

Chlorination: Phytotoxicity and effects on the production and quality of *Lactuca sativa* var. Mantecosa grown in a closed, soil-less system

(With 5 Figures & 1 Table)

Cloración: fitotoxicidad y efectos sobre la producción y calidad de *Lactuca sativa* var. Mantecosa creciendo en un sistema de producción cerrado, sin suelo

(With 5 Figures & 1 Table)

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Abstract. The addition of chlorine constitutes an economical disinfection method for closed, soil-less systems. Three quantities of sodium hypochlorite (0.55, 5.5 and 11 ppm) were applied to closed, soil-less greenhouse-grown lettuce (*Lactuca sativa*), to study the effect on its production (fresh weight and dry matter) and some commercial and nutritional quality factors (phytotoxicity, Vitamin C, nitrates). Sodium hypochlorite was weekly added within the nutrient solution, and the chemical properties (pH, EC and chlorides) of the recycled solution were measured. Damage to leaves was evaluated 15 days before harvest. Chlorination produced different effects regarding both production and quality. All treatments presented plants with excellent commercial weight, although quantities of 0.55 and 5.5 ppm presented a 17% greater weight. However, this improvement was statistically not significant. Three different groups were observed for the phytotoxicity effects: the 11 ppm dose showed 42% of damage to leaves, while leaf damage was 22% for the 0.55 and 5.5 ppm quantities, and 15% for the control. The addition of sodium hypochlorite did not affect (significantly) Vitamin C and dry matter production. All treatments presented

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nitrate contents exceeding the allowed values; this fact was related with the nitrate composition of the nutrient solution. The chemical properties of the nutrient solution were adequate for the species. This study suggests the 0.55 and 5.5 ppm quantities as a positive option regarding plant weight. Further research should be developed to adjust fertilization, and to diminish the phytotoxicity symptoms.

Key words: chlorination, *Lactuca sativa*, nitrates, phytotoxicity, vitamin C.

Resumen. En los cultivos sin suelo con recirculación la adición de cloro constituye un método económico de desinfección. Tres dosis de hipoclorito de sodio (0,55; 5,5 y 11 ppm) se agregaron semanalmente a la solución nutritiva, con la finalidad de estudiar el efecto sobre la producción (peso fresco y seco) y algunos factores de calidad comercial y nutricional (fitotoxicidad, vitamina C y nitratos) de un cultivo de lechuga (*Lactuca sativa*). Se evaluaron las propiedades químicas pH, conductividad y cloruros. La cloración produjo diferentes efectos en la producción y calidad. Todos los tratamientos presentaron plantas con excelente tamaño comercial. Las dosis de 0,5 y 5,5 ppm presentaron un peso fresco un 17% mayor que las otras dosis, aunque la diferencia no fue estadísticamente significativa. Para la fitotoxicidad se observaron tres diferentes grupos. La dosis de 11 ppm mostró un 42 % de daño en hojas, mientras que el daño producido por las dosis de 0,55 y 5,5 ppm sólo fue de un 22 %, y en el control un 15 %. La adición de hipoclorito de sodio no afectó (significativamente) la vitamina C y la producción de materia seca. Todos los tratamientos presentaron valores de nitratos que excedieron los permitidos, lo que se atribuyó a la composición de nitratos de la solución nutritiva. Las propiedades químicas de la solución nutritiva fueron adecuadas para la especie. Este estudio sugiere que dosis de 0,55 y 5,5 ppm constituyen una opción positiva para lograr mejores pesos frescos en las plantas, siendo necesario nuevos ensayos a fin de ajustar la fertilización y disminuir los síntomas de fitotoxicidad.

Palabras clave: Cloración, *Lactuca sativa*, nitratos, fitotoxicidad, vitamina C.

INTRODUCTION

Soil-less growing systems present advantages in greenhouse plant production over conventional systems. These systems can provide water and nutrients to the plant without restricting root development. Therefore, they can promote plant growth and achieve higher yields. Recently, the demand for environmentally friendly growing systems has boosted in many agricultural and horticultural crops, both in developed and developing countries.

