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Ecotoxicology of Pig Slaughterhouse Waste Using *Lactuca sativa* L., *Raphanus sativus* L., and *Oryza sativa* L.

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ABSTRACT: Pork is the most consumed animal protein around the world. The production levels are significant, which results in the generation of large amounts of slaughter waste. Such waste is often disposed of improperly in agricultural areas, causing environmental imbalance by the contamination of soil and water sources with metals and pathogenic organisms. This study evaluates the phytotoxic effects of pig slaughterhouse waste *in natura* and after stabilization processes on lettuce (*Lactuca sativa* Linnaeus, 1753), radish (*Raphanus sativus* Linnaeus, 1753), and rice (*Oryza sativa* Linnaeus, 1753), in addition to shoot nutrient contents. To do this, the waste was evaluated through phytotoxicity tests on lettuce, radish, and rice plants *in natura* (PSWin) as well as after aerated composting (PSWa), natural composting (PSWn), and vermicomposting (PSWv). The evaluations were done through germination, root length, plant development, and shoot nutrient analysis. We found that PSWin and PSWa negatively affected germination, root length, and plant development. Shoot nutrient contents varied greatly among treatments, some of which were above, below, or within the recommended limits. Based on these results, we infer that pig slaughterhouse waste *in natura* and after aerated composting is phototoxic to lettuce, radish, and rice plants. Phosphorus and sulfur exhibited contents above those recommended in all the treatments for lettuce, radish, and rice. On the other hand, potassium and calcium contents were below the recommended thresholds.

Keywords: pig farming, phytotoxicity, lettuce, radish, rice.

INTRODUCTION

Pork is currently the most consumed animal protein around the world, with significant production levels (Oliveira et al., 2017). In 2018, 44.20 million pigs were butchered in Brazil, representing an increase of 1.02 million heads compared to 2017 (IBGE, 2019). The southern region of Brazil accounted for 66.8 % of the country's pig slaughter in 2018, followed by the southeastern (18.7 %), midwestern (14.5 %), northeastern (0.9 %), and northern (0.1 %) regions. These data indicate that the Brazilian pork production is likely to continue to grow in the next decade, resulting in a growing number of slaughterhouses with increased amounts of waste.

In agricultural areas, such waste is frequently disposed of improperly on the soil surface, without prior knowledge of its composition. This may result in the contamination of soil and water sources by heavy metals such as copper (Cu) and zinc (Zn), which are important components of feed dietary supplement and antibiotic formulations (Santos, 2010). Also, although some metals are necessary nutrients for plants and living organisms, they represent a risk if they exceed tolerable concentrations and may negatively impact the ecosystem and human health (Rodrigues et al., 2009). Moreover, slaughterhouse waste often contains pathogenic organisms, mainly coliform bacteria (total and fecal) and intestinal parasites (helminth eggs and larvae), which also make it impossible to use them directly for fertilization in food production areas (Pedrosa et al., 2013).

Nevertheless, there has been increasing pursuit for the development of strategies and actions that contribute to sustainability in all sectors, including the industrial sector (Briedis et al., 2011). Agroindustrial wastes are potentially interesting and abundant, and their adequate use reduces the environmental impact (Bassaco et al., 2015). Thus, strategies such as the treatment and reuse of waste should be based on a chemical and physical characterization, indicating its contaminant potential. However, it is also essential to evaluate its biological implications and possible interactions with the environment (Costa et al., 2008; Kalcikova et al., 2011).

