Received: 2 February 2023 | Revised: 19 May 2023 | Accepted: 23 May 2023

DOI: 10.1111/asj.13849

RESEARCH ARTICLE

Animal Science Journal WILEY

Hazards of swine slurry: Heavy metals, bacteriology, and overdosing—Physicochemical models to predict the nutrient value

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Funding information
This work was supported by the Xunta de Galicia (Unidad Mixta de Investigación, Desarrollo e Innovación sobre el Sector Cárnico) and by the pre-doctoral grant of Miguel Fernández-Labrada from "Programa de ayudas a la etapa predoctoral" of the Xunta de Galicia (Consellería de Educación, Universidade e Formación Profesional) (grant number ED481A-2020/130).

Abstract

In this work, 124 samples of slurry from 32 commercial farms of three animal categories (lactating sows, nursery piglets, and growing pigs) were studied. The samples were collected in summer and winter over two consecutive years and analyzed for physicochemical properties, macronutrient and micronutrient, heavy metals, and major microbiological indicators. The results were found to be influenced by farm type and to deviate especially markedly in nursery piglets, probably as a consequence of differences in pig age, diet, and management. The main potential hazards of the slurries can be expected to arise from their high contents in heavy metals (Cu and Zn), especially in the nursery piglet group, and from the high proportion of samples testing positive for *Salmonella* spp. (66%). Linear and nonlinear predictive equations were developed for each animal category and the three as a whole. Dry matter, which was highly correlated with N, CaO, and MgO contents, proved the best predictor of fertilizer value. Using an additional predictor failed to improve the results but nonlinear and farm-specific equations did. Rapid on-site measurements can improve the accuracy of fertilizer value estimates and help optimize the use of swine slurry as a result.

KEYWORDS
electrical conductivity, fertilizer value, pig slurry, prediction model, relative density

1 | INTRODUCTION

Pork consumption continues to grow, and so does the number of bred pigs (OECD/FAO, 2022). According to FAOSTAT (2020), Spain is the fourth world producer of swine, with more than 34 million heads (EUROSTAT, 2021). Intensively bred swine are usually held in stables, where they produce large amounts of slurry containing substantial amounts of fertilizing nutrients (Penha et al., 2015), as well as organic matter of use for maintaining soil fertility (Ferreira et al., 2021). However, excessive amounts of slurry can detract from fertilizing efficiency and pose environmental problems through volatilization of ammonia (Matsunaka et al., 2008), leaching of nitrates or eutrophication by leached N and P (Sørensen & Jensen, 2013). Additional hazards associated to swine slurry can arise from (a) too high contents in heavy metals accumulating in soil and crops (Drescher et al., 2021; Tang et al., 2020) or leaching to ground and underground water (da Rosa Couto et al., 2016) and (b) their containing pathogenic bacteria (Hutchison et al., 2004; Nag et al., 2021) that can survive over long periods in slurry—or in soil after application—(Marszałek et al., 2019; Tran et al., 2020) and cause animal or even human diseases (Venglovsky et al., 2018). Whether a given slurry has a favorable or unfavorable impact depends largely on its composition (Antezana et al., 2016), its application method and rate (Brandão et al., 2020; Lovanh et al., 2010), and the environmental conditions during and after application (Carozzi et al., 2013; Maris et al., 2021). Unfortunately, most farmers do not know the exact composition of the slurries they use (Department for Environment Food & Rural Affairs, 2021; Scottish Government, 2022) and tend to ensure that they will meet the needs of their crops by using too large amounts (Scottish Government, 2022), thereby increasing the risk of an unfavorable environmental impact (Díez et al., 2006; Hernández et al., 2013).

The composition of slurry can be easy established from laboratory physicochemical analyses. This, however, is often an unattractive choice for farmers as it takes time and money (Moral et al., 2005). The fertilizer value of a slurry can also be rapidly estimated by using instrumental techniques such as near infrared spectroscopy (NIRS) (Horf et al., 2022; Sørensen et al., 2007), which, however, is only affordable by specialist laboratories or large agrarian corporations.

Some authors have developed regression equations to estimate the nutrient contents of swine slurries from easily measured parameters. For example, the P content of a slurry is closely related to its density (Moral et al., 2005; Singh & Bicudo, 2005; Yang et al., 2006) and dry matter content (Scotford, Cumby, Han, & Richards, 1998; Scotford, Cumby, White, et al., 1998; Zhu et al., 2003). Equations using electrical conductivity as a predictor have also provided accurate estimates of N and K contents (Antezana et al., 2016; Martínez-Suller et al., 2008; Moral et al., 2005; Yang et al., 2006). The equations, however, are not applicable to all slurries as their properties depend on the particular animal species and age, farm facilities, nutrition regime, and geographical region (Antezana et al., 2016; Martínez-Suller et al., 2008; Suresh & Choi, 2011).

The primary aims of this work were (a) to extract physical, chemical, and microbiological information from swine slurry; (b) to examine the variability of their properties in terms of year season (spring and autumn) and animal category (growing pigs, lactating sows, and nursery piglets); and (c) to relate easily measured parameters such as pH, electrical conductivity, dry matter, and relative density to their fertilizer value.

2 | MATERIAL AND METHODS

2.1 | Sampling

Slurry samples were obtained from 32 farms in Galicia, NW Spain. Of the 32 farms, 20 were growing pigs (GP, weight > 22 kg), 6 lactating sows (LS), and another 6 nursery piglets (NP, 6–22 kg).

The selected farms were all representative in size and management system of the body of intensive farms in the region. LS, NP, and GP farms were rearing 400–2000, 6000–10000 and 1500–4000 heads at the time under similar conditions in the three groups.

Each target farm was sampled four times (two in winter and another two in summer over two consecutive years). Samples were directly obtained from storage pits after turning over and homogenization and were held in 1 L tightly closed containers that were kept in a cool box for transfer to the laboratory. Once there, they were stirred and split into two portions each. One portion was used for fresh measurements and the other was freeze-dried for subsequent analysis.

2.2 | Analyses

Each fresh slurry portion was used to determine dry matter (DM) by drying to constant weight in a stove at 105°C, relative density (RD) with a hygrometer after stirring and 15 s of stabilization (Chescheir et al., 1985), pH, and electrical conductivity (EC) by potentiometry in undiluted, unfiltered slurry.

