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Contamination of soil and pineapple fruits under fertilization with sewage sludge

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A B S T R A C T

Sewage sludge is rich in organic matter and nutrients for the plants, and can be used in pineapple production, but there are few studies with scientific evaluation of possible inorganic and biological contaminants in pineapple plantations. In view of the above, this study aimed to evaluate the contamination of the soil and in fruits of pineapple fertilized with sewage sludge. The study was conducted in the municipality of Janaúba-MG. Treatments were arranged in a randomized block design, with four replicates, in a 2 x 5 factorial scheme, consisting of two different fertilizers: sewage sludge (SS) and mineral fertilizers (MF), and five pineapple varieties (Pérola, Vitória, Smooth Cayenne, MD-2, Fantastic IAC). Nitrogen was adopted as the base element to calculate the sewage sludge dose in the pineapple crop. Fertilization with sewage sludge increased the Zn and Cu contents in the soil and the Zn content in the leaves of the pineapple plants. Sewage sludge did not change the contents of As, Pb, Ba, Cu and Zn in the fruit, and Cr, Ni and Cd contents were not detected by the method used. Pineapple fruits did not show microbiological contamination above the limits established by the Brazilian legislation.

Palavras-chave:
Ananas comosus var. *comosus*
biossólido
coliformes
elementos tóxicos

Contaminação do solo e frutos de abacaxizeiros sob adubação com lodo de esgoto

R E S U M O

O lodo de esgoto é rico em matéria orgânica e nutrientes às plantas, e pode ser utilizado na produção de abacaxi, porém são escassos estudos com avaliação científica de possíveis contaminantes inorgânicos e biológicos nos seus cultivos. Diante do exposto, objetivou-se com este trabalho, avaliar a contaminação do solo e frutos de abacaxizeiros adubados com lodo de esgoto. O estudo foi conduzido no município de Janaúba-MG. Os tratamentos foram dispostos no delineamento em blocos casualizados, com quatro repetições, num esquema fatorial 2 x 5, consistindo de dois adubos distintos: lodo de esgoto (LE) e adubação mineral (AM) e, cinco variedades de abacaxizeiros (Pérola, Vitória, Smooth Cayenne, MD-2, IAC Fantástico). O N foi adotado como elemento base para o cálculo da dose de lodo de esgoto na cultura do abacaxizeiro. A adubação com lodo de esgoto aumentou os teores de Zn e Cu no solo e o teor de Zn na folha dos abacaxizeiros. O lodo de esgoto não alterou os teores de As, Pb, Ba, Cu e Zn no fruto e os teores de Cr, Ni e Cd não foram detectados pelo método utilizado. Os frutos de abacaxizeiro não apresentaram contaminação microbiológica acima dos limites estabelecidos pela legislação brasileira.

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Introduction

The sludge generated in sewage treatment has physico-chemical properties that make it apt for use as organic fertilizer in agriculture. Nevertheless, the lack of characterization of the sludge regarding the presence of pollutants and the possible contamination of soil and plants lead to inadequate disposal of this material in landfills.

The Brazilian National Environmental Council (CONAMA) through the Resolution 375 of 2006 adopted new measures on the use of sewage sludge in agriculture, defining new concentrations of metals allowed in sewage sludge and in the soil and level of pathogenic organisms, to use this waste in agriculture (Brasil, 2006).

Pineapple cultivars have great variability regarding size, leaf architecture and fruit height in the plant (Caetano et al., 2015). These differences in the features may be determinant, making it easier or more difficult the contact of the fruit with the waste applied in the soil. In contrast, pineapple cultivation with sewage sludge may reduce production costs and provide adequate destination to these wastes, decreasing the environmental impact favored by the production of chemical fertilizers and inadequate disposal.

Considering the possibility of increment in the contents of heavy metals in the soil and contamination of fruits (Zuba Junio et al., 2011; Nascimento et al., 2014) as well as the possibility of microbiological contamination (Rocha et al., 2003; Bastos et al., 2009), complementary studies are necessary to ensure the use of this waste in agriculture, especially in the pineapple crop.

In this context, the present study aimed to evaluate the contamination of soil and fruits of pineapple under sewage sludge fertilization.