Ecotoxicological testing with terrestrial organisms is one of the tools to evaluate the harmful effects of agroindustrial wastes, such as those of pig slaughterhouses. Via such tests, the toxicity of the wastes on the organisms can be evaluated via bioassays (Pandard et al., 2006; Chiochetta, 2013). Toxicity is a property that reflects the potential of a substance to cause harm to a living organism and depends on the concentration and properties of the chemical substance to which the organism is exposed as well as the time of exposure (Rand, 2000). Some plants are bioindicators of toxic substances, and several studies have shown the efficiency of lettuce (*Lactuca sativa* Linnaeus, 1753), radish (*Raphanus sativus* Linnaeus, 1753), and rice (*Oryza sativa* Linnaeus, 1753) in toxicity testing (Souza et al., 2014; Zhang et al., 2014; Alvarenga et al., 2016; Caetano et al., 2016), wherein the phytotoxicity of these plants can be determined based on seed germination, root length, and plant development (OECD, 1984a).

Franco et al. (2017) have evaluated the effects of different doses of sanitary landfill leachate on the germination of lettuce seeds and observed a 100 % inhibition of germination with 1.0 mL of the pollutant. In evaluating the efficiency of the electrolytic process in treating effluents from the textile industry through bioassays with seeds of lettuce (*Lactuca sativa* Linnaeus, 1753), arugula (*Eruca sativa* Linnaeus, 1753), and cucumber (*Cucumis sativus* Linnaeus, 1753), Moraes and Bidoia (2015) found that electrolytic treatment was successful and indicated a low toxicity to the organisms tested. According to Souza et al. (2005), plant seeds are highly suitable for bioassays, mainly because the germination process begins when the seeds are rehydrated. Thus, physiological changes occur at an early stage, and the seeds become highly sensitive when exposed to substances that may cause stress, consequently affecting germination.

Although still less frequent than animal testing, studies using plants as indicators of toxicity have increased in recent years (Priac et al., 2017), mainly in the monitoring of the toxicity of water and soil pollutants. The advantages using plants lie in the great variety of evaluation parameters such as seed germination, root length, biomass gain, and plant growth (Žaltauskaitė and Čypaitė, 2008), making plants versatile bioindicators because they are sensitive to a variety of aspects, yielding results that allow us to evaluate the ecotoxicological effects of the environment (D'Abrosca et al., 2008).

In this context, this study evaluated the phytotoxic effects of pig slaughterhouse waste *in natura* and after stabilization processes on lettuce (*Lactuca sativa* Linnaeus, 1753), radish (*Raphanus sativus* Linnaeus, 1753), and rice (*Oryza sativa* Linnaeus, 1753), in addition to shoot nutrient contents.

MATERIALS AND METHODS

The pig slaughterhouse waste (PSW) used in this study was obtained from a pig slaughterhouse in the northern region of the state of Rio Grande do Sul (RS). This slaughter waste is composed of the material that is found inside the intestine of the pigs (feed leftovers) and hairs. To carry out the ecotoxicological tests through plant phytotoxicity, the PSW underwent a stabilization process through composting and vermicomposting.

Stabilization of pig slaughterhouse waste

This stage of the study was conducted in the experimental area of the Universidade Estadual do Rio Grande do Sul (UERGS) in the municipality of Três Passos, which is located at the Escola Técnica Estadual Celeiro (ETEC) in Bom Progresso, RS (27° 33' S and 53° 51' W).

Three systems can be applied to stabilize PSW: aerated composting - with the turning of the compost pile twice a week during the entire stabilization period; natural composting - without the turning of the compost pile; and vermicomposting - earthworms were inoculated after 150 days of aerated composting, when the previously composted PSW showed no further changes in temperature. Earthworms of the species *Eisenia Andrei* Bouche (1972) were inoculated at the surface of the compost pile in the morning at a ratio of 5,000 worms per m^2 of bed.