In addition, this fresh portion was used for microbiological analyses that were carried out less than 24 h after sampling. The tests were performed from a 10 g aliquot of fresh slurry diluted with 90 mL of buffered peptone water, followed by further 10-fold serial dilutions (1 mL previous dilution + 9 mL buffer solution). One milliliter of each dilution was inoculated in 3 M™ Petrifilm™ *E. coli*/Coliform Count Plates to determine (a) colony forming units (CFU) of total coliforms, after incubation at 30°C for 24 h, according to ISO 4832:2006 (ISO, 2006); (b) CFU of 24-h thermotolerant (fecal) coliforms by incubation at 44°C for 24 h, according to NF V08-060 (04/2009) (AFNOR, 2009); and (c) CFU of *Escherichia coli* after incubation at 37°C for 24 h according to ISO 16649-2:2001 (ISO, 2001). Aerobic mesophilic bacteria were determined according to ISO 4833:2013 (ISO, 2013) inoculating 1 mL of dilution on agar plates and incubating them at 30°C for 72 h. Volumes of 100 μL of 10-fold dilutions were used to determine *Enterococcus* spp. with Kanamycin Aesculin Azide Agar Base (Dehydrated) from Thermo Scientific™, after 24 h of incubation at 37°C, using the manufacturer's version of the method of Mossel et al. (1973) and *Salmonella* spp. according to Leifson (1935) after 48 h of incubation at 37°C. Thus, the minimum detection limits resulted in 100 CFU·mL^{-1} for *Salmonella* spp. and *Enterococcus* spp. and 10 CFU·mL^{-1} for the other bacteria.

Biological oxygen demand after 5 days (BOD_5) was determined with BOD System 6 equipment from Velp Scientifica and chemical oxygen demand (COD) according to APHA (1999).

Each freeze-dried slurry portion was used to determine total C and N on a LECO 2000 combustion analyzer following grinding to <1 mm particles and P, K, Ca, Mg, Na, B, Fe, Mn, Cd, Cr, Cu, Hg, Ni, Pb, and Zn by ICP-MS after microwave-assisted digestion with nitric acid on an ETHOS 900 Labstation (USEPA, 2007).

2.3 | Statistical analysis

Data were subjected to basic descriptive analysis (minimum and maximum values, mean, and deviation) with Microsoft Excel® v. 2018 and also to statistical analysis with SPSS Statistics v. 25 from IBM Corp. (Armonk, NY, USA).

Those results exhibiting homoscedastic variances in Levene's test were examined for significant differences between farm types by one-way analysis of variance (ANOVA). In the presence of differences, data were subjected to Tukey's post hoc HSD test. Dunnett's T3 test was used instead with non-homoscedastic variances. Differences between sampling dates were sought with Student's *t* test. Also, Pearson's test was used to identify bivariate correlations between measured parameters and N, P_2O_5, K_2O, CaO, and MgO contents. Nonlinear and simple and multiple regression equations were used to identify those variables predicting the previous contents with the highest accuracy in terms of R^2 by using pH, EC, DM, and RM as independent variables.

3 | RESULTS

3.1 | Slurry composition

As can be seen in Table 1, the mean pH of the slurries was 7.11 and scarcely variable within groups (CV < 0.5%). There were differences in pH between types of farm, however, with a mean of 6.02 for nursery piglets (NP), 7.31 for lactating sows (LS), and 7.37 for growing pigs (GP). The content in dry matter (DM) averaged at 5.65% but ranged widely (0.41%–16.97%). This was also the case with the relative density (RD), which ranged from 0.99 to 1.07 $kg{\cdot}m^{-3}$. The electrical conductivity (EC) differed between farm types and was higher for GP than it was for LS. Student's *t* test revealed the absence of significant differences in DM and EC between the two sampling seasons (winter and summer).

The mean contents in highly soluble nutrients such as K, Mg, and Na were highest in the GP group (6.87, 1.08, and 2.34 $mg{\cdot}kg^{-1}$, respectively), intermediate in the LS group (6.21, 0.80, and 2.11 $mg{\cdot}kg^{-1}$, respectively), and lowest in the NP group (4.43, 0.60, and 1.37 $mg{\cdot}kg^{-1}$, respectively). Also, the mean Ca contents of GP and LS slurries (2.23 and 2.21 $mg{\cdot}kg^{-1}$, respectively) were significantly higher than was that of NP slurries (1.15 $mg{\cdot}kg^{-1}$). There were no significant differences in N or P contents between groups. There was high variability in the contents of macronutrients (particularly P and K, with CV > 70%).

As regards fertilizer value, GP slurries contained increased amounts ($kg{\cdot}m^{-3}$) of N, CaO, and MgO relative to the others. Although the K_2O and P_2O_5 contents followed the same trend, they did not differ significantly between groups. The contents in P_2O_5 exhibited the highest variability (4.91 ± 6.39 $kg{\cdot}m^{-3}$); also, they were the highest, followed by those of K_2O, N, CaO, and MgO (3.52 ± 3.18, 3.29 ± 2.31, 1.75 ± 1.62, and 1.03 ± 1.05 $kg{\cdot}m^{-3}$, respectively).

Regarding heavy metals, Cu and Zn were present at very high and worrying levels in NP slurries (1029 and 4678 $mg{\cdot}kg^{-1}$, respectively)—much higher indeed than those of GP slurries (320.6 and 1231 $mg{\cdot}kg^{-1}$, respectively) and LS slurries (121.1 and 847.7 $mg{\cdot}kg^{-1}$, respectively). All other metals were present at levels below the tolerated limits set by Regulation (EU) 2019/1009 (2019).

3.2 | Bacteriology

The most abundant bacterial group in the slurries was that of aerobic mesophiles, with a mean of 7.03 Log $CFU{\cdot}mL^{-1}$ and no significant differences between farm types. Also, there was little variability within and between groups. The mean total coliform level was 4.80 Log $CFU{\cdot}mL^{-1}$ and also did not differ between groups. That of fecal coliforms was 3.88 Log $CFU{\cdot}mL^{-1}$ but differed between groups, with 4.77 ± 0.67, 3.78 ± 1.85, and 3.64 ± 1.18 Log $CFU{\cdot}mL^{-1}$ for LS, GP, and NP, respectively. *Escherichia coli* levels were also similar among groups, with 4.61 ± 0.64, 3.26 ± 1.93 and 3.38 ± 1.26 Log $CFU{\cdot}mL^{-1}$ for LS, NP, and GP, respectively.

Salmonella spp. levels were considerably higher in NP slurries than they were in GP slurries (3.60 ± 1.89 vs. 1.91 ± 1.86 Log $CFU{\cdot}mL^{-1}$) but similar to those in LS slurries (3.44 ± 1.50 Log $CFU{\cdot}mL^{-1}$). *Salmonella* spp. were below the minimum detection limit (100 $CFU{\cdot}mL^{-1}$) from 13%, 17%, and 44% of all LS, NP, and GP samples, respectively.

Enterococcus spp. levels averaged at 5.27 ± 0.88 Log $CFU{\cdot}mL^{-1}$ and differed little among farm types. A Student's *t* test was used to look for differences in bacteria levels for seasons of year (spring or autumn). The sampling seasons did not cause significant differences except for slightly higher levels in *Enterococcus* spp. in winter than in summer (5.43 vs. 5.11 Log $CFU{\cdot}mL^{-1}$).

