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Physicochemical and Phytotoxic Characterisation of Residual Sludge from the Malting of Barley

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Abstract

Residual sludge that results from the treatment of malt house effluents produced during the malting of barley in two malt houses from the Pampa (Argentina) was evaluated to determine its fertilising potential and capability for improving soil as a way to reuse or recycle this material. It should be noted the content of organic matter and nitrogen with a high content of cellulose and hemicellulose and mostly organic nitrogen. The fraction of hydrolysable, non-distillable nitrogen is a good indicator of the degree of oxidisability. It is also remarkable the content of P, K and Ca, Mg, Cu and Zn. However, the amount of trace metals, which do not have a function in biological processes, was very low. *Dactylis glomerata* L. and *Phalaris bulbosa* L. were utilised as seeds in the phytotoxicity tests, with a percentage of germination of less than 90% when the concentration of sludge was increased. Their characteristics and germination test indicate that this residual sludge constitutes a suitable amendment for agricultural soils.

Keywords: Sludge; Nutrients; Heavy metals; Phytotoxicity; Germination index

Introduction

The most commonly used grain for the production of beer is malting barley, and the initial stage of the manufacturing process includes the production of barley malt. The process of malting barley (*Hordeum vulgare* L.) includes the following main steps: a) cleaning and storage of the grains, b) immersion of the grains in water and subsequent germination, and c) transformation of the green malt (the product of germination) into dry malt. The malting process is the enzymatic conversion of starch into maltose [1].

The typical technology used in the treatment of the effluents from malt houses and breweries includes a primary stage for the mechanical separation of the coarse solid waste (grains, rootlets, etc.) and a secondary biological stage (which can be anaerobic or aerobic) for the removal or degradation of the residual organic load.

When using aerobic processes, activated sludge technology is generally used [2]. The excess residual sludge for a conventional activated-sludge treatment is the range of 0.4 to 0.6 kg of sludge per kg of biochemical oxygen demand (BOD) removed [3].

The reuse (recycling) of biosolids as agricultural amendment reduces the disposal of these materials in landfills [4]. These materials have a proven effectiveness as soil amendments in arid / semiarid zones [5].

The malting sludge (the surplus activated sludge) generally does not contain toxic heavy metals in substantial quantities. The main advantage of using sewage sludge as an organic amendment is that the essential nutrients, especially N, are found in an organic form in the sludge, and they are released gradually, allowing them to be used more efficiently by cultivated plants. This is in contrast with conventional chemical fertilisers, in which the essential nutrients are in an inorganic form and are soluble in water, whereby they leach below the root zone [1].

The materials used as soil amendments can contain compounds that negatively affect plant development [6]. Therefore, it is necessary to evaluate beforehand the phytotoxicity [7] of the amendments, and thus, germination bioassays should be performed [8].

Germination indices are widely used as phytotoxicity indicators of both compost [8-10] and organic waste [9-11]. Moreover germination percentages of less than 90% are used by some researchers as early indicators of phytotoxicity [10].

The objective of this work is to characterize and assess the potential phytotoxicity of the sludge of two malting barley to estimate the effects that can cause use as soil amendment, because this operation is being carried out in the south of the Buenos Aires province (Argentina).

Materials and Methods

Material and sampling

In this work, the residual sludge from two barley malt houses was studied. Sludge A was from a malt house located 10 km from Bahia Blanca (Argentina) with a production capacity of 300 Mg of malt per day, and sludge B was from malt house B located 200 km from Bahia Blanca (Argentina) with a production capacity of 250 Mg of malt per day.

The properties of malt house sludge, as in the case of sewage sludge [12], do not follow normal distributions due to typical variations in the malting of barley. In these cases, the median or geometric mean constitutes the typical or average values that best represent the system under analysis.

The sampling of sludge was performed according to the procedure indicated by the EPA [13]. To obtain a representative sample, multiple samples were taken over one week, and they were formed into a composite sample. The procedure was repeated for five consecutive

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weeks. The composite samples were stored in amber glass receptacles and kept in a refrigerator (temperature<4°C).