To each of the three systems, 500 kg of PSW were added and arranged in piles of 2.0 × 1.0 × 1.5 m (L × W × H); the moisture of each compost pile was adjusted to 60 % (Inácio and Miller, 2009). A plastic tarpaulin was placed on top of the piles to avoid excessive loss or gain of moisture inside each pile and to keep the temperature regulated according to the phases of the composting process. During the entire periods of composting and vermicomposting, the temperature was monitored every two days, and the pH(H_2O) was monitored every seven days. Characterization of the pig slaughterhouse waste was done before the stabilization processes, using a representative sample of the waste. In PSWa, PSWn, and PSWv, the characterization was carried out at the end of the 365 days of the stabilization processes, when five subsamples were randomly collected within the pile of each treatment to form a composite sample. The waste was biologically and chemically characterized in the Soil Analysis Laboratory of the Universidade Federal do Rio Grande do Sul and in the Laboratory of Bacteriology and Laboratory of Veterinary Parasitology of the Universidade Federal de Santa Maria, respectively (Table 1).

Ecotoxicological tests

The ecotoxicological tests were carried out in a laboratory of the Universidade Estadual do Rio Grande do Sul (UERGS) in Três Passos, Rio Grande do Sul, based on the guidelines of the Organization for Economic Co-operation and Development (OECD) - guideline for the testing of chemicals No. 208 (OECD, 1984b). The bioindicator plants used in these

Table 1. Chemical and biological characterization of pig slaughterhouse waste from the northern region of the state of Rio Grande do Sul (RS) in 2017

Determination[1]	Treatments				Methodology
	PSWin	PSWa	PSWn	PSWv	
pH	5.20	5.80	6.90	6.50	Potentiometry/1:5 (solid/aqueous extract)
CE (mS cm^{-1})	1.28	3.20	0.42	2.83	IN SDA No. 17, May 21, 2007
CEC ($mmol_c$ kg^{-1})	609.00	670.00	658.00	754.00	IN/MAPA 28/July 27, 2007
Organic C (g kg^{-1})	480.00	290.00	400.00	310.00	Wet combustion/Walkley-Black
N (g kg^{-1})	14.00	42.00	34.00	44.00	Kjeldahl/0.01 % detection limit
P (g kg^{-1})	7.80	13.00	14.00	16.00	Nitric-perchloric acid wet digestion/ICP-OES
K (g kg^{-1})	1.30	3.60	1.80	4.40	
Ca (g kg^{-1})	16.00	47.00	31.00	42.00	
Mg (g kg^{-1})	1.80	5.70	3.00	6.00	
S (g kg^{-1})	2.00	1.60	4.20	3.10	
Cu (g kg^{-1})	0.06	0.206	0.16	0.19	
Zn (g kg^{-1})	0.39	1.30	1.10	1.20	
Fe (g kg^{-1})	1.40	13.00	4.10	5.00	
Mn (g kg^{-1})	0.13	0.47	0.24	0.26	
Na (g kg^{-1})	0.83	2.40	1.20	2.90	
Al (g kg^{-1})	0.49	5.40	2.50	6.80	
Cd (g kg^{-1})	<0.0002	0.0002	<0.0002	<0.0002	
Cr (g kg^{-1})	0.006	0.018	0.018	0.02	
Ni (g kg^{-1})	0.003	0.016	0.011	0.015	
Pb (g kg^{-1})	0.006	0.011	0.008	0.009	
As (g kg^{-1})	<0.002	<0.002	<0.002	<0.002	
Se (g kg^{-1})	<0.004	<0.004	<0.004	<0.004	
Hg (g kg^{-1})	0.000001	0.000002	< 0.000001	0.000001	Cold vapor wet digestion/EPA method 7471 A
TBC (CFU mL^{-1})	4.5×10^7	3×10^7	1×10^6	1.1×10^7	Total bacterial plate count
TCC (CFU mL^{-1})	3×10^7	$<1 \times 10^6$	$<1 \times 10^6$	$<1 \times 10^6$	Total coliform plate count
TFCC (CFU mL^{-1})	1×10^6	$<1 \times 10^6$	$<1 \times 10^6$	$<1 \times 10^6$	Fecal coliform plate count
Parasites	Coccidian oocysts	Coccidian oocysts and cysts *Entamoeba* sp.	Negative	Negative	Modified Bailenger method