3.3 | Correlations

Nutrient contents in fresh slurry ($kg{\cdot}m^{-3}$) were significantly correlated with dry matter (DM) and relative density (RD) in all cases, with greater Pearson's *r* values for DM (Table 2). DM and RD were also significantly correlated ($r = 0.489$, $p < 0.001$; Table 3).

pH was significantly correlated with organic C and also with metals such as Cu or Zn. However, there were no such correlations within groups (see Data S1) except for pH and C ($r = -0.518$, $p < 0.001$) and pH and COD ($r = -0.223$, $p < 0.05$) in the GP group. There was thus no direct correlation, but rather common causality probably due to a proportional effect of the group factor on these parameters.

Electrical conductivity (EC) exhibited low, but significant, correlation with soluble elements such as N ($r = 0.210$, $p < 0.05$), K ($r = 0.242$, $p < 0.01$), and Na ($r = 0.268$, $p < 0.01$). Dry matter (DM) was negatively correlated with the contents in K ($r = -0.387$, $p < 0.001$) and Na ($r = -0.438$, $p < 0.001$), and so was RD with K ($r = -0.201$, $p < 0.05$) and Na ($r = -0.220$, $p < 0.05$).

Other correlations worth noting were those between N and Na ($r = 0.293$), N and K ($r = 0.263$), Na and K ($r = 0.936$), and Ca and Mg ($r = 0.699$). There were additional correlations between microelements (Mn, Cd, Cr, and Zn).

TABLE 1 Physicochemical properties of slurries from each type of farm.

	Lactating sows ($n = 6$)					Nursery piglets ($n = 6$)					Growing pigs ($n = 24$)					All samples	
Parameter	Mean		Min	Max	SD	Mean		Min	Max	SD	Mean		Min	Max	SD	Mean	SD
Physicochemical properties																	
pH	7.31	b	6.88	7.62	0.20	6.02	a	5.62	6.66	0.27	7.37	b	6.35	8.41	0.36	7.11	0.61
EC ($dS{\cdot}m^{-1}$)	11.96	a	4.30	20.66	4.31	13.8	ab	4.25	22.80	5.79	16.82	b	1.55	29.20	6.00	15.34	5.99
RD ($kg{\cdot}m^{-3}$)	1.017	a	1.007	1.037	0.009	1.021	a	1.003	1.055	0.014	1.019	a	0.990	1.070	0.013	1.019	0.012
DM (%)	4.19	a	0.89	11.78	3.00	5.77	a	1.11	14.35	3.51	6.07	a	0.41	16.97	3.79	5.65	3.65
C (%)	37.49	a	23.10	49.33	6.48	49.49	b	39.50	57.91	5.87	37.04	a	22.11	51.54	6.82	39.46	8.14
BOD_5 ($mg\ O_2{\cdot}L^{-1}$)	6963	a	74	29872	6824	10790	a	900	28728	6322	9067	a	300	37499	9535	8996	8580
COD ($mg\ O_2{\cdot}L^{-1}$)	19545	a	3723	79840	17 062	39335	b	1080	99768	28511	23729	a	2550	78528	17295	25870	20754
Macronutrients (% d.m.)																	
N	5.07	a	2.99	15.56	2.39	5.24	a	3.92	7.44	0.84	6.24	a	3.01	14.31	2.17	5.83	2.09
P	3.11	a	1.56	9.84	2.01	2.77	a	0.36	11.21	2.17	3.73	a	0.74	20.64	3.14	3.43	2.80
K	6.21	ab	1.02	15.43	4.66	4.43	a	0.60	11.52	3.14	6.87	b	1.73	19.42	4.46	6.29	4.35
Ca	2.21	b	0.70	4.21	0.79	1.15	a	0.48	1.78	0.29	2.23	b	0.66	5.31	0.94	2.02	0.93
Mg	0.80	ab	0.15	1.78	0.44	0.60	a	0.08	1.65	0.35	1.08	b	0.07	2.33	0.62	0.94	0.58
Na	2.11	ab	0.38	5.83	1.55	1.37	a	0.09	2.88	0.77	2.34	b	0.45	6.19	1.45	2.12	1.41
Micronutrients and heavy metals ($mg{\cdot}kg^{-1}$)																	
B	67.11	a	11.71	460.1	90.01	75.40	b	15.35	201.0	44.79	71.6	ab	24.77	265.3	34.47	71.47	50.76
Fe	1801	a	358.0	4099	1102	2111	a	652.6	3223	773.4	1819	a	340.2	4951	1083	1871	1035
Mn	443.2	a	73.84	1006	238.5	574.0	ab	290.2	1368	230.9	609.3	b	92.56	1549	298.7	571.5	281.9
Cd	0.33	a	0.06	0.63	0.15	0.37	a	0.07	0.80	0.17	0.41	a	0.07	1.38	0.21	0.39	0.19
Cu	121.1	a	33.96	333.5	69.37	1029	c	355.6	2128	446.6	320.6	b	35.95	1316	190.9	416.0	390.9
Cr	10.11	a	2.36	25.65	6.14	9.93	a	4.28	19.11	4.43	8.79	a	4.06	21.18	2.98	9.25	4.03
Hg	0.05	a	0.00	0.35	0.09	0.02	a	0.00	0.18	0.04	0.03	a	0.00	0.20	0.04	0.03	0.05
Ni	8.16	a	1.90	26.39	5.74	8.49	a	2.35	17.74	4.85	9.09	a	1.86	20.84	4.10	8.81	4.57
Pb	0.97	b	0.00	3.99	0.94	1.22	b	0.00	3.54	1.00	0.55	a	0.00	1.95	0.44	0.75	0.73
Zn	847.7	a	128.7	2030	490.0	4678	b	1674	9352	2071	1231	a	152.6	2689	530.2	1806	1713
Bacteriology (Log $CFU{\cdot}mL^{-1}$)																	
Aerobic mesophilic	7.31	a	6.32	8.03	0.52	7.12	a	5.36	8.73	1.00	6.92	a	5.48	8.10	0.57	7.03	0.68
Total coliforms	4.98	b	3.68	6.27	0.70	4.84	a	0.00	6.69	1.66	4.73	a	3.07	7.09	0.76	4.80	0.98
Fecal coliforms	4.77	a	3.63	6.05	0.67	3.78	ab	0.00	5.69	1.85	3.64	a	0.00	5.60	1.18	3.88	1.32
E. coli	4.61	a	3.24	5.76	0.64	3.26	b	0.00	5.42	1.93	3.38	b	0.00	5.57	1.26	3.59	1.41
Enterococcus spp.	5.55	a	4.51	6.90	0.67	5.80	a	2.96	7.73	1.15	5.03	a	2.96	6.98	0.74	5.27	0.88
Salmonella spp.	3.44	ab	0.00	5.53	1.50	3.60	b	0.00	6.07	1.89	1.91	a	0.00	5.57	1.86	2.52	1.95

TABLE 1 (Continued)