Material and Methods

The experiment was carried out in the experimental area located in the municipality of Janaúba, MG, Brazil (15º 43' 47.4" S; 43º 19' 22.1" W; 516 m). According to Köppen's classification, the climate of the region is "Aw" (tropical with dry winter). Mean annual rainfall and temperature are 850 mm and 26 °C, respectively.

The soil of the area is classified as eutrophic Red Latosol, with medium texture, according to the Brazilian Soil Classification System, and its chemical and physical attributes in the 0-20 cm layer were determined according to the methodology of EMBRAPA (1997): pH (H_2O) = 5.60; OM = 1.50 dag kg^{-1}; P = 2.10 mg dm^{-3} and K = 120.00 mg dm^{-3} (Mehlich-1); Al^{3+} = 0 $cmol_c$ dm^{-3}, Ca^{2+} = 1.60 $cmol_c$ dm^{-3} and Mg^{2+} = 0.60 $cmol_c$ dm^{-3} (1 mol L^{-1} KCl); H + Al = 1.50 $cmol_c$ dm^{-3} (0.5 mol L^{-1} calcium acetate, pH 7.0); Zn = 3.40 mg dm^{-3} (Mehlich-1); Fe = 18.30 mg dm^{-3}; Mn = 15.50 mg dm^{-3}; Cu = 1.90 mg dm^{-3}; B = 0.50 mg dm^{-3} (Extractor: $BaCl_2$); Sand = 53.30%; Silt = 24.30% and Clay = 22.40%. Treatments were arranged in 2 x 5 factorial scheme and consisted of two types of fertilization (sewage sludge-SS and mineral fertilization-MF) and five pineapple cultivars (Pérola, Vitória, Smooth Cayenne, MD-2 and IAC Fantástico). The experimental design was randomized blocks with four replicates.

In both treatments, 3 g $plant^{-1}$ of P_2O_5 were applied at planting as single superphosphate. As top-dressing, 15 g $plant^{-1}$ of N and K_2O were applied as urea and potassium sulfate, respectively. This top-dressing fertilization was split into four applications, as proposed by Spironello et al. (2004). In the treatment with sewage sludge, nitrogen fertilization was based on crop demand and on the available N content (mineral forms of N-NH_4^+ and N-NO_3^-), originally and in the mineralizable organic fraction (MOF) of 20%. Sewage sludge was applied on soil surface, at the base of the canopy, split into four portions applied at 60-day intervals (according to the top-dressing mineral fertilization).

The sewage sludge used in the study came from a UASB reactor. After digestion, the sludge accumulated at the bottom of the reactor is disposed in drying beds where it undergoes solarization. Metal contents in the sludge for As, Cd, Ba, Cr, Cu, Hg, Ni, Pb, Se and Zn corresponded to 41.0, 39.0, 1,300.0, 1,000.0, 1,500.0, 17.0, 420.0, 300.0, 100.0 and 2,800.0 mg kg^{-1}, respectively. The limit for agricultural use established by CONAMA (Brasil, 2006) for Cd, Cr, Cu, Ni, Pb and Zn are, respectively 5.2, 143.1, 14.0, 22.1, 69.7 and 959.0 mg kg^{-1}.

Pineapples were planted in furrows at double row spacing of 0.90 x 0.40 x 0.20 m (totaling 76,923 plants ha^{-1}), as recommended by Cardoso et al. (2013) for the cultivar Vitória. The experimental units consisted in three double rows (six rows per plot) and ten plants per row, totaling 60 plants per plot. Twelve plants from the central double row were used for evaluations.

At 12 months after the first fertilization, soil samples (four subsamples to form a composite sample) were collected in the 0-0.20 cm layer, 0.10 m away from the planting row. These samples were air-dried, pounded to break up clods, sieved through a 2 mm mesh to obtain air-dried fine earth (ADFE) and characterized regarding contents of As, Ba, Cd, Pb, Cr, Ni, Cu and Zn, according to the methodology of USEPA (1999). The contents were determined after digestion using 0.50 g of soil sample and 10 mL of nitric acid.