[1] Average of two replicates per treatment. CE = electrical conductivity; TBC = total bacterial count; TCC = total coliform count; TFCC = total fecal coliform count; PSWin = pig slaughterhouse waste *in natura*; PSWa = aerated composting; PSWn = natural composting; PSWv = vermicomposting.

phytotoxicity tests were lettuce, radish, and rice. Seeds were purchased from the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI). Five treatments with eight replicates were used in a completely randomized experiment to evaluate both the lethal effects caused by PSW through non-seed germination and the sublethal effects of the presence of substances that may affect plant emergence and development. The treatments were as follows: T1 (pig slaughterhouse waste *in natura* - PSWin); T2 (pig slaughterhouse waste after aerated composting - PSWa); T3 (pig slaughterhouse waste after natural composting - PSWn); T4 (pig slaughterhouse waste after vermicomposting - PSWv); T5 (washed sand - control).

The assays were conducted in a BOD incubator with a temperature of 27 ± 2 °C and illumination of approximately 1,000 lux. For each treatment, 300 g of waste were placed in 0.5-L plastic pots, with 10 seeds per pot. Subsequently, the moisture of each experimental unit was adjusted to 60 % of field capacity.

Germination percentage and root length were evaluated in four replicates per treatment seven days after plant emergence for lettuce and radish and 15 days for rice. Total plant length, the total number of leaves, fresh and dry shoot weight, fresh and dry root weight, and shoot nutrient contents were evaluated 14 days after sowing for lettuce and radish and 20 days for rice. Shoot nutrient analysis was carried out according to the methodology described by Tedesco et al. (1995).

The results of the phytotoxicity tests (germination percentage, root length, and plant development) were submitted to analysis of variance (ANOVA) and complementary orthogonal contrast tests using the Dunnett test ($p \leq 0.05$). We compared treatments versus control and treatments versus treatments, using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Ecotoxicological tests

The average values and the results of the orthogonal contrasts on germination percentage, root length, and plant development of lettuce, radish, and rice subjected to pig slaughterhouse waste are shown in tables 2 and 3, respectively.

Lettuce

For lettuce, the control treatment had the highest germination percentage, followed by PSWv and PSWn, respectively. We observed no significant differences among the three treatments when compared by orthogonal contrasts. However, PSW *in natura* (PSWin) and after aerated composting (PSWa) caused a significant inhibition in germination (100 and 90 %, respectively). Germination is a biological phenomenon

Table 2. Average values of germination (G), root length (RL), total plant length (TPL), number of leaves (NL), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), and dry root weight (DRW) for lettuce, radish, and rice

Treatments	G	RL	TPL	NL	FSW	FRW	DSW	DRW
	%	cm		mg				
Lettuce								
Control	100	3.20	8.13	3.00	21.89	2.88	1.57	0.62
PSWin	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PSWa	10	1.68	7.02	2.25	12.88	1.72	1.33	0.26
PSWn	94	3.11	10.75	3.00	42.23	2.38	2.06	0.39
PSWv	96	4.09	11.60	4.25	101.56	4.65	4.83	0.33
Radish								
Control	75	4.76	13.68	1.75	108.18	4.01	6.23	0.59
PSWin	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PSWa	12	1.20	5.07	1.00	23.47	1.54	4.92	0.22
PSWn	80	5.54	15.47	2.75	307.02	5.90	12.67	0.30
PSWv	84	6.18	15.91	2.75	366.96	5.95	18.07	0.43
Rice								
Control	92	9.11	29.74	2.69	53.58	76.12	8.75	11.26
PSWin	30	2.30	19.14	3.17	85.79	42.09	18.67	3.53
PSWa	43	0.55	9.64	1.66	24.17	41.94	3.85	2.57
PSWn	86	1.63	15.31	1.93	38.45	49.56	7.84	2.68
PSWv	92	8.45	22.37	2.13	49.90	55.6	8.23	3.76

PSWin = pig slaughterhouse waste *in natura*; PSWa = aerated composting; PSWn = natural composting; PSWv = vermicomposting.