Parameter	Lactating sows ($n = 6$)				Nursery piglets ($n = 6$)				Growing pigs ($n = 24$)				All samples	
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	SD
Fertilizer value (kg·m^{-3})														
N	1.99 a	0.47	4.93	1.33	3.07 ab	0.46	6.31	1.78	3.75 b	0.12	12.99	2.52	3.29	2.31
P_2O_5	3.15 a	0.32	10.85	3.09	3.40 a	0.38	11.56	3.01	5.89 a	0.11	46.80	7.60	4.91	6.39
K_2O	2.88 a	0.14	15.96	3.45	2.43 a	0.30	6.15	1.45	4.04 a	0.27	21.30	3.38	3.52	3.18
CaO	1.45 ab	0.09	6.51	1.41	0.94 a	0.13	2.33	0.60	2.08 b	0.08	9.03	1.79	1.75	1.62
MgO	0.65 ab	0.02	2.31	0.63	0.58 a	0.02	1.45	0.40	1.27 b	0.01	6.15	1.20	1.03	1.05

Note: Different letters in each row denote significant differences at $p \leq 0.05$.
Abbreviations: BOD, biological oxygen demand; COD, chemical oxygen demand; d.m., dry matter; DM, dry matter; EC, electrical conductivity; RD, relative density.

As regards bacteria, they were positively correlated with parameters connected to organic matter, as *Enterococcus* spp. with C ($r = 0.245$), total coliforms with COD ($r = 0.300$), and *Enterococcus* spp. with COD ($r = 0.379$). On the other hand, fecal coliforms, *E. coli*, and *Enterococcus* spp. were negatively correlated with EC ($r = -0.240$, $r = -0.265$, and $r = -0.180$, respectively), and so were aerobic mesophilic bacteria, *Enterococcus* spp., and total coliforms with Hg ($r = -0.309$, $r = -0.286$, and $r = -0.197$, respectively). All groups of bacteria exhibited moderate to high correlations with each other.

The number of variables was reduced by using principal component analysis (PCA), the factor matrix thus obtained being subjected to varimax rotation. The results are summarized in Table 4. Three different principal components were selected that jointly accounted for 45.1% of the overall variance (PCA_1 for 20.2%, PCA_2 for 14.8%, and PCA_3 for 10.2%). PCA_1, which discriminated the NP group (Figure 1), was correlated positively with Cu, Zn, C, and COD and negatively with pH, Na, Ca, and K (Figure 2). PCA_2 accounted for bacteria content, and PCA_3, which accounted for nothing in particular, was positively correlated with Ca, Mg, Mn, and Cd and negatively correlated with K and Na. None of the factors discriminated the LS and GP groups.

3.4 | Regression equations

As can be seen in Table 5, dry matter (DM) was the most common predictor in the regression equations. Only relative density (RD) was a better predictor for N content in LS and NP slurries. The equations exhibited close fitting, with R^2 values up to 0.889. The goodness-of-fit of some equations was improved by adding a second predictor (pH or EC), but only slightly (less than 0.05 except when adding EC to DM to predict the MgO content in NP slurries, which increased R^2 by 0.143). The equations for the individual farm type generally exhibited better goodness-of-fit than the overall model for the three groups. The improvement amounted to 0.067 and 0.039 R^2 units with linear and nonlinear regression equations, respectively.

The nonlinear equations providing the best fit were of the exponential type—by exception, inverse equations performed better with the N content of LS slurries. In most cases, using nonlinear equations improved R^2 by up to 0.095 units by exception, it failed to increase R^2 in predicting the K_2O and MgO contents of LS slurries and the MgO contents of GP slurries. As with the linear equations, DM was the most common predictor for the nonlinear ones, with EC as the best for estimating K_2O in most cases.

Nitrogen was the individual macronutrient exhibiting the best fitting in nondiscriminated samples ($R^2 = 0.845$ with linear equations and $R^2 = 0.870$ with exponential equations). This allowed the fertilizer value of the slurries to be estimated with a mean error less than 26% and 22%, respectively. With a single mean value (3.29 kg N·m^{-3}), the error rose to 134%. The mean errors in the P_2O_5, K_2O, CaO, and MgO contents of non-discriminated samples as estimated with linear equations were 66.2%, 69.4%, 69.4%, and 116%, respectively, whereas those made with nonlinear equations were 50.6%, 66.2%,

TABLE 2 Pearson correlation matrix between easily determined parameters and macronutrient contents.

	N ($kg·m^{-3}$)	P_2O_5 ($kg·m^{-3}$)	K_2O ($kg·m^{-3}$)	CaO ($kg·m^{-3}$)	MgO ($kg·m^{-3}$)
pH	−0.101	0.049	0.047	0.120	0.106
EC ($dS·m^{-1}$)	−0.045	−0.156	0.147	−0.228**	−0.141
RD ($kg·m^{-3}$)	0.502***	0.392***	0.229*	0.406***	0.428***
DM (%)	0.903***	0.630***	0.442***	0.829***	0.784***

Abbreviations: DM, dry matter; EC, electrical conductivity; RD, relative density.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
***Significant at $p < 0.001$.

38.0%, and 77.0%, respectively. The errors ensuing from the use of a single mean value were much greater: 257% for P_2O_5, 119% for K_2O, 204% for CaO, and 518% for MgO.

4 | DISCUSSION

The mean pH of the slurries was lower than previously reported values (Antezana et al., 2016; Moral et al., 2005; Suresh & Choi, 2011), possibly, as suggested by Beccaccia et al. (2015), as a result of the water supply (6.57 ± 0.61) being more acidic than, for example, those measured by Moral et al. (2005): 7.5–7.9. Water is in fact a major component of slurries, and we found significant correlation between pH in the water supply and in the slurries ($r = 0.357$, $p < 0.05$).

Electrical conductivity (EC) was slightly lower and spanned a narrower range than those reported elsewhere (Antezana et al., 2016; Suresh & Choi, 2011; Yagüe et al., 2012). Also, it was significantly higher in GP slurries than in the others. These results are consistent with those of previous studies and were probably a consequence of increased dietary salt and protein contents (Moral et al., 2005). One other potentially influential factor was lower dilution of the slurries by effect of the animals wasting less water (Palhares, 2016. Consistent with this assumption, GP slurries exhibited the highest contents in DM; however, the data were so variable that they concealed any significant differences between groups. In fact, the highest DM content was 41 times the lowest. Previous studies (Antezana et al., 2016; Martínez-Suller et al., 2008; Suresh & Choi, 2011; Yagüe et al., 2012) revealed similar or even greater variability (up to 60 times). So wide variability in water content resulted in also wide variability in nutrient contents. Therefore, an accurate knowledge of the DM content of slurry is crucial with a view to assessing its fertilizer value.

Dry matter (DM) can be estimated through relative density (RD). The two were moderately but significantly correlated here. In any case, RD spanned a wider range (0.990 and 1.070 $kg·m^{-3}$) than elsewhere, where it never exceeded 1.04 $kg·m^{-3}$ (Moral et al., 2005; Suresh et al., 2009; Suresh & Choi, 2011; Zhu et al., 2003).