Twelve leaves were collected at the beginning of flower induction and six fruits were collected at maturity stage, in each plot, to determine the total contents of As, Ba, Cd, Pb, Cr, Ni, Cu and Zn. In leaves and fruits, digestion was carried out with 0.10 g of sample and 10 mL of nitric acid in the microwave digester Mars 6 (CEM). Readings of As, Ba, Cd, Pb, Cr, Ni, Cu and Zn were performed by atomic absorption spectrophotometry (Varian - AAS 240 FS), using the atomization method for the elements Ba, Cd, Pb, Cr, Ni, Cu and Zn operating in the flame mode, with air-acetylene (99.99% purity acetylene), whereas Ba determination required the use of air-nitrous oxide (99.99% purity nitrous oxide). Arsenic was analyzed in the hydride generator system coupled to atomic absorption, through the reaction with $NaBH_4$ and hydrochloric acid. Argonium was used as carrier gas. The flame used for atomization was composed by air-acetylene (99.99% purity acetylene).

The fruits collected for microbiological analysis were placed in sterile plastic bags and immediately removed from the area for separation of peel and pulp. In the samples of pineapple peel and pulp, coliforms at 35 and 45 °C (or thermotolerant microorganisms) were analyzed by the most probable number (MPN) method, described by Silva et al. (2010).

Soil data were subjected to analysis of variance by F test and, when significant at 0.05 probability level, means were compared by Scott-Knott test. Statistical analyses were carried out using the Software SISVAR® (Ferreira, 2011) and the results related to the microbiological evaluation were subjected to descriptive analysis.

Results and Discussion

Fertilization had significant effect ($p \leq 0.05$) on Zn and Cu contents in the soil and Zn content in the leaf (Table 1). However, sludge application did not change As, Ba and Pb contents in soil and plant. Although present in the sludge, Cd, Cr and Ni contents remained below the detection limit of the analytical method used.

Fertilization with sewage sludge caused greater increments in Cu and Zn contents in the soil (10.31 and 16.86 mg kg^{-1}, respectively) compared with mineral fertilization, which led to contents of 7.06 mg kg^{-1} of Cu and 8.79 mg kg^{-1} of Zn (Table 2). The North American norm through the United States Environmental Protection Agency (USEPA, 1999) considers as safe maximum Cu and Zn contents of 100 and 250 mg dm^{-3}, respectively.

Considering the amount of sludge applied to the soil and the Cu and Zn content in the sludge, the applied amounts did not exceed the theoretical cumulative load allowed by the CONAMA resolution nº 375, of August 2006 (Brasil, 2006). Increments in Cu and Zn contents have been frequently reported in soils treated with sewage sludge (Zuba Junio et al., 2011; Balkhair & Ashraf, 2015). In other studies aiming at reducing the phytoavailability of these and other elements, Donner et al. (2012) and Contin et al. (2015) cited, as an alternative, the use of biochar, iron chloride and calcium oxide to mitigate the toxic effects of these elements.

Regardless of the treatments, the mean contents of As, Ba and Pb were 0.11, 0.04 and 0.04 mg kg^{-1}, respectively. These values were below those reported in the literature. Kabata-

Table 1. Summary of analysis of variance for As, Cd, Ba, Cr, Cu, Ni, Pb, Zn contents in the soil, leaf and fruit of pineapple plants (CTV) subjected to sewage sludge and mineral fertilizations (FRT)