Table 3. Results of the contrasts for germination (G), root length (RL), total plant length (TPL), number of leaves (NL), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), and dry root weight (DRW) for lettuce, radish, and rice

Contrasts	G	RL	TPL	NL	FSW	FRW	DSW	DRW
Lettuce								
C × all	*s*	*s*	*s*	*s*	*s*	*ns*	*ns*	*s*
C × PSWin	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
C × PSWa	*s*	*s*	*s*	*s*	*ns*	*ns*	*ns*	*s*
C × PSWn	*ns*	*ns*	*s*	*ns*	*s*	*ns*	*ns*	*ns*
C × PSWv	*ns*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWin × PSWa	*s*	*s*	*s*	*s*	*ns*	*s*	*s*	*ns*
PSWin × PSWn	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
PSWin × PSWv	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWa × PSWn	*s*	*s*	*s*	*s*	*s*	*ns*	*ns*	*ns*
PSWa × PSWv	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWn × PSWv	*ns*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
Radish								
C × all	*s*	*s*	*s*	*ns*	*ns*	*ns*	*ns*	*s*
C × PSWin	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
C × PSWa	*s*	*s*	*s*	*ns*	*ns*	*s*	*ns*	*s*
C × PSWn	*ns*	*ns*	*ns*	*s*	*s*	*s*	*s*	*ns*
C × PSWv	*ns*	*ns*	*ns*	*s*	*s*	*s*	*s*	*ns*
PSWin × PSWa	*ns*	*ns*	*s*	*s*	*ns*	*ns*	*s*	*ns*
PSWin × PSWn	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWin × PSWv	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
PSWa × PSWn	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWa × PSWv	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*ns*
PSWn × PSWv	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*s*	*ns*
Rice								
C × all	*s*	*s*	*s*	*s*	*ns*	*s*	*ns*	*s*
C × PSWin	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
C × PSWa	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
C × PSWn	*ns*	*s*	*s*	*s*	*ns*	*s*	*ns*	*s*
C × PSWv	*ns*	*ns*	*s*	*s*	*ns*	*s*	*ns*	*s*
PSWin × PSWa	*ns*	*ns*	*s*	*s*	*s*	*ns*	*s*	*ns*
PSWin × PSWn	*s*	*ns*	*ns*	*s*	*s*	*ns*	*s*	*ns*
PSWin × PSWv	*s*	*s*	*ns*	*s*	*s*	*ns*	*s*	*ns*
PSWa × PSWn	*s*	*ns*	*s*	*ns*	*ns*	*ns*	*ns*	*ns*
PSWa × PSWv	*s*	*s*	*s*	*s*	*s*	*ns*	*s*	*ns*
PSWn × PSWv	*ns*	*s*	*s*	*ns*	*ns*	*ns*	*ns*	*ns*

s = significant; ns = not significant through orthogonal contrasts using the Dunnett test ($p<0.05$). C = Control; PSWin = pig slaughterhouse waste *in natura*; PSWa = aerated composting; PSWn = natural composting; PSWv = vermicomposting.

and determined by a range of specific conditions, such as environmental conditions (Marcos Filho et al., 1987).

The treatment PSWv had the highest average values for root length (RL) and the plant development parameters TPL, NL, FSW, FRW, and DSW. This means that overall, it promoted better plant development and presented significant differences for most parameters in comparison to the other treatments. According to Vig et al. (2011), vermicomposting is the most suitable alternative in the integrated management of solid waste, mainly because these wastes are not used *in natura* and can be transformed into valuable organic compost.

Radish

For radish, PSWv, PSWn, and the control treatment showed the highest average values for germination percentage, without any significant differences among these treatments. Also, PSW *in natura* (PSWin) and after aerated composting (PSWa) caused a significant inhibition in germination (100 and 88 %, respectively).