Overall, the contents in macronutrient and micronutrient, and those in heavy metals, are consistent with those reported by other authors (Abubaker et al., 2015; Antezana et al., 2016; Möller & Stinner, 2009; Moral et al., 2005; Pantelopoulos & Aronsson, 2021). On the other hand, the P contents are higher than usual for swine slurries with the sole exception of those reported by Suresh and Choi (2011). Also, the N contents are lower than usual, whereas the K and Na contents are slightly higher than previously reported values but span similar ranges.

NP slurries were markedly different from LS and GP slurries. PCA_1 accounted for the differences, explained 20% of the total variance and discriminated the NP group from the other two. NP slurries had the highest pH values, probably because of their high contents in volatile fatty acids (VFA) and/or low contents in ammonia nitrogen ($N\text{-}NH_4$) (Paul & Beauchamp, 1989). In fact, Antezana et al. (2016) previously found NP slurries to contain increased levels of VFA and decreased levels of $N\text{-}NH_4$. NP slurries had significantly lower contents in K, Ca, and Mg and also in P here. At early growth stages, pigs are fed mineral-richer diets than lactating sows and growing pigs (NRC, 2012; Rostagno et al., 2017). However, nutrition efficiency is much higher in young pigs than it is in adult pigs (Creech et al., 2004; Fix et al., 2010). This results in an increased proportion of nutrients being absorbed and a decreased proportion excreted. An identical conclusion was previously drawn by Antezana et al. (2016). There were also differences in Cu and Zn levels between slurry groups. Thus, NP slurries had the highest contents in both metals, which exceeded the tolerated limit for organic fertilizers set by Regulation (EU) 2019/1009 (2019) (300 $mg·kg^{-1}$ for Cu and 800 $mg·kg^{-1}$ for Zn) by a factor of up to 3. Continuous use of slurries with high Cu and Zn contents can lead to accumulation in soil, and also on plants growing on it, thereby threatening animal, human, and environment health (Provolo et al., 2018; Tang et al., 2020). While the Cu and Zn contents of the studied slurries can be worrisome, they are very similar to others found in previous work (Antezana et al., 2016; Moral et al., 2005; Pantelopoulos & Aronsson, 2021). This is due to addition of Cu and Zn in amounts that exceed their nutritional requirements, as they are known to promote growth and prevent diarrhea (Bonetti et al., 2021; Hill et al., 2001). The differences in Cu and Zn contents between farm types are consistent with the fact that supplies of these two elements are reduced during fattening and suppressed from sows' diets (Hill & Spears, 2000; Reese & Hill, 2010).

All other heavy metals analyzed were present at levels below the legally tolerated limits. Such levels decreased in the following

TABLE 3 Pearson correlation matrix between slurry properties.

	pH	EC	RD	DM	C	BOD_5	COD	N	P	K	Ca	Mg	Na	B	Fe	Mn
EC	0.152ns															
RD	−0.116ns	0.093ns														
DM	−0.147ns	−0.162ns	0.489***													
C	−0.661***	−0.106ns	0.109ns	0.270**												
BOD_5	−0.080ns	0.002ns	0.079ns	−0.020ns	0.252**											
COD	−0.347***	0.053ns	0.067ns	0.276**	0.509***	0.310***										
N	0.127ns	0.210*	−0.006ns	−0.074ns	−0.145ns	0.056ns	−0.114ns									
P	0.086ns	−0.079ns	0.112ns	0.140ns	0.082ns	0.199*	−0.077ns	0.027ns								
K	0.314***	0.242**	−0.220*	−0.438***	−0.616***	−0.098ns	−0.356***	0.263**	−0.051ns							
Ca	0.330***	−0.197*	0.031ns	0.390***	−0.062ns	−0.110ns	0.007ns	−0.034ns	0.256**	−0.386***						
Mg	0.173*	−0.016ns	0.134ns	0.379***	0.094ns	0.043ns	0.078ns	−0.083ns	0.307***	−0.508***	0.699***					
Na	0.336***	0.268**	−0.201*	−0.387***	−0.612***	−0.101ns	−0.359***	0.293***	−0.109ns	0.936***	−0.33***	−0.429***				
B	−0.061ns	0.133ns	−0.009ns	−0.132ns	−0.158ns	−0.154ns	−0.098ns	0.091ns	0.009ns	0.239**	−0.103ns	−0.025ns	0.208*			
Fe	−0.089ns	−0.161ns	0.206*	0.217*	0.107ns	0.162ns	−0.007ns	−0.106ns	0.231**	−0.45***	0.179*	0.306***	−0.487***	−0.296***		
Mn	−0.084ns	−0.119ns	0.076ns	0.430***	0.183*	−0.008ns	0.193*	−0.147ns	0.228**	−0.623***	0.62***	0.621***	−0.615***	−0.115ns	0.495***	
Cd	0.025ns	0.004ns	−0.052ns	0.186*	0.310***	0.124ns	0.366***	−0.093ns	−0.011ns	−0.508***	0.341***	0.476***	−0.453***	0.004ns	0.098ns	0.509***
Cu	−0.637***	−0.104ns	−0.002ns	0.062ns	0.542***	0.186*	0.440***	−0.101ns	−0.143ns	−0.374***	−0.248**	−0.037ns	−0.367***	0.047ns	0.165ns	0.258**
Cr	−0.079ns	−0.155ns	−0.020ns	0.133ns	0.056ns	−0.055ns	0.207*	−0.156ns	−0.212*	−0.4***	0.189*	0.255**	−0.337***	−0.049ns	0.237**	0.321***
Hg	0.042ns	−0.057ns	−0.156ns	−0.120ns	−0.036ns	−0.054ns	−0.107ns	−0.026ns	0.02ns	0.133ns	0.021ns	−0.036ns	0.079ns	0.430***	−0.04ns	−0.050ns
Ni	0.088ns	0.043ns	−0.063ns	−0.138ns	−0.261**	−0.230**	0.042ns	−0.031ns	−0.427***	0.108ns	−0.039ns	−0.042ns	0.155ns	0.250**	−0.208*	−0.004ns
Pb	−0.297***	−0.233**	0.035ns	−0.054ns	0.078ns	−0.151ns	−0.006ns	0.054ns	−0.358***	−0.191*	−0.107ns	−0.159ns	−0.191*	0.032ns	0.043ns	0.061ns
Zn	−0.681***	−0.081ns	0.050ns	0.075ns	0.578***	0.108ns	0.465***	−0.135ns	−0.158ns	−0.384***	−0.254**	−0.058ns	−0.367***	0.029ns	0.155ns	0.203*
Aerobic mesophilic	0.073ns	−0.115ns	−0.074ns	0.012ns	0.030ns	−0.132ns	0.266**	−0.166ns	−0.215*	−0.206*	0.038ns	0.04ns	−0.192*	−0.279**	0.105ns	0.112ns
Fecal \coliforms	0.039ns	−0.240**	0.076ns	−0.102ns	−0.050ns	0.057ns	0.066ns	−0.061ns	0.026ns	−0.001ns	−0.009ns	−0.097ns	−0.009ns	−0.180*	0.043ns	−0.085ns
Total coliforms	0.003ns	−0.120ns	−0.036ns	−0.007ns	0.064ns	0.155ns	0.300***	−0.149ns	−0.059ns	−0.141ns	0.022ns	−0.036ns	−0.154ns	−0.144ns	0.018ns	0.092ns
E. coli	0.157ns	−0.265**	0.063ns	−0.098ns	−0.101ns	0.052ns	−0.012ns	−0.043ns	0.037ns	−0.011ns	0.065ns	−0.005ns	0.012ns	−0.156ns	0.029ns	−0.074ns
Enterococcus spp.	−0.065ns	−0.180*	0.013ns	−0.006ns	0.171ns	0.245**	0.379***	−0.103ns	0.047ns	−0.174*	−0.054ns	−0.035ns	−0.177*	−0.291***	0.105ns	0.042ns
Salmonella spp.	−0.132ns	−0.123ns	−0.009ns	−0.025ns	0.119ns	0.164ns	0.149ns	−0.055ns	0.042ns	−0.109ns	−0.039ns	−0.044ns	−0.14ns	−0.116ns	0.144ns	0.042ns