Sustainable production and development would enable an appropriate use of the resources and the environment (Ikeda et al., 2002). Closed soil-less systems seem to be an adequate technique to reduce the environmental impact caused by draining away the nutrient solution, and for saving water, fertilizers and then economical resources. When recirculation is used, this system has high water and fertilization use efficiency and low environmental impact (Gonnella et al., 2003). However, the risk of dispersing root infecting pathogens with a recycled nutrient solution needs to be avoided (Jarvis, 1992; Stanghellini & Rasmussen, 1994; Hong et al., 2003; Schmitzler, 2004). Propagation of diseases in these systems is favoured by the more appropriate environmental conditions to pathogens and the presence in the recycled solution of pathogens morphologically more adapted (waterborne pathogens).

Lactuca sativa L. is, among the vegetables, one of the major leafy vegetables cultivated in soil-less systems in Argentina.

Within the disinfection methods (Garibaldi et al., 2004), chlorination is a chemical method used to prevent, reduce or eliminate the pathogens present in the nutrient solution (Braun & Supkoff, 1994; Van Os, 2000; Poncet et al., 2001; Newman, 2004). Some pathogens usually affect root systems and progress to minimal foliar symptoms until the plant dies. In these cases, prevention is the best strategy for eliminating or minimizing the initial inoculum (Kong et al., 2004). This method of low cost and easy application is based on the oxidation property of hypochlorous acid, and takes into account the chlorine demand to reduce the pathogens, the residual chlorine (chlorine in the solution after the reaction) and the time of contact (time for the application effectivity) (<http://www.ext.nodak.edu/ext-pubs/hòqual/watsys/aeI046whtm>; Newman, 2004). In general, chlorine has been used for killing microorganisms in drinking water. Chlorine at a concentration of 1-2 ppm (residue chlorine) could kill zoospores of *Pytaceae* fungi within 1 minute; concentrations of 100 ppm could kill the mycelia (Smith, 1979). However the residue chlorine at a concentration of 10 ppm has affected plant growth, and could result in root damage when used for any extended time period (Ikeda et al., 2002). Experimental works inform that different quantities and application times were selected for (1) their

effectiveness to control pathogens, (2) its phytotoxicity and (3) effects on productivity (Frink & Bugbee, 1987; Pierna Inistta & Diaz Gil, 2001; Escobar et al., 2003). The chemical instability of the chlorinated compounds constitutes a major problem of chlorination, resulting in a loss and subsequent change of concentration. One of the main forms of chlorine used includes sodium hypochlorite (NaOCl). This compound, when mixed with water, forms sodium hydroxide (NaOH) and hypochlorous acid (HClO). The last one constitutes the available and active chlorine. The hypochlorous acid is what kills pathogens. In high pH solutions, most of the hypochlorous acid dissociates to form hypochlorite ion (OCl⁻) which is not an effective sanitizer. A pH around 6.5-7.5 will maintain about 80% of Chlorine as active chlorine or HClO (<http://edis.ifas.ufl.edu/HS336>; Newman, 2004).

The phytotoxicity of chlorine makes it necessary to quantify the effects on the quality of edible plant species.

The ingestion of nitrates through either food or water produces health problems in humans due to the formation of toxic compounds: nitrites (as Methahemoglobinemia) and nitrosamines (Carcinogenic) (Wright & Davidson, 1964; Maynard et al., 1976; Walters & Smith, 1981; Craddock, 1983; Vajagic et al., 1986; Preussmann, 1988; Somogyi, 1988).

The importance of nitrate content in vegetables is underlined in the European Commission Regulation N° 563/2002 (http://www.food.gov.uk/food_surv.htm). It sets the maximum nitrate levels for lettuce and spinach in the different seasons and cultivation methods. The JECFA (Joint FAO/WHO Expert Committee on Food Additives) has set the Acceptable Daily Intake (ADI) of nitrate between 0 and 3.65 mg/kg of body weight (WHO, 1995).

According to this ADI, only 100g of raw vegetables (with a NO₃⁻ concentration of 2500 mg/kg f.w.) accounts for an intake of 250 mg NO₃⁻. Hence, if a 60-kg person consumes this amount alone, the ADI for NO₃⁻ will be exceeded by 14%.

Therefore, an important quality feature of leaf vegetables is their low nitrate content, even more for ready-to-use vegetables. Product cutting

(Caponigro et al., 1997) and packaging, especially airtight packages, can cause an increase of nitrite content. This is even more dangerous than nitrate (Achtzehn & Hawat, 1970).