Sludge characterization

The characterisation of malt house sludge was performed via the evaluation of the physical, chemical, and biological properties. The pH was measured in a sample suspended in double-distilled water (at a sample: water ratio 1:2.5) with a pH meter. The electrical conductivity (related to the content of soluble salts) was measured with a conductivity metre. The dry matter was calculated as the weight difference after evaporation at 110°C.

The methodology indicated in [14] was used to determine the organic matter content in the sludge. The fractionation of the sludge organic matter was performed according to the the method proposed by [15], although the water-soluble compounds were extracted using the procedure recommended in [16].

Kjeldahl nitrogen (N_{Kj}) was determined as per [17], and the amount of nitrates + nitrites was determined by visible absorption spectrophotometry using Hach equipment (DR Model 2010) as described in Method 1686 [18]. Nitrogen ammonium was determined by EPA. Method 350.1 [19].

The organic nitrogen compounds present in the sludge was fractionated by the [20] method.

After performing acid digestion of the sludge samples using nitric/perchloric acid, the total phosphorus content and the concentrations of Na, Mg, K, Ca, Al, Mn, Co, V, Cr, Ni, Cd, Pb, Se, S and Ba were determined. For all of these compounds, Method 200.7 of the EPA [21] was used for analysis, with inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

The oxidation kinetics were also measured by determining the pH of the mixture resulting from the addition of hydrogen peroxide [22].

Germination test

The inhibition of elongation in the radicle and hypocotyl are very sensitive sub lethal indicators for the evaluation of toxic effects in plants, providing complementary information to germination studies.

Five replicates of each germination experiment were performed in petri dishes with seeds from different plants (monocots and a dicot) orchard grass seed, Porto variety (*Dactylis glomerata* L.), alfalfa, 6-Victoria group SP INTA variety (*Medicago sativa* L.), Phalaris (*Phalaris bulbosa* L.), and tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv.), according with international recommendations [23].

In all cases, sludge A and B and distilled water were added at sludge dilutions of 10%, 50%, and 100% and were compared with controls (only distilled water). Fifty seeds were added to each plate, with the exception of tall wheatgrass, for which 25 seeds per plate were used. The temperature of the germination chamber was 28 °C (+/- 2°C) in all cases.

The seeds were kept in water while stirring to achieve uniform moisturisation and were subsequently separated by centrifugation and filtration. They were then incubated in an oven at 28°C for 24 hours, after which germination was interrupted by adding 1 mL of ethyl alcohol. Next, the number of germinated seeds per plate was counted, and the root length of each germinated seed was measured. Seeds with at least 1 mm of root length were considered to have germinated. The results are expressed as the percentage of germination.

$$\%\ \text{Germination} = \frac{N^{\circ}\text{germinated seeds in the sample}}{N^{\circ}\text{germinated seeds in the control}} \times 100$$

Statistical analysis

One-factor analysis of variance (ANOVA) was performed to test for significant differences in certain variables. In all cases, triplicate samples were used to determine the physical and chemical properties to take into account the minimum requirements of the t test (Student), which requires at least three replicates for each case. For the phytotoxicity tests, assays were performed with five replicates for each dilution factor of each sludge type (three levels and a control for each type of sludge). The modified Shapiro-Wilks test was applied to previous tests to verify the approximation to the normal distributions for the variables under study. As a nonparametric alternative to the analysis of variance (ANOVA), in some cases, the Kruskal-Wallis test was used with the method of Pair Comparisons to compare the treatments or the Wilcoxon test was used. For the statistical analysis of oxidation kinetics data, also it was used the Durbin-Watson statistic to detect the presence of autocorrelation in the residuals from a regression analysis.

Results and Discussion

Physicochemical characterisation

The obtained results are shown in Table1. Sludge B was obtained from a malt house that includes a dehydration step (belt filter) in its effluent treatment process; hence, there is a difference in the dry-matter content of the sludge. In the activated-sludge processes of effluent treatment, the residual sludge contains 5 to 20 g kg^{-1} of solids [24].