The treatments PSWv and PSWn showed the highest average values for root length and plant development. There were no significant differences when compared to each other, except for SDM. In a study by Souza et al. (2014), who evaluated the toxicity of two soils with the presence of pesticide residues on lettuce and radish bioindicator plants, there were no significant differences in germination percentage between the control and the tested treatments. However, there was a negative interference with plant development in one of the soil samples, which indicates a phytotoxic effect.

Rice

For rice, the average germination percentage was highest in the control treatment, followed by PSWv and PSWn, without significant differences among the treatments. Similar to the other crops, PSWin and PSWa caused a significant inhibition in germination (70 and 57 %, respectively).

The control treatment had the highest average values for root length and plant development (TPL, FRW, and DRW). Analyzing the orthogonal contrasts, the control treatment showed significant differences in plant development (TPL, NL, FRW, and DRW) when contrasted with PSWv, in root length and plant development (TPL, NL, FRW, and DRW) when contrasted with PSWn, and in root length and plant development (all parameters) when contrasted with PSWin and PSWa.

Based on the results of the three bioindicator plants presented above, we found that, overall, PSWin and PSWa negatively affected germination percentage and root length. According to Sobrero and Ronco (2008), the periods of germination, root development, and the first days of seedling growth are dominated by several physiological processes, which may be compromised by the presence of toxic substances. This suggests the occurrence of a phytotoxic effect, attributable to possible contamination found in PSW *in natura* and after aerated composting, although the results of the chemical analyses were within the legal standards according to Brasil (2009) and Brasil (2016a). However, for the parameters pH and EC (Table 1), this was not the case when comparing the results of the biological analyses and the thresholds established by the legislation (Brasil, 2016b). According to Pablos et al. (2011), there is a correlation between physical-chemical properties and ecotoxicological tests. Young et al. (2012) found inhibition of root lengthening in lettuce plants and associated this with the high EC value found in the sanitary landfill leachate.

A phytotoxic effect was expected for PSW *in natura* because of the more acidic pH and the biological contamination (Table 1). However, this negative effect was also found in the treatment that underwent aerated composting (PSWa), with inhibition of germination in lettuce, radish, and rice by 90, 88, and 57 %, respectively. Furthermore, low average values of root length and plant development were found in PSWa. According to Sobrero and Ronco (2004), toxic effects can be determined via seed germination and root growth. In our study, the results suggest a phytotoxic effect, which can be attributed to the high electrical conductivity value found in the PSWa treatment (Table 1), associated with secondary compounds that may have been formed during aerated composting (Tang et al., 2018). According to Wilson (1984), electrical conductivity

(EC) is indicative of the concentration of ionized salts in the solution and represents a parameter for estimating the salinity of the materials and the osmotic potential of the solution. Thus, it is a direct contributor to seed plasma membrane damage following salt stress (Ueda et al., 2016).

Tang et al. (2018) have investigated the phytotoxic effect of sewage sludge after stabilization on *Ipomoea hederacea* and *Helianthus annuus*, plant species which have also been recommended by the OECD (1984b), and found high values of electrical conductivity and a positive correlation with the low percentage of plant germination. According to Bafana et al. (2011), the increase in toxicity may be a result of by-products formed, such as aromatic amines, which are known toxic compounds. Composting, which was one of the waste treatment processes used in this study, also involves extremely complex biochemical transformations promoted by different soil microorganisms, which may differ in terms of their community compositions depending on the composting method (Inácio and Miller, 2009). Gerber et al. (2017) have evaluated the phytotoxicity of untreated and treated effluents from a pig slaughterhouse on cucumber and lettuce seeds and found that, although the phytotoxic effects of the treated effluent were lower in comparison to the raw effluent, the percentage of germination of both plants subjected to each of the effluents was less than 80 %. Thus, both effluents were phytotoxic to the bioindicator plants.