The carcinogenic effects of nitrosamine compounds could not be proved in humans exposed to diets with large nitrate contents (fish, lettuce, spinach) (Van Manen et al., 1998). The absence of a relation between the presence of these compounds and the risk of cancer in humans (Gangoli et al., 1994) could be related to the fact that vegetables have Vitamin C. Therefore, the intake of nitrates through water (no Vitamin C) constitutes a greater risk than its intake from vegetables (Hotchkiss et al., 1992).

Vitamin C is one of the most important vitamins present in vegetables and fruits, contributing more than 90% of human vitamin C requirements. Therefore, this vitamin results one of the most important nutritional quality factors. Many biological processes, like the inhibition of the nitrosamine synthesis and the reduction of cardiovascular and carcinogenic diseases are attributed to Vitamin C (Harris, 1996).

Lactuca sativa is a species with great ability to accumulate nitrates (Richardson & Hardgrave, 1992). There exists an antagonism between the effects of nitrates and vitamin C (Graifenberg et al., 1993). Then, low contents of nitrates and high levels of vitamin C are selected as parameters for good quality foods.

Chlorination is an economical disinfection method. However, the chemical changes of the associated recycled nutrient solution are complex, and differ with seasonal and location factors. Soil-less cultures are widespread, but there is a lack of information on the effects of chlorination on some crops.

Our objective was to characterize the production and quality of *Lactuca sativa* cultivar Mantecosa growing in a closed soil-less system after different quantities of sodium hypochlorite were added to the nutrient solution, as a disinfection method.

MATERIALS AND METHODS

Mantecosa, Butterhead or loose-head type of lettuce (*Lactuca sativa*) was grown during the spring (October) period, in a 240 m² greenhouse of metallic structure, covered with polyethylene and provided with lateral ventilation. It was located in the research area of the Floriculture Institute (INTA-CASTELAR), Buenos Aires, Argentina.

Pots were filled with perlite and received individual drip irrigation with a nutrient solution, following the plant needs. They were placed on black plastic-covered wood benches with an inclination of 5 % to enable draining of the nutrient solution to a storage tank. One lettuce plant was placed in each pot.

Irrigation and fertilization were performed adding the Enshi solution (CETTEFFHO), which provides macronutrients [252 mg/l N (9% as NH₄⁺), 312 mg/l K, 54 mg/l P, 48 mg/l Mg, 65 mg/l S and 169 mg/l Ca] and micronutrients (0.26 mg/l Co, 0.54 mg/l Zn, 0.48 mg/l Fe, 0.35 mg/l Mo, 0.78 mg/l Mn, 0.32 mg/l Cu and 0.2 mg/l B). This solution was prepared with water previously subjected to reverse osmosis (pH: 6.58; EC: 0.016 dS/m² and Cl⁻: 19.56 mg/l).

The Chlorine stock solution was prepared with 100 ml of commercial sodium hypochlorite (55.5%) in 10 liters of water, previously subjected to reverse osmosis. The quantities for each treatment were prepared by dilution of the stock solution. Three different quantities of sodium hypochlorite, and a control without sodium hypochlorite, were applied to four treatments. The corresponding quantities for each treatment were: 0.55 ppm (0.015 meq/l) with 1 ml stock/liter solution; 5.5 ppm (0.15 meq/l) with 10 ml/l, and 11ppm (0.30 meq/l) with 20 ml/l. One week after placing the plants in the pots, the first chlorination was applied. It continued throughout all the culture, and within the fertilization and irrigation solution. To improve the collection of the lixiviated material, a 5% slope was allowed to conduct it towards the storage tank of the nutrient solution.

Controls of the electrical conductivity (conductimeter), pH (potenciometry) and chlorides (volumetric) of the re-circulating nutrient solution were

weekly performed. This allowed to keep the water volume and the chlorine and fertilizer quantities at the initial levels for each treatment.

Fifteen days before harvest, symptoms of phytotoxicity were monitored evaluating the relation: number of leaves with necrosis/ total number of leaves per plant affected by chlorine x 100.

At harvest (45 days after sowing), plant fresh and dry matter were determined using a thermo-ventilated oven at 70 °C until constant weight was reached.

Determinations of Vitamin C content in the fresh material (AOAC, 1980) and nitrate content in the dry material (Cataldo et al., 1975) evaluated effects on plant quality.