Component	Sludge A	Sludge B
pH	6.57a	7.48a
EC (mS cm^{-1})	2.9b	1.2a
Dry matter (DM) (g kg^{-1})	19a	126b
Organic matter(g kg^{-1} DM)	835.5a	860a
Ash (g kg^{-1} DM)	164.5a	140a
N (g kg^{-1} DM)	65a	82a
P (g kg^{-1} DM)	1.23a	8.2b
K (g kg^{-1} DM)	1.7a	6.1b
Ca (g kg^{-1} DM)	0.516a	4.6b
Na (g kg^{-1} DM)	1.01a	4.9b
Mg (g kg^{-1} DM)	5b	2.3a
V (mg kg^{-1} DM)	3.8a	8.2a
Cr (mg kg^{-1} DM)	3a	1.5a
Cu (mg kg^{-1} DM)	42a	11.1a
Zn (mg kg^{-1} DM)	376a	453a
Fe (mg kg^{-1} DM)	11,525a	7,885a
Al (mg kg^{-1} DM)	481a	1,543a
B (mg kg^{-1} DM)	89a	96a
Mn (mg kg^{-1} DM)	50a	125a
Co (mg kg^{-1} DM)	0.2a	0.2a
Mo (mg kg^{-1} DM)	1.2a	1.9a
Ni (mg kg^{-1} DM)	3.7a	2.9a
Pb (mg kg^{-1} DM)	1a	1.4a
Se (mg kg^{-1} DM)	<0.1a	<0.1a
S (mg kg^{-1} DM)	9,440a	5,440a
Ba (mg kg^{-1} DM)	59a	83a

In each row, values followed by the same letter do not differ significantly (p>0.05)

Table 1: General physicochemical characteristics of barley malting sludge.

The residual activated sludge from brewery-effluent treatment plants contain between 130 and 140 g kg^{-1} of dry matter when they include dehydration processes without chemical additives such as lime [1]. Other natural organic amendments of animal origin have similar dry-matter content; for example, cow manure contains between 5 and 10 g kg^{-1} [25] and swine manure contains 15 g kg^{-1}. In some cases, such as for poultry manure, the dry-matter content depends on the collection system used, with typical values of 10 g kg^{-1} [26].

The pH values of sludge differ slightly but always fluctuate around neutral. This variation is due to the differences in the biological processes carried out in each effluent treatment plant and to the adjustment of the chemical parameters in the flocculation processes.

The residual activated sludge from effluent treatment has pH values of approximately 7 [27]. The pH of animal manures vary by source, such as 7.1 for poultry [26], between 5.1 and 5.9 for swine [28], 5.2 for cattle and 9.4 for horses [29].

The organic matter content in the sludge was high and similar to that found in manures and fertilisers without treatment or in very high-quality compost [30]. The C/N ratios were 7.47 and 6.10 for malting sludges A and B, respectively. Manures have higher values for the C/N ratio: 19 for cattle, 29 for sheep, and between 13 and 15 for swine [31]. Sewage sludge has similar values according to different researchers: 7.8 [32] and between 6.1 and 7.34 [33]. When comparing the C/N ratio of sludge with plant (vegetable) waste, malting sludge resembles alfalfa waste (*Medicago sativa* L.), whose C/N ratio varies between 14 and 16 or red clover waste (*Trifolium pratense* L.), whose C/N ratio is 11.7 [34,35].

The organic matter content in residual activated sludge from sewage-effluent treatment varies between 700 and 900 g kg^{-1}, depending on the origin of the effluents and on the type of biological treatment in place [24].

Sludge from biological treatments contains varying concentrations of the nutrients required by plants for growth. The contents of both micro- and macro-elements present in the chemical composition of malting sludge were similar to the contents in barley, the grain used in the malting process [36].

The amount of macronutrients in the malting sludge is relatively low. There is a greater variability of P in sludge B, which is due to the addition of phosphate as a nutrient in the biological treatment process. When compared to other types of sludge, it appears that the P level of sewage varies from 1.1 to 5.5 g kg^{-1} [37], whereas brewery effluents contain from 1.32 to 1.76 g P kg^{-1} [1].