(Continues)

TABLE 3 (Continued)

	Cd	Cu	Cr	Hg	Ni	Pb	Zn	Aerobic mesophilic	Fecal coliforms	Total coliforms	*E. coli*	*Enterococcus* spp.
EC												
RD												
DM												
C												
BOD_5												
COD												
N												
P												
K												
Ca												
Mg												
Na												
B												
Fe												
Mn												
Cd												
Cu	0.450***											
Cr	0.456***	0.348***										
Hg	−0.005ns	−0.109ns	−0.117ns									
Ni	0.187*	0.153ns	0.526***	−0.077ns								
Pb	0.079ns	0.287***	0.382***	−0.039ns	0.376***							
Zn	0.339***	0.919***	0.319***	−0.120ns	0.145ns	0.336***						
Aerobic mesophilic	0.076ns	0.087ns	0.245**	−0.309***	0.078ns	0.039ns	0.057ns					
Fecal coliforms	−0.180*	−0.110ns	−0.037ns	−0.146ns	−0.079ns	−0.113ns	−0.087ns	0.501***				
Total coliforms	0.095ns	0.165ns	0.055ns	−0.197*	−0.019ns	−0.039ns	0.182*	0.576***	0.595***			
E. coli	−0.143ns	−0.205*	−0.007ns	−0.103ns	0.002ns	−0.121ns	−0.199*	0.473***	0.822***	0.514***		
Enterococcus spp.	0.115ns	0.225*	0.112ns	−0.286**	−0.115ns	−0.041ns	0.208*	0.640***	0.673***	0.705***	0.583***	
Salmonella spp.	0.028ns	0.167ns	0.080ns	−0.081ns	−0.171ns	0.011ns	0.188*	0.446***	0.522***	0.607***	0.411***	0.596***

Abbreviations: BOD, biological oxygen demand; COD, chemical oxygen demand; DM, dry matter; EC, electrical conductivity; ns, not significant; RD, relative density.

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

TABLE 4 Pearson correlations between the principal components for slurry properties, composition, and bacteria concentrations.

	PCA_1	PCA_2	PCA_3
pH	−0.820***		0.176*
EC		−0.183*	
RD			
DM	0.307***		0.374***
C (d.m.)	0.788***		0.188*
BOD_5	0.224**		
COD	0.630***	0.199*	0.191*
N (d.m.)	−0.234**		−0.202*
P (d.m.)			0.208*
K (d.m.)	−0.504***		−0.644***
Ca (d.m.)	−0.346***		0.799***
Mg (d.m.)			0.844***
Na (d.m.)	−0.531**		−0.587***
B (d.m.)			
Fe (d.m.)	0.168*		0.264**
Mn (d.m.)	0.240**		0.759***
Cd (d.m.)	0.324***		0.695***
Cu (d.m.)	0.853***		
Cr (d.m.)	0.210*		0.385***
Hg (d.m.)		−0.196*	
Ni (d.m.)			
Pb (d.m.)	0.206*		
Zn (d.m.)	0.875***		
Aerobic mesophilic		0.748***	
Fecal coliforms		0.822***	−0.178*
Total coliforms		0.829***	
E. coli	−0.244**	0.752***	
Enterococcus spp.	0.240**	0.833***	
Salmonella spp.	0.226**	0.721***	

Abbreviations: BOD, biological oxygen demand; COD, chemical oxygen demand; d.m., dry matter; DM, dry matter; EC, electrical conductivity; RD, relative density.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
***Significant at $p < 0.001$.

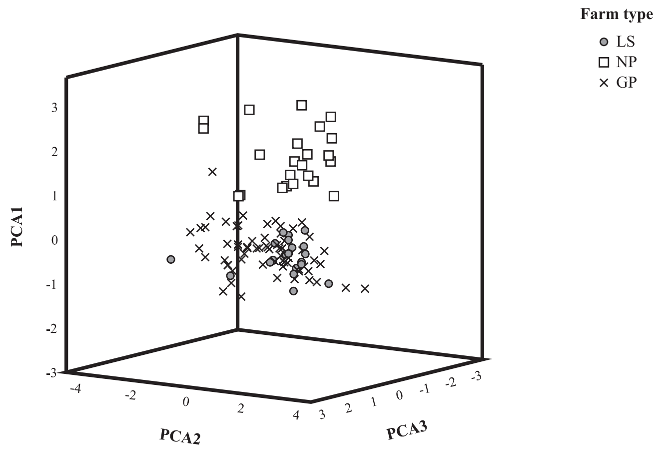

FIGURE 1 Principal component analysis (PCA) scores plot for different types of farms. GP, growing pigs, LS, lactating sows, NP, nursery piglets.

sequence: Cr > Ni > Pb > Cd > Hg. This sequence, and the specific levels of each metal, is consistent with previous reports (Antezana et al., 2016; Leclerc & Laurent, 2017; Tang et al., 2020).