A completely randomized experimental design with four treatments was used with 20 replicates/treatment, to examine the effects of the independent variable treatment (four treatments: 1- control; 2- 0.55 mg/l; 3- 5.5 mg/l and 4- 11 mg/l) on dependent variables. One-way ANOVA was performed on fresh weight, dry matter, vitamin C, nitrates, pH, EC and chlorides, and significant differences were examined using the LSD test ($p < 0.05$). With this purpose, SPSS software was used (Field, 2000).

RESULTS

Phytotoxicity. Damage to leaves caused by the addition of sodium hypochlorite showed three different groups, presenting values from 15.7 to 42.7 %. Lowest ($p < 0.05$) leaf damage was found in the control treatment, while it was intermediate at the 0.55 and 5.5 mg/l quantities (22%) (Fig.1). The third group, with 11mg/l of sodium hypochlorite, showed the greatest ($p < 0.05$) damage to leaves (42%, Fig. 1).

Production of *Lactuca sativa*, cultivar Mantecosa

Fresh weight. The 0.55 and 5.5 mg/l treatments presented larger values of fresh weight (mean=17%) than the other treatments. However, this increase was not statistically significant ($p > 0.05$) (Fig. 2).

Fig. 1. Phytotoxic effects of different quantities of Sodium Hypochlorite on *Lactuca sativa* cultivar Mantecosa, grown in a closed, soil-less culture. Histograms are the mean values for each treatment. Vertical bars are ± 1 S.D. Different letters represent significant differences (LSD, $p \leq 0.05$).

Fig. 1. Efectos fitotóxicos de diferentes cantidades de Hipoclorito de Sodio sobre *Lactuca sativa* cultivar Mantecosa, que creció en un medio de cultivo cerrado sin suelo. Los histogramas son los valores promedio para cada tratamiento. Las barras verticales representan ± 1 D.S. Letras distintas indican diferencias significativas (LSD, $p \leq 0,05$).

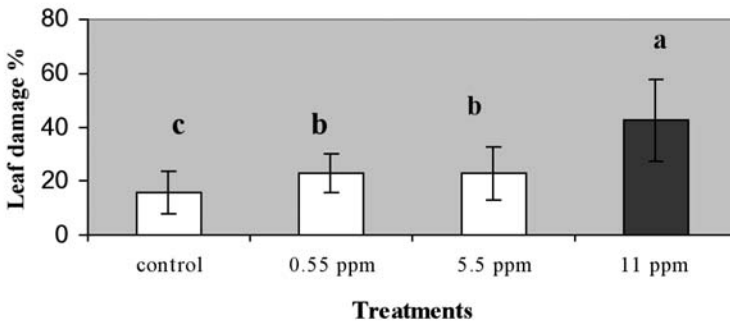


Fig. 2. Effect of different chlorination quantities on fresh weight (FW) of *Lactuca sativa* cultivar Mantecosa. Histograms are the mean values for each treatment. Vertical bars are ± 1 S.D. Equal letters indicate lack of significant differences (LSD, $p > 0.05$).

Fig. 2. Efecto de diferentes cantidades de cloro en el peso fresco de *Lactuca sativa* cultivar Mantecosa. Los histogramas son los valores promedio para cada tratamiento. Las barras verticales representan ± 1 D.S. Letras iguales indican falta de diferencias significativas (LSD, $p > 0,05$).

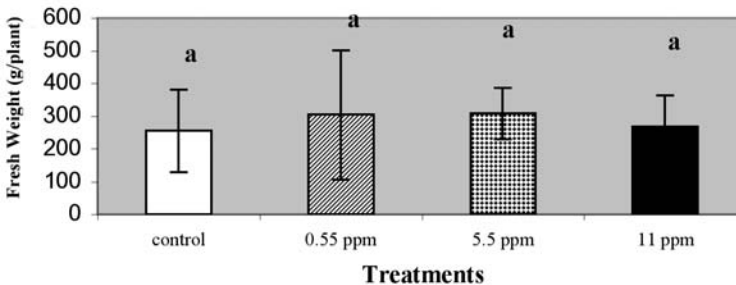


Fig. 3. Effect of different Sodium Hypochlorite quantities on dry matter production of *Lactuca sativa* cultivar Mantecosa. Histograms are the mean ($n=18$) values for each treatment. Vertical bars are ± 1 S.D. Equal letters represent lack of significant differences (LSD, $p > 0.05$).