The difference in the concentrations of Na, K and Ca is explained by the differences in the quality of water used in the malting process. The K content of sewage sludge varies from 0.08 to 1.1% [37], and that of residual sludge from breweries between 0.41 and 0.5% [1], which is on the same order of magnitude as the K content in malting sludge.

The Mg content of the sludge was similar to values found in sewage sludge, which range from 0.03 to 1.1 g kg^{-1} [37] and is analogous to the range for activated sludge of brewery effluent, 0.3 to 0.42 g kg^{-1} [1].

The S content coincided with the typical values in sewage sludge [12], whose range is 0.06 to 0.11 g kg^{-1}.

In malting sludge, varying amounts of micro-elements that are essential for plant and animal life were found; a few were found at relatively high levels (Fe and Al), but most were at lower concentrations, on the order of mg kg^{-1}, similar to the values reported by [37] for sewage sludge. The higher levels of Fe were most likely due to the use of ferric chloride in the wastewater treatment process.

The Zn content was slightly higher than the values found in sludge from brewery effluents, 200 mg Zn kg^{-1} [1], and was similar to the lower limit of the typical range for sewage sludge, 108 mg Zn kg^{-1} [13]. The other values published for the Zn content in sewage sludge are higher than those found in malting sludge [12,37].

Cu content was low compared to values published for both brewing effluent sludge, 110 mg kg^{-1} [1], and sewage sludge, whose typical range is from 85 to 2900 mg kg^{-1} [13]. This is due to the low Cu content in the barley seed that comes from the soils of the Province of Buenos Aires, where, according to availability criteria proposed by some researchers, some areas are deficient in Cu [38]. In manure from swine, the content of Cu and Zn are variable; the published values are 30 to 50 mg kg^{-1} for Cu and 45 to 65 mg kg^{-1} for Zn [39]. However, the Cu level in sewage sludge exceeds that of malting sludge [37].

The levels of B, Mn, Al, and Ba in malting sludge were within the range indicated by the EPA [13] for these compounds in sewage sludge and were also analogous to the typical values of brewery effluent sludge [1]. For Mn, various authors have measured higher concentrations in sewage sludge [37].

The concentrations of Se, Pb, Co, Cr, Ni, and Mo were lower than those found in sewage sludge [37].

Malting sludge contains V, another micronutrient necessary for animal life. The V can replace Mo as a specific catalyst in N fixation [39,40] and could thereby act as an essential micronutrient for certain plants.

The organic components in the sludge included microbial cellular material and its decomposition products and chemical compounds from the biologically treated wastewater (proteins, polysaccharides, fats, lipids, etc.). Most of the organic carbon (more than 75%) in the sludge was determined to be insoluble in water and mainly consisted of cellulose and hemicellulose fractions [1].

In Table 2, the fractionation of both the organic matter and the nitrogen compounds are detailed.

The concentrations of both organic nitrogen and inorganic nitrogen (ammonium and nitrate) depend on the type of physical, chemical, and biological treatment used at each malt house and the subsequent handling process.

Most of the organic nitrogen was contained in the solid fraction of the sludge and was thus not modified by the drying or dehydration processes. In contrast, the concentration of the inorganic nitrogen, which is very soluble in water, was reduced upon the removal of water. For the inorganic forms of nitrogen present in malting sludge, the nitrate concentrations outweighed the ammonium forms due to the aerobic wastewater treatment process.

Fractions	Component	Malthouse A	Malthouse B
Inorganic N	$N\text{-}NH_4^+$	0.07a	0.04a
	$N\text{-}NO_3^-$	0.08a	0.09a
Organic N	$N\text{-}NH_4^+$	8.43a	9.88 a
	N-Hexosamine	2.94 a	3.96 a
	N-a-amino acid	9.75 a	11.83 a
	N-hydrolyzable, no distillable	14.36 a	21.57 b
	N-not hydrolysable	29.37 a	34.63 a
Organic matter	Water-soluble	21.15 a	22.59 a
	Liposoluble	10.26 a	11.29 a
	Cellulose and Hemicellulose	52.05 a	46.83 a
	Lignin	16.54 a	19.29 a

In each row, values followed by the same letter do not differ significantly (p>0.05)

Table 2: Fractionation of carbonaceous and nitrogenous compounds (g kg^{-1}).