SV	DF	Mean square							
		As	Cd	Ba	Cr	Cu	Ni	Pb	Zn
					Soil				
FRT	1	1.2E-4^{ns}	-	1.0E-5^{ns}	-	15.9**	-	5.0E-5^{ns}	650.44**
CTV	4	2.5E-4^{ns}	-	7.0E-6^{ns}	-	0.88^{ns}	-	3.0E-6^{ns}	19.92^{ns}
Block	3	9.8E-5^{ns}	-	9.0E-6^{ns}	-	0.36^{ns}	-	4.0E-6^{ns}	20.41^{ns}
FRT x CTV	4	2.7E-5^{ns}	-	2.5E-7^{ns}	-	0.16^{ns}	-	1.0E-6^{ns}	7.74^{ns}
Residual	27	9.7E-5	-	7.0E-6	-	0.98	-	5.0E-6	8.66
CV (%)		8.61	-	6.32	-	12.59	-	5.10	23.12
					Leaf				
FRT	1	1.5E-3^{ns}	-	1.8E-5^{ns}	-	5.85^{ns}	-	4.8E-5^{ns}	462.50*
CTV	4	1.3E-3^{ns}	-	3.9E-5^{ns}	-	2.25^{ns}	-	9.0E-5^{ns}	311.68^{ns}
Block	3	1.7E-3^{ns}	-	3.2E-5^{ns}	-	2.14^{ns}	-	1.2E-4^{ns}	425.41^{ns}
FRT x CTV	4	3.1E-3^{ns}	-	3.9E-5^{ns}	-	1.46^{ns}	-	9.4E-5^{ns}	444.72^{ns}
Residual	27	2.5E-3	-	4.8E-5	-	1.37	-	1.1E-4	178.21
CV (%)		28.79	-	12.31	-	10.26	-	6.90	30.31
					Fruit				
FRT	1	1.0E-4^{ns}	-	9.0E-6^{ns}	-	0.44^{ns}	-	4.0E-6^{ns}	0.19^{ns}
CTV	4	3.8E-3^{ns}	-	1.4E-6^{ns}	-	1.46^{ns}	-	2.0E-5^{ns}	89.14^{ns}
Block	3	1.6E-2^{ns}	-	7.5E-4^{ns}	-	4.49^{ns}	-	3.0E-4^{ns}	483.25^{ns}
FRT x CTV	4	2.0E-4^{ns}	-	4.5E-5^{ns}	-	6.35^{ns}	-	1.9E-5^{ns}	124.95^{ns}
Residual	27	3.6E-3	-	2.5E-5	-	2.49	-	1.0E-5	204.29
CV (%)		15.92	-	3.53	-	16.22	-	23.14	12.25

^{ns}Not significant; **;*Significant at 0.01 and 0.05, respectively by F test

Table 2. As, Ba, Cd, Pb, Cr, Ni, Cu and Zn contents in the soil, leaf tissue and fruit of pineapple plants subjected to sewage sludge (SS) and mineral fertilizations (MF)

Metals	As	Ba	Cd	Pb	Cr	Ni	Cu	Zn
	mg kg^{-1}							
				Contents in the soil				
SS	0.11a	0.04a	< DL	0.04a	< DL	< DL	10.31a	16.86a
MF	0.11a	0.04a	< DL	0.04a	< DL	< DL	7.06b	8.79b
Mean	0.11	0.04	-	0.04	-	-	7.71	12.75
				Contents in the leaf				
SS	0.10a	0.16a	< DL	0.15a	< DL	< DL	23.72a	45.72a
MF	0.09a	0.17a	< DL	0.15a	< DL	< DL	20.31a	32.29b
Mean	0.10	0.17	-	0.15	-	-	22.98	43.21
				Contents in the fruit				
SS	0.13a	0.14a	< DL	0.14a	< DL	< DL	21.72a	43.16a
MF	0.13a	0.14a	< DL	0.14a	< DL	< DL	23.41a	40.34a
Mean	0.13	0.14	-	0.14	-	-	21.31	42.18

Means followed by the same lowercase letter in the column do not differ by Scott-Knott test at 0.05 probability level; < DL - Below the detection limit; SS - Sewage sludge; MF - Mineral fertilization