Nutrient content

The total nutrient contents in shoots of lettuce, radish, and rice are shown in table 4.

Lettuce

For lettuce, the following nutrients were within the ranges recommended by van Raij et al. (1997) for contents of total P, K, Ca, Mg, S, Cu, Zn, Mn, and Fe:Mg in PSWn and PSWv, Cu and Mn in PSWv, and Zn in PSWn. These treatments had values between 4.0-6.0 g kg^{-1} for Mg, 0.005-0.025 g kg^{-1} for Cu, 0.03-0.15 g kg^{-1} for Mn, and 0.03-0.10 for Zn. For P and S, the values were above those suggested by the author (4.0-7.0 g kg^{-1} for P and 1.5-2.5 g kg^{-1} for S) in all the treatments, especially for S in PSWa. According to Furtini Neto et al. (2000), S is an essential macronutrient for the development of cultivated plant species, and the lack of S may affect yields of several crops. However, S in excess can also drastically affect plant biological responses and may be harmful to the shoots, the stem, and the roots, causing the reduction of chlorophyll as well as a loss of trunk and root biomass. Excessive S levels may also impair the transport of water inside the plant, in addition to making some nutrients unavailable to the environment, thus compromising plant germination and development (Brena, 2009).

On the other hand, K and Ca were below the ranges recommended by van Raij et al. (1997) in all the treatments. When evaluating shoot nutrient contents in lettuce at 34 days with phosphate fertilization, Kano et al. (2012) also found values within the limits recommended by van Raij et al. (1997).

Radish

For radish, Silva et al. (1999) recommend shoot contents of P, K, Ca, Mg, Cu, Zn, Mn, and Fe of 3.0-7.0 g kg^{-1}, 40.0-75.0 g kg^{-1}, 30.0-45.0 g kg^{-1}, 5.0-12.0 g kg^{-1}, 0.005-0.025 g kg^{-1}, 0.02-0.25 g kg^{-1}, 0.05-0.25 g kg^{-1}, and 0.05-0.2 g kg^{-1}, respectively. However, the nutrients that remained within the limits recommended by the author in this study were Mg in all the treatments, Mn in PSWn and PSWv, and Fe in PSWn. The following nutrients showed levels above those recommended: P and Cu in all the treatments, Zn in PSWn and PSWv, Mn in the control, and Fe in the control and in PSWv. On the other hand, the following nutrients were below those recommended: K and Ca in all the treatments, Cu, Zn, and Fe in PSWa, and Zn in the control and PSWa.

Table 4. Total nutrient contents in shoots of lettuce and radish (evaluated 14 days after sowing) and rice (evaluated 20 days after sowing)

Nutrients[(1)]	Treatments				
	Control	PSWin	PSWa	PSWn	PSWv
	g kg^{-1}				
	Lettuce				
P[(2)]	8.50	0.00[(3)]	8.60	9.40	7.10
K	4.40	0.00	6.70	30.30	28.20
Ca	1.10	0.00	0.50	0.70	0.70
Mg	7.70	0.00	6.80	4.10	4.90
S[(4)]	8.60	0.00	45.30	5.20	2.90
Cu	<DL[(5)]	0.00	<DL	0.10	0.02
Zn	<DL	0.00	<DL	0.10	0.13
Mn	<DL	0.00	<DL	0.37	0.13
Fe	<DL	0.00	<DL	0.41	0.30
	Radish				
P	8.20	0.00	17.70	11.30	11.50
K	3.30	0.00	5.70	21.90	21.10
Ca	1.90	0.00	2.10	2.80	1.40
Mg	5.20	0.00	12.50	10.40	10.70
S	12.10	0.00	20.80	9.30	9.20
Cu	0.05	0.00	<DL	0.02	0.02
Zn	0.07	0.00	<DL	0.26	0.21
Mn	0.61	0.00	<DL	0.09	0.06
Fe	0.35	0.00	<DL	0.08	0.21
	Rice				
P	5.80	9.20	10.50	17.60	11.30
K	8.90	9.30	11.70	12.70	13.30
Ca	0.50	0.20	0.60	0.80	0.30
Mg	4.70	5.30	3.90	5.30	4.90
S	2.60	2.30	3.10	3.10	2.70
Cu	0.03	0.02	0.05	0.03	0.04
Zn	0.09	0.05	0.11	0.13	0.17
Mn	1.53	0.06	0.23	0.08	0.09
Fe	0.21	0.07	0.26	0.05	0.11