The organic nitrogen in the sludge originated from the biological treatments and contained mainly amino acid-type compounds and a small fraction of hexosamines and amides, which originate from plant protein materials. Here, the malting barley gave rise to a very high fraction of organic nitrogen, consistent with the high content of cellulose and hemicellulose from the fractionation of the organic matter. Sludge A contained more cellulose and hemicellulose and less lignin than sludge B. The organic nitrogen content in both sludge samples exceeded 90% of the total. Published values of organic N in biosolid waste from sewage sludge indicate a similar relationship, > 94% of the total N was organic N [41].

The contents of the inorganic nitrogen fractions were similar to the published values for N-NH_4^+ and N-NO_3 in raw manure, non-stabilised sewage sludge, or mature compost [30]. The total nitrogen content of the sludge was high and bolsters its use as amendment. The total nitrogen content of sewage sludge varies between 10 and 100 g per kg of dry matter [33,41]. Activated sludge from biological treatments of brewery effluents contain between 70 and 80 g of nitrogen per kg of dry matter [1]. Manures generally have total N contents lower than that of malting sludge; only swine manure has a total N content that exceeds that of malting sludge.

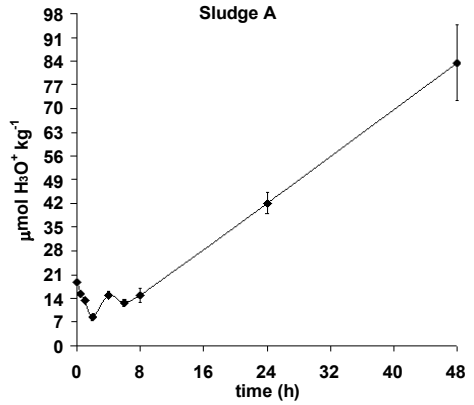

Figure 1: Oxidation kinetics of malting sludge A (cmol H_3O^+ kg^{-1}).

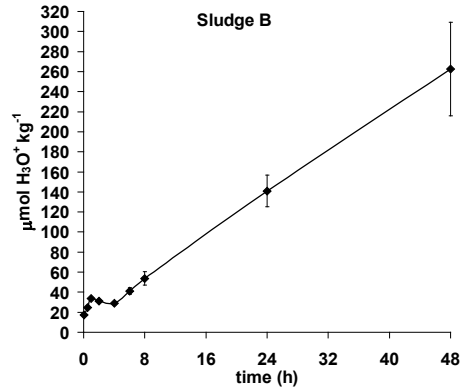

Figure 2: Oxidation kinetics of malting sludge B (cmol H_3O^+ kg^{-1}).

	Sludge A	Sludge B
Equation	$y = e^{(-6.6984 + 0.040434 x)}$	$y = e^{(-5.9127 + 0.0514 x)}$
Correlation coefficient	0.8983	0.9343
R-Squared	80.7%	87.3%
R-Squared (adjusted for degrees of freedom)	79.93%	86.79%
Standard error of the test	0.30928	0.3069
Statistical D of Durbin-Watson	0.8295 (P = 0.0001)	0.94317 (P = 0.0006)

Table 3: Statistical analysis of oxidation kinetics data.

Organic matter oxidation kinetics

To study the chemical composition of the lignins, both degradative and non-degradative physicochemical methods were employed. Chemical degradation was complemented by non-degradative methods (spectroscopy). Traditional oxidative methods, such as sequential oxidation using hydrogen peroxide, potassium permanganate, etc., allowed for the characterisation of the basic aromatic components of the lignins and lignocelluloses; thus, the same oxidation products generated by other methods of biological degradation were obtained using chemical methods [42].

Studies were carried out on the degradative oxidation of the malting sludge samples using hydrogen peroxide. The change in acidity indicated a greater or lesser tendency of oxidisability of the organic matter in the sludge. Higher concentrations of H_3O^+ corresponded to a more rapid, and therefore easier, oxidation of the organic matter in the sludge and signified a rapid mineralisation.

The results obtained for the oxidation of the malting sludge (Figures 1 and 2) confirmed the data from the fractionation of the contained organic matter.

