this work, the use of 3% HCl completely eliminated the levels of K, Ca, Mg, and Cu, and significantly reduced the levels of Fe and Mn (above 70%) and Zn (above 80%).

For future research aiming to advance knowledge and develop robust, standardized methodologies, it is suggested to investigate and compare other methods of washing and purification, whether they involve chemical, physical, or physicochemical processes. Additionally, studying alternative substrates or composite mixtures beyond sand is recommended.

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