[(1)] Average of two replicates per treatment. [(2)] Hydrogen peroxide/sulfuric acid digestion. [(3)] 0 % germination. PSWin = pig slaughterhouse waste *in natura*; SWa = aerated composting; PSWn = natural composting; PSWv = vermicomposting. [(4)] Nitric-perchloric acid digestion. [(5)] Below the detection limit.

Rice

For rice, Malavolta et al. (1997) recommend shoot contents of P, K, Ca, Mg, S, Cu, Zn, Mn, and Fe of 2.5-4.0 g kg^{-1}, 25.0-35.0 g kg^{-1}, 7.5-10.0 g kg^{-1}, 5.0-7.0 g kg^{-1}, 1.5-2.0 g kg^{-1}, 0.01-0.02 g kg^{-1}, 0.025-0.035 g kg^{-1}, 0.1-0.15 g kg^{-1}, and 0.2-0.3 g kg^{-1}, respectively. In this study, the nutrients that remained within the values recommended by these authors were: Zn in all the treatments (except PSWn), Mn and Fe in PSWin, PSWn, and PSWv, Mg in PSWin and PSWn, and Cu in PSWin. In contrast, the following showed values above those recommended: P, S, and Cu in all the treatments (except Cu in PSWin), Fe and Mn in the control and PSWa, and Zn in PSWn. The nutrients K, Ca, and Mg showed levels below those recommended in all the treatments (except Mg in PSWin and PSWn).

In our study, P and S (except S in radish, for which no reference values are available) showed contents above those recommended by the aforementioned authors for all three crops and in all the treatments. On the other hand, K and Ca were below those values recommended for all three crops and in all the treatments. According to Faquin and Andrade (2004), if there is excess or deficiency of a nutrient in plants, this will manifest in visible symptoms that are typical for each element, because each element always exerts the same function in any kind of plant. Furthermore, there was also a great variability among the treatments in regard to the nutrients which were above, below, or within the limits recommended for each crop. This suggests that this variability is attributed to the fact that the pig slaughterhouse waste *in natura* presents a variable chemical composition for the different stabilization processes, as shown in the chemical characterization (Table 1).

According to Trani et al. (2013), organic materials may vary in composition according to their origin, moisture content, and stabilization process. These materials have numerous effects on the soil, such as improving soil structure, water infiltration, and aeration. Also, according to the same authors, these materials promote the reduction of abrupt changes in soil temperature, which interferes with biological processes and plant nutrient uptake. However, despite the advantages of using organic waste, one of the challenges to overcome is the imbalance of nutrients in relation to crop needs (Westerman and Bicudo, 2005).

With this study, we further confirmed the importance of stabilizing pig slaughterhouse waste before using it in agricultural fields. Furthermore, our results highlight the importance of evaluating the toxicity of this waste under different treatment methods. Thus, it is crucial to continue the advancement of ecotoxicological testing with a greater diversity of bioindicators to solve this environmental issue.

CONCLUSIONS

Pig slaughterhouse waste *in natura* and after aerated composting had phytotoxic effects on lettuce, radish, and rice plants.

Phosphorus and sulfur contents were above those recommended in all the treatments for all three plant species, while potassium and calcium contents were below the recommended thresholds.

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