Both sludge samples began the oxidation process in similar conditions, but B was oxidised more intensely than A due to the greater content of hydrolysable N.

After performing the corresponding hypothesis tests, we can conclude that the lines are parallel ($p > 0.48$) but not equal ($p < 0.001$), the sludge B is more readily degradable and degradation rates are similar (Table 3).

Seed germination bioassays

In the biophytotoxicity tests with the seeds, we measured the percentage of seeds germinated with malting sludge at the dilutions indicated and compared the results with the control (Table 4).

For both sludges, only orchard grass seed (*Dactylis glomerata* L.) and phalaris (*Phalaris bulbosa* L.) show signs of phytotoxicity (delay/inhibition) during germination. For sludge A, both orchard grass and phalaris show significant decreases for the 100% dilution.

The germination percentage was less than 90% when the concentration of sludge exceeded 10%. Sludge B also showed significant decreases in the rate of germination of orchard grass (*Dactylis glomerata* L.) for the 100% dilution, whereas the germination of phalaris did not decrease significantly between the 100% and 50% dilutions. The germination of orchard grass (*Dactylis glomerata* L.) was less than 90% when the dilution exceeded 50%, and that of phalaris (*Phalaris bulbous* L.) was less than 90% when the amount of sludge was greater than 10%. Neither alfalfa (*Medicago sativa* L.) nor tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv.) were indicators for the biophytotoxicity of this type of sludge, given that their germination was favoured. Sludge A had a greater effect on the germination of orchard grass (*Dactylis glomerata* L.) than sludge B, possibly due to the higher salt content in sludge B. Consistent with the results published by other researchers, a higher level of salinity in the sludge can inhibit germination [43]. The germination percentages of alfalfa (*Medicago sativa* L.) and tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv.) seeds were greater

Sludge A	G (%) *Medicago sativa*	G (%) *Agropyron elongatum*	G (%) *Dactylis glomerata*	G (%) *Phalaris bulbosa*
100%	139 a,I	70 b,I	19 a,II	31 a,II
50%	164 a,I	82 b,I	50 a,I	61 a,I
10%	173 a,I	88 b,I	123 b,I	93 b,I
Sludge B	**G (%) *Medicago sativa***	**G (%) *Agropyron elongatum***	**G (%) *Dactylis glomerata***	**G (%) *Phalaris bulbosa***
100%	108 a,II	114 b,II	50 b,I	70 c,II
50%	119 a,II	114 b,II	108 a,II	72 c,II
10%	153 a, I	214 b,I	108 a,I	100 c,I

In each column, values for the same species followed by the same letter do not differ significantly (p>0.05). In each row, values for the same dilution followed by the same Roman numeral do not differ significantly (p>0.05).

Table 4: Germination (G) of seeds.

when sludge was used compared to distilled water only (control). Both of these species have higher salt-tolerance thresholds than orchard grass [44], which could explain this behaviour.

For both sludge samples, a germination-promoting effect was observed for alfalfa; this effect was much more prominent in the lowest dilutions (10 - 50%). Sludge B, which has a lower salinity level, had the same effect on tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv.).

Conclusions

The levels of essential nutrients (N, P and K) and micronutrients (particularly Ca, Mg, Cu and Zn) are high, while the amounts of trace metals that do not have a function in biological processes are very low. The fractions of hydrolysable-N, non-distillable N are an indicator of a greater ease of oxidation. Approximately 50% of the organic matter of malting sludge is cellulose and hemicellulose. Within the chemical composition of malting sludge, Ba stands out as a trace metal without a specific biological function. The phytotoxic effects of malting sludge are mainly attributed to its higher salinity and its lower stabilisation. *Dactylis glomerata* L. and *Phalaris bulbosa* L. were good phytotoxic indicators for this type of residual sludge. Sludge from malting-effluent treatment plants has a lower concentration of toxic metals than sewage sludge and could thereby be applied as soil amendments in moderate to high doses, constituting an excellent "a priori" amendment for agricultural production according with local legislation of each country and environmental assessment for aquifers at agricultural land in contact through the leachate.

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