Because they were raw slurries, their levels of fecal contamination indicators were high relative to other organic fertilizers such as digestates and composts. However, specific populations were similar in number to those found in other raw slurries. Such was the case with *Salmonella* spp. which was present in a considerable proportion of samples (66%) compared to previous reports (5% to 71%) (Caballero-Lajarín et al., 2015; Hutchison et al., 2004; Watabe et al., 2003) although the detection limit of the method used here was higher than that of other possible methods. European legislation requires the absence of *Salmonella* spp. from organic fertilizers, and *E. coli* and *Enterococcus* spp. levels not to exceed 3 Log CFU·mL^{-1} (Regulation [EU] 2019/1009, 2019). Only one of the 124 samples examined fulfilled all three requirements. Therefore, in order to use pig slurry as an organic fertilizer in accordance with this regulation, a sanitization treatment would be necessary in addition to storage (Skowron et al., 2013). If raw slurry is used, it will be important to avoid direct contact with the edible organ (for example, using hanging tubes or injection to the application) and to ensure safety periods that guarantee its safe consumption (Nicholson et al., 2004).

Some authors have found bacterial survival in slurries to decrease with increasing temperature (Goss et al., 2013; Nicholson et al., 2005; Tian et al., 2021). In this work, the factor sampling season influenced the levels of *Enterococcus* spp.—which were lower in the warm season—but not those of the other bacteria groups. Electrical conductivity (EC) was significantly correlated, in a negative manner, with fecal coliforms, *E. coli*, and *Enterococcus* spp. Suresh et al. (2009) previously found negative correlation between EC and *Salmonella* spp. in swine slurry. Elevated salinity is known to adversely affect the survival of various bacterial groups (Anderson et al., 2005; Bordalo et al., 2002). Elements such as K and Na, which are primarily found in dissolved form in slurries (Masse et al., 2005), were also negatively correlated with aerobic mesophilic bacteria and *Enterococcus* spp. Because correlations between bacterial groups were all high, it made no sense to use more than one group as indicator of fecal contamination. In fact, PCA_2 gathered all studied bacterial groups in a single variable and accounted for 14.67% of the total variance.

Dry matter (DM) is usually an accurate indicator of nutrient contents as it accounts for most of the variability due to dilution (Antezana et al., 2016). However, it takes a long time to measure because it requires waiting for the slurry to dry. In any case, DM is easy to measure and requires no skilled staff or dedicated equipment. Using it as a predictor provided regression equations very closely

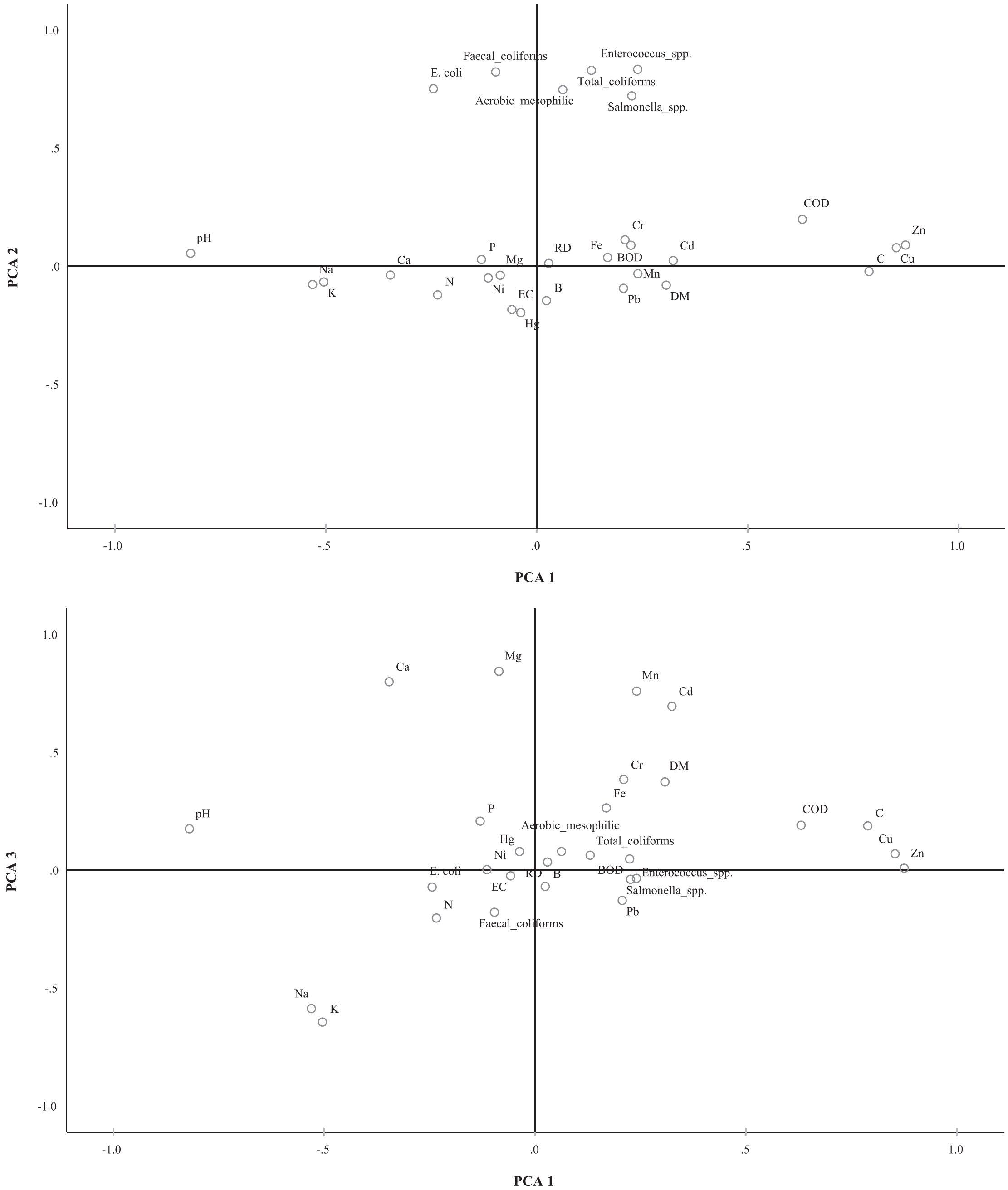

FIGURE 2 Loading plots for different variables on PCA_1, PCA_2, and PCA_3. BOD, biological oxygen demand; COD, chemical oxygen demand; DM, dry matter; EC, electrical conductivity; RD, relative density.

fitting N contents and closely fitting CaO and MgO contents. The goodness-of-fit was similar to that obtained in previous studies (Martínez-Suller et al., 2008; Suresh & Choi, 2011). By contrast, P_2O_5 contents were poorly predicted, especially if one considers that they are highly correlated with DM and RD (Moral et al., 2005; Suresh & Choi, 2011; Yagüe et al., 2012). DM also provided poor predictions of K_2O. A good prediction of K_2O content was only achieved when EC was used as the sole predictor of the model. This is because most of the K is in dissolved form in the slurries (Masse et al., 2005). Moral et al. (2005) and Yang et al. (2006) also found EC to be the best predictor for K_2O.

As expected, using an additional predictor resulted in improved fitting; however, the improvement was so small that it does not warrant purchase of a second measuring set or the delay in measuring another variable. Moral et al. (2005), Suresh and Choi (2011), and Yagüe et al. (2012) also obtained very modest improvements by using a second predictor, with an increase in R^2 of only 0.025, 0.06, and 0.076, respectively.