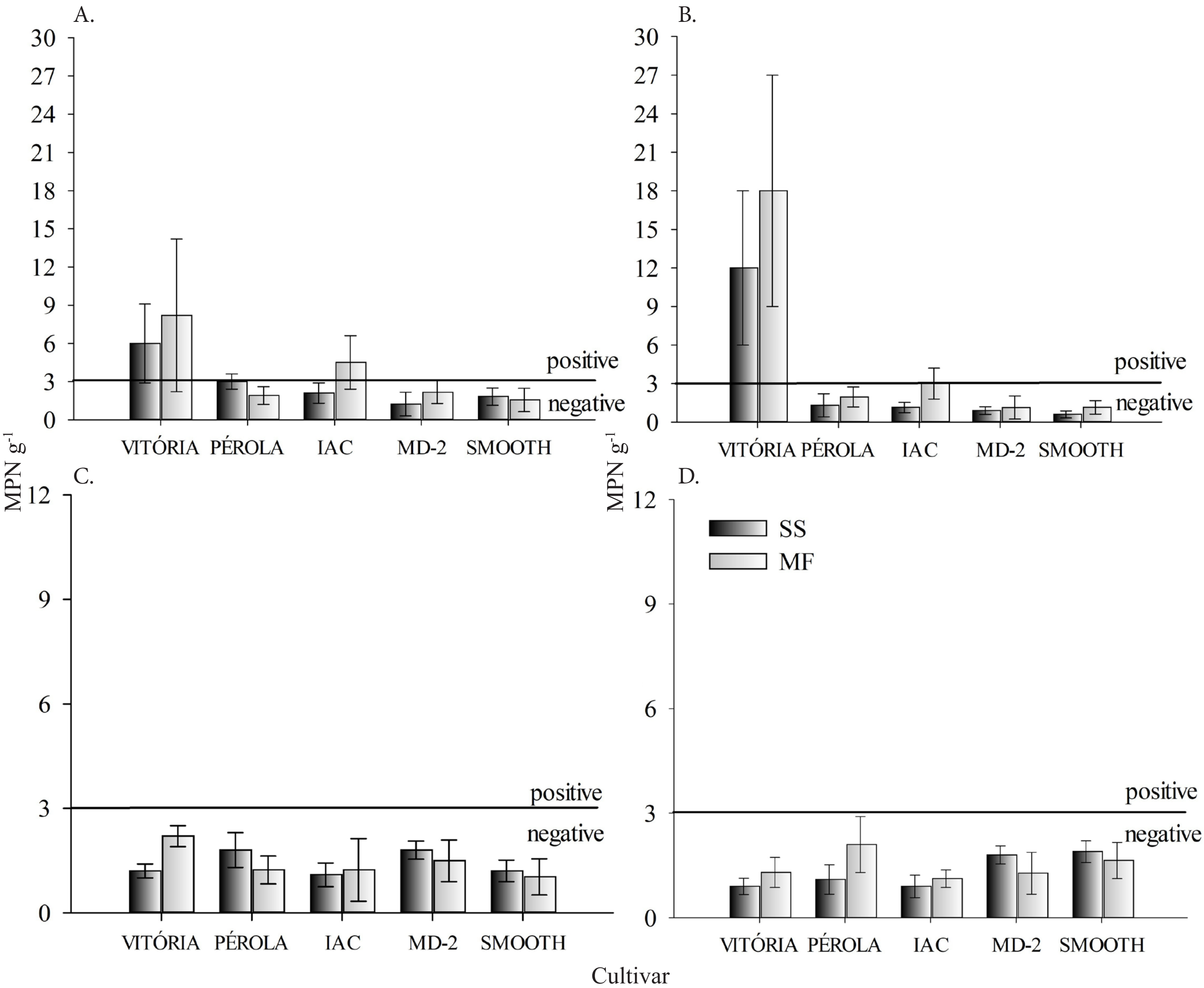
Pendias & Pendias (1992) report that Ba content in the soil, on a global scale, varies from 19 to 2,368 mg kg^{-1} and As content in agricultural soils varies from 0.1 to 40 mg kg^{-1}, and the most common value is 6 mg kg^{-1} of soil.

Sewage sludge fertilization increased the leaf Zn contents in comparison to the treatment with mineral fertilization (Table 2). Increments of Zn contents in the leaf tissues of plants grown in soils fertilized with sewage sludge have also been observed by Zuba Junio et al. (2011) in corn (*Zea mays*), Muhammad et al. (2013) in field mustard (*Brassica campestris*), Sridhar et al. (2014) in tomato (*Solanum lycopersicum*). As, Ba and Pb contents did not differ between the types of fertilization, and their mean values were 0.10, 0.17 and 0.15 mg kg^{-1}, respectively. According to Kabata-Pendias & Pendias (1992), these values are considered as normal in plant tissues. In contrast, Cr, Ni and Cd contents were below the detection limit.