Fig. 3. Efecto de diferentes cantidades de hipoclorito de sodio sobre la producción de materia seca de *Lactuca sativa* cultivar Mantecosa. Los histogramas son los valores promedio ($n=18$) para cada tratamiento. Las barras verticales representan ± 1 D.S. Letras iguales indican falta de diferencias significativas (LSD, $p > 0,05$).

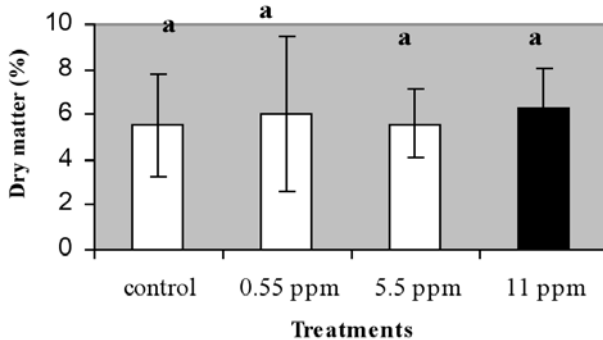


Fig. 4. Effect of different Sodium Hypochlorite quantities on nitrate content of *Lactuca sativa* cultivar Mantecosa. Histograms are the mean values for each treatment. Vertical bars are ± 1 S.D. Equal letters represent lack of significant differences (LSD, $p > 0.05$).

Fig. 4. Efecto de diferentes cantidades de hipoclorito de sodio sobre el contenido de nitratos en *Lactuca sativa* cultivar Mantecosa. Los histogramas son los valores promedio para cada tratamiento. Las barras verticales representan ± 1 D.S. Letras iguales indican falta de diferencias significativas (LSD, $p > 0,05$).

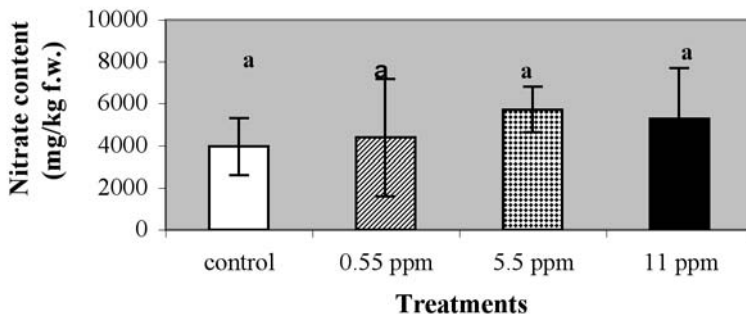
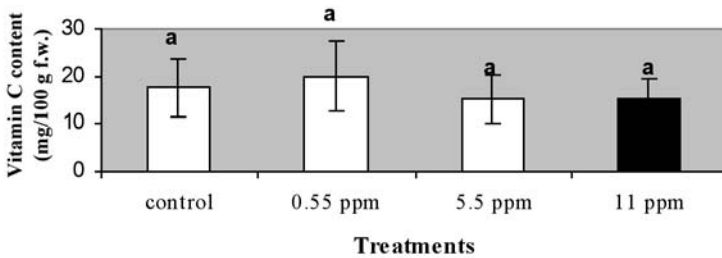


Fig. 5. Effect of different Sodium Hypochlorite quantities on Vitamin C content of *Lactuca sativa* cultivar Mantecosa. Histograms are the mean values for each treatment. Vertical bars are ± 1 S.D. Equal letters represent lack of significant differences (LSD, $p > 0.05$).

Fig. 5. Efecto de diferentes cantidades de hipoclorito de sodio sobre el contenido de Vitamina C en *Lactuca sativa* cultivar Mantecosa. Los histogramas son los valores promedio para cada tratamiento. Las barras verticales representan ± 1 D.S. Letras iguales indican falta de diferencias significativas (LSD, $p > 0,05$).



Production of dry matter. Dry matter production was 5.52 – 6.27 % of fresh weight, and there was no significant differences ($p > 0.05$) among treatments (Fig. 3).

Quality of Mantecosa

Nitrate contents. On a fresh weight (f.w.) basis, there were no significant differences ($p > 0.05$) in nitrate content among treatments (Fig. 4).

Vitamin C contents. There were no significant differences ($p > 0.05$) among treatments for vitamin C contents (Fig. 5).