TABLE 5 Predictive equations for N, P_2O_5, K_2O, CaO, and MgO contents, all in $kg{\cdot}m^{-3}$.

Linear regression equations	R^2	Nonlinear regression equations	R^2
Lactating sows			
$N = 0.1031 + 0.4680\ DM$	0.799***	$N = 85.8350 - (85.497\ RD^{-1})$	0.419**
$N = -81.4164 + 81.7870\ RD$	0.419***		
$P_2O_5 = -0.2923 + 0.8220\ DM$	0.638***	$P_2O_5 = 0.5604\ DM^{1.1052}$	0.726***
$P_2O_5 = 0.406 - 182.7384\ DM$	0.406**	$P_2O_5 = 60.6055\ RD^{0.6687}$	0.416**
$K_2O = 0.2117 + 0.6375\ DM$	0.307**	$K_2O = 0.7809\ DM^{0.6998}$	0.245*
$K_2O = -1.7656 + 0.7280\ DM + 0.1336\ EC$	0.329*		
$CaO = -0.1027 + 0.1808\ DM$	0.880***	$CaO = 0.2127\ DM^{1.2697}$	0.865***
$MgO = -0.1761 + 0.2044\ DM$	0.860***	$MgO = 0.0602\ DM^{1.5157}$	0.748***
Nursery piglets			
$N = 0.2331 + 0.5016\ DM$	0.889***	$N = 0.5017\ DM^{1.0337}$	0.953***
$N = -87.4663 + 88.6394\ RD$	0.471**		
		$P_2O_5 = 0.0.7491\ DM^{0.7610}$	0.368***
$K_2O = -0.2395 + 0.1937\ EC$	0.600***	$K_2O = 0.0687\ EC^{1.3276}$	0.754***
$CaO = 0.1371 + 0.1401\ DM$	0.681***	$CaO = 0.1470\ DM^{1.0475}$	0.866***
$CaO = -0.1986 + 0.1244\ DM + 0.0286\ EC$	0.716***		
$MgO = 0.1092 + 0.085\ DM$	0.487***	$MgO = 0.0507\ DM^{1.3219}$	0.654***
$MgO = -0.2531 + 0.0798\ DM + 0.0276\ EC$	0.630***		
Growing pigs			
$N = 0.1876 + 0.5944\ DM$	0.849***	$N = 0.5781\ DM^{1.0292}$	0.880***
$P_2O_5 = -1.3227 + 1.1809\ DM$	0.415***	$P_2O_5 = 0.4607\ DM^{1.2595}$	0.741***
$K_2O = 2.1038 + 0.2926\ DM$	0.209***	$K_2O = 1.4206\ EC^{0.536}$	0.420***
$CaO = -1.0906 + 0.5669\ DM$	0.805***	$CaO = 0.1917\ DM^{1.2681}$	0.884***
$CaO = -0.2214 + 0.5591\ DM - 0.0473\ EC$	0.821***		
$MgO = -0.3297 + 0.2621\ DM$	0.790***	$MgO = 0.0616\ DM^{1.5157}$	0.726***
All samples			
$N = 0.0942 + 0.5708\ DM$	0.838***	$N = 0.5488\ DM^{1.0213}$	0.870***
$N = -0.5184 + 0.5799\ DM + 0.0365\ EC$	0.845***		
$P_2O_5 = -0.970 + 1.037\ DM$	0.396***	$P_2O_5 = 0.5050\ DM^{1.1645}$	0.682***
$K_2O = -0.093 + 0.270\ DM + 0.118\ EC$	0.271***	$K_2O = 1.1417\ EC^{0.5675}$	0.307***
$CaO = -0.6669 + 0.4406\ DM$	0.689***	$CaO = 0.1882\ DM^{1.2181}$	0.827***
$CaO = -5.786 + 0.481\ DM + 0.696\ pH$	0.713***		
$MgO = -0.2653 + 0.2304\ DM$	0.659***	$MgO = 0.0590\ DM^{1.4848}$	0.715***
$MgO = -2.391 + 0.233\ DM + 0.295\ pH$	0.709***		

Abbreviations: DM, dry matter (%); EC, electrical conductivity ($dS{\cdot}m^{-1}$); RD, relative density ($kg{\cdot}m^{-3}$).
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
***Significant at $p < 0.001$.

Nonlinear regression has scarcely been used to predict fertilizer value. In fact, only Suresh et al. (2009) have reported exponential or polynomial equations with a high goodness-of-fit. Nonlinear predictive models are probably not more difficult to use by farmers than are linear models as they only require measuring certain parameters and substituting their values into a simple equation. This can be an effective way of improving the goodness-of-fit of predictive models and reducing errors in extreme values.

Likewise, using separate equations for each type of farm might further improve fitting with no added complications for farmers since each farm typically raises swine at a single rearing stage. Martínez-Suller et al. (2008) previously obtained average improvements in R^2 of 0.115 units; also, they confirmed that using specific equations for each type of farm led to more accurate predictions of slurry fertilizer value by effect of their encompassing the variability due to differences in diet or animal age.

5 | CONCLUSIONS

The wide range of dry matter (DM) content spanned by the studied slurries resulted in widely variable nutrient levels. The best predictive models were those based on this parameter, which exhibited high goodness-of-fit, especially for N, CaO, and MgO. By exception, electrical conductivity (EC) was a better predictor for K_2O. P_2O_5 estimates were less accurate, but using a predictive model invariably reduced errors from the mean value. Therefore, a thermobalance in combination with the proposed models can provide a rapid, accurate method for estimating the fertilizer value of slurries with a view to adjusting their application rate.

Slurry composition (K, Ca, and Mg), pH, and EC differed among farm types (LS, NP, and GP). Using specific equations for each group can absorb some of the variability observed between groups providing more accurate estimates of fertilizer value without more effort or involvement on the part of farmers. So can using non-linear equations instead of linear equations. On the other hand, the slightly greater accuracy obtained with an additional predictor does not warrant the added expenses and delay of using additional equipment for a second set of measurements.

The main risks in using swine slurries arise from not accurately knowing which specific nutrients, and in what amounts, are added to the soil, the typically high contents in Cu and Zn, and a high likelihood of their containing *Salmonella* spp.

ACKNOWLEDGMENTS

Authors would like to thank the use of RIAIDT-USC analytical facilities.

CONFLICT OF INTEREST STATEMENT

The authors report there are no competing interests to declare.

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How to cite this article: Fernández-Labrada, M., López-Mosquera, M. E., García, L., Barrio, J. C., & López-Fabal, A. (2023). Hazards of swine slurry: Heavy metals, bacteriology, and overdosing—Physicochemical models to predict the nutrient value. *Animal Science Journal*, *94*(1), e13849. https://doi.org/10.1111/asj.13849