Sewage sludge fertilization did not promote increments in As, Ba, Pb, Cu and Zn contents in the fruit, compared with mineral fertilization, and their mean values were 0.13, 0.14, 0.14, 21.31 and 42.18 mg kg^{-1}, respectively (Table 2). Pb contents in the fruit were below the limit established by ANVISA, which is 0.3 mg kg^{-1}, whereas As contents were slightly above the limit established by the Brazilian norm (0.10 mg kg^{-1}) in fresh fruits (Brasil, 2013). However, in canned pineapple, the FAO/WHO (FAO, 2011) establishes maximum Pb content of 1 mg kg^{-1}. In tomato fruits fertilized with sewage sludge, Waqas et al. (2015) found up to 0.5 mg kg^{-1} of As and Pb, and the limits established by the Chinese norm are 0.05 and 9.0 mg kg^{-1}, respectively.

For the elements Ba, Cu and Zn, the Brazilian norm does not yet provide critical reference levels. Cu and Zn contents were below the maximum limits allowed by the FAO/WHO, which are 40 and 60 mg kg^{-1}, respectively. Availability in the soil and bioaccumulation of metals are driven by a series of factors, such as pH, organic matter, solubility, salinity and parent material (Kumar & Chopra, 2014; Peña et al., 2015). Thus, these data suggest more studies on the availability and bioaccumulation in fruits, to establish safe levels in food for human consumption.

There was no contamination by total coliforms in the peel and pulp (MPN $g^{-1} < 3$) of fruits of the cultivars Pérola, IAC Fantástico, MD-2 and Smooth Cayenne (Figures 1A and B). However, contamination occurred in the peel and pulp of the cultivar Vitória in both treatments and, therefore, it



MPN - Most probable number; Bar - Standard error of the mean (n = 4)

Figure 1. Microbiological analysis of pineapple fruits under sewage sludge and mineral fertilizations. (A) total coliforms in the peel, (B) total coliforms in the pulp, (C) thermotolerant coliforms in the peel, (D) thermotolerant coliforms in the pulp

was assumed not to be due to the sewage sludge, but to the occurrence of fruit borer (*Strymon megarus*). Fruit borer can cause necrosis and expose pineapple pulp to microbial contamination from the environment.

Contamination by thermotolerant microorganisms was negative (MPN $g^{-1} < 3$) in all treatments (Figure 1C and D). According to Sousa et al. (2006), the survival of pathogenic organisms present on the surface of the crops depends on environmental factors such as sunlight, temperature and relative air humidity, among others. These authors also highlight that, in general, these conditions are unfavorable to the pathogen. Furthermore, the last fertilization with sewage sludge in the pineapple crop is usually performed at early flowering, when there are no fruits; thus, there is no possibility of contact between the waste and the fruit.

Despite the positive contamination by total coliforms observed in the cultivar Vitória, the levels did not exceed the limit established by ANVISA, which is 500 MPN g^{-1} of thermotolerant coliforms for fruit sample.

In study evaluating two scenarios of exposure for the use of sewage sludge: (i) risk to the consumer, due to consumption of raw vegetables (leafy and roots) and (ii) risk to the worker, Bastos et al. (2009) found that highest risks seem to be more related to occupational health (risk to the worker) than to consumer's health and, among the pathogens considered, to the transmission of virus, helminths, protozoa and lastly bacteria. Sidhu & Toze (2009) point out the efficiency of stabilization with calcium oxide and solarization to reduce the microbial load, possibly being a promising alternative to make possible the use of sewage sludge in agriculture.

Conclusions

1. Fertilization with sewage sludge increased Cu and Zn contents in the soil and Zn content in pineapple leaves.

2. Sewage sludge did not alter As, Pb, Ba, Cu and Zn contents in the fruit, and Cr, Ni and Cd contents were not detected by the method used.

3. Contamination by total coliforms and thermotolerant coliforms was not detected in fruits of the cultivars Pérola, IAC Fantástico, MD-2 and Smooth Cayenne.

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