Chemical properties of the recycled nutrient solution

Ph of the solution. The 11 ppm treatment showed a significantly higher ($p \leq 0.05$) pH than the other treatments (Table 1).

Electrical conductivity of the solution. Electrical conductivity was higher ($p \leq 0.05$) in the 5.5 than in the 11 ppm treatment (Table 1)

Table 1. Chemical Properties of the recycled solution with addition of different Sodium hypochlorite quantities. Different letters within any column indicate significant differences among treatments (n=22) (LSD, $p \leq 0.05$).

Tabla 1. Propiedades químicas de la solución reciclada con el agregado de diferentes cantidades de hipoclorito de sodio. Letras diferentes dentro de cualquier columna indican diferencias significativas entre tratamientos (n =22) (LSD, $p \leq 0,05$).

Treatments	pH	EC (ds/m ²)	Chlorides (meq/l)
Control	5.36 ± 0.70 b	2.62 ± 0.23 ab	0.90 ± 0.06 c
0.55 ppm	5.72 ± 0.81 b	2.62 ± 0.44 ab	0.99 ± 0.11bc
5.5 ppm	5.91 ± 0.74b	2.67 ± 0.45 a	1.06 ± 0.14b
11 ppm	6.14 ± 0.52 a	2.41 ± 0.48 b	1.29 ± 0.07a

Contents of Chlorides in the solution. The highest ($p \leq 0.05$) chloride content was observed in the 11 ppm treatment (Table 1).

DISCUSSION

The chlorine dose of 11 ppm caused a higher amount of chlorotical and necrotic tissues on leaves, affecting the commercial quality of plants. Symptoms of chlorosis and necrosis were observed on lower leaves in the plant. Chlorosis was developed first at the leaf tips, and as toxicity progressed, it moved towards the leaf edges. This is a typical response to phytotoxicity by chlorine (<http://www.geocities.com/pbarrosvanc/tema01.html>). Our results were similar to those of Fink & Bugbee (1987). They found a general lack of vigor and foliage chlorosis on vegetable seedlings when residual chlorine reached 18 mg/l.

No symptoms of disease by natural infections were observed during the period of the experiment.

The different sodium hypochlorite quantities did not affect plant weight production. Values of plant fresh weight were similar to those cited

by Fernandes et al. (2002). There was a negative correlation between fresh weight and dry matter production.

All the nitrate values exceeded those allowed (3500 mg/kg) by the European legislation for spring, greenhouse-grown lettuce [European Commission Regulation N°.563/2002 (http://food.gov.uk/food_surv.htm)].

These values could be caused by a high dose of nitrogen fertilization, since there is a direct relationship between the quantities of nitrogen supplied by the fertilizer and the leaf nitrate content (Saez Sironi et al., 2002). Also, the nitrate – ammonium ($\text{NO}_3^- / \text{NH}_4^+$) relation in the nutrient solution could have affected the levels of leaf nitrates. The solution presented 9 % of nitrogen as ammonium. Other experiments showed that contents of 20 % nitrogen as ammonium in the solution diminished the amount of leaf nitrates (Van Der Boon et al., 1990).

The range of values reported for vitamin C contents is very wide. Geigy (1975) observed values of 31 mg/100 g of f.w., while Souci (1981) and Comenge (1962) mentioned 8-21 mg/100 g of f.w. Watt & Merrill (1975) also reported values of 18 mg/100 g of f.w.

Mantecosa variety presented vitamin C values (15-20 mg/100 g of f.w.) similar to those cited by Watt & Merrill (1975). The vitamin C content showed a negative correlation with fresh weight (Drews et al., 1997), and a positive correlation with dry matter. This could be related to the incidence of light on the vitamin C contents and fresh weight production. Light presence is related to a greater photosynthesis and carbohydrate production, and therefore, to a greater production of dry matter. Vitamin C synthesis is also favoured by the presence of light (Blom- Zandstra & Lampe, 1985).

Although there was an increase of pH in the recycled solution before the addition of increasing chlorine quantities, values did not surpass the range recommended for the species by Van Der Boon et al. (1990), and they were suitable for chlorination. The electrical conductivity maintained adequate range values (Reed, 1999), and they were similar to those presented

by Magnani & Oggiano (1997). The chloride content showed values from 0.90-1.29 meq/l. The largest contents corresponded to the largest sodium hypochlorite quantities added, although they did not exceed the adequate values for the species (Reed, 1999).

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