

Effects of biowaste-based amendments on soil's humic substances and biochemical properties

Efeitos da utilização de corretivos derivados de biorresíduos nas substâncias húmicas e nas propriedades bioquímicas do solo

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ABSTRACT

The use of biowaste-based amendments to increase soil organic carbon (SOC) stock in agroecosystems could be an important agricultural practice, with potential to counteract SOC depletion. Despite the overall benefits to soil properties, potential impacts can be observed on soil biota, due to the biowaste-based amendments composition and/or instability, which can be assessed evaluating specific soil biochemical properties. A field study was set-up for two years, with *Lolium multiflorum* Lam, to evaluate the effect of biowaste-based amendments on soil microbial and biochemical properties. Dewatered sewage sludge (SS; 6, 12 and 24 t dry matter ha⁻¹), municipal solid waste compost (MSWC), and agricultural wastes compost (AWC), with application doses calculated to deliver the same amount of organic matter (OM) per unit area, were tested. Generally, enzymatic activities (dehydrogenase, β -glucosidase, acid-phosphatase, cellulase and protease), potential nitrification, and soil organic matter fractions (humins, humic acids (HA) and fulvic acids (FA)), increased with the treatment doses, and from the first to the second year of application. For some properties, like humins and HA, potential nitrification and dehydrogenase activity, their increase was more notorious following both composts application, especially in the second year, favoured by the application of more stable OM, while other properties were more responsive to SS application, with more active organic matter (e.g., FA, β -glucosidase, acid-phosphatase).

Keywords: Soil organic carbon, organic amendments, sewage sludge, compost, soil enzymatic activities, potential nitrification.

RESUMO

O uso de corretivos orgânicos derivados de resíduos para aumentar o armazenamento de carbono orgânico do solo (SOC) em agroecossistemas poderá constituir uma prática agrícola importante, com potencial para neutralizar o esgotamento do SOC. Apesar dos benefícios gerais para as propriedades do solo, podem ocorrer potenciais impactos na biota do solo, devido à composição e/ou falta de estabilidade da matéria orgânica dos corretivos orgânicos utilizados, o que pode ser avaliado através de propriedades bioquímicas específicas do solo. Foi montado um ensaio de campo, por dois anos, com *Lolium multiflorum* Lam, para avaliar os efeitos da aplicação de corretivos derivados de resíduos orgânicos nas propriedades microbianas e bioquímicas do solo. Lama residual urbana desidratada (SS; 6, 12 e 24 t de matéria seca ha⁻¹), composto de resíduos sólidos urbanos (MSWC) e composto de resíduos agrícolas (AWC), com doses de aplicação calculadas para fornecer a mesma quantidade de matéria orgânica (OM) por unidade de área, foram testadas. Em geral, as atividades enzimáticas (desidrogenase, β -glucosidase, fosfatase ácida, celulase e protease), nitrificação potencial e frações de matéria orgânica do solo (humina, ácidos húmicos (HA) e ácidos fúlvicos (FA)), aumentaram com as doses de tratamento, e do primeiro para o segundo ano de aplicação. Para algumas propriedades, como humina e HA, nitrificação potencial e atividade da desidrogenase, o aumento foi mais marcado após a aplicação de ambos os compostos, principalmente no segundo ano, favorecidas pela aplicação de OM mais estável, enquanto outras propriedades foram mais responsivas à aplicação de SS, com mais matéria orgânica ativa (e.g., FA, β -glucosidase, fosfatase ácida).

Palavras-chave: carbono orgânico do solo, corretivos orgânicos, lamas residuais urbanas, composto, atividades enzimáticas do solo, nitrificação potencial.

INTRODUCTION

In Portugal, as in other Mediterranean countries, where soil organic matter (SOM) in agroecosystems are quite below critical levels recommended by the UNCCD (United Nations Convention to Combat Desertification), the use of biowaste-based soil amendments seems an attractive option, because this practice enables valuable components to be reused (e.g., OM, N, P, K), avoiding, at the same time, the landfilling of organic wastes. However, there are associated threats, namely of soils contamination with potentially toxic trace elements (PTEs), organic contaminants, and pathogenic microorganisms, and their limit values have been established for sewage sludge (Decree-Law n.º 276/2009), and biowaste-based fertilizers (Decree-Law n.º 30/2022 and Portaria n.º 185/2022). Because of that, it is important to thoroughly analyse the risks of each specific material, considering its physicochemical characteristics, to compare the results with established limit values (Alvarenga *et al.*, 2015), and its maturation/stability status (Alvarenga *et al.*, 2016). However, despite the importance of their characterization, it is very important to evaluate the effects of the application of these biowaste-based amendments in field experiments, which can deliver more robust results, and the potential cumulative effects of their application to soil (Alvarenga *et al.*, 2017). Generally, these studies emphasise the effects on soil physicochemical properties, more related with soil fertility aspects (e.g., pH, OM content, total and available nutrients' concentrations, cation exchange capacity), or on the accumulation of pollutants (e.g., PTEs, persistent organic contaminants), both in soil and in the crop. These studies are very important, but they often disregard effects on other soil properties, like microbial and biochemical properties, that are good indicators of soil overall health (Bhaduri *et al.*, 2022). For instance, dehydrogenase activity, as well as potential nitrification, are considered good indicators, representative of the active microbial population of a soil, while exoenzymes, such as hydrolases, can be used to assess the biological cycling of the elements, such as N, P, and C. All in one, they can be classified as metabolic soil bio-indicators, to evaluate ecosystem restoration and sustainability (Bhaduri *et al.*, 2022).

Considering the importance that SOM represents, as a property, to the agroecosystem, influencing soil fertility, nutrient cycling, water retention, and gases exchange (Weil & Brady, 2017), it is very important to evaluate the implications to the SOM when organic materials with different characteristics, namely regarding chemical composition (lignin, suberin), stability and maturity, are used as soil amendments (Alvarenga *et al.*, 2016). The particulate fraction (free organic matter, FOM) is separated previously by density, and after is done via an alkaline extraction of the soil, obtaining the so-called humic substances (HS), composed by three fractions, operationally defined considering their pH-dependent solubility in aqueous solutions (humins, humic acids (HA) and fulvic acids (FA)) (Weil & Brady, 2017). Humins, insoluble in alkaline solutions, is formed by highly condensed, larger molecules, complexed with clays, resistant to decomposition, therefore considered the most stable fraction of SOM. On the other hand, HA (high molecular weight, up to 300,000 Da) and FA (lower molecular weight, 2,000-50,000 Da), both soluble in alkaline solutions, are composed by more reactive macro-molecules, HA more stable and less soluble than FA (Benites *et al.*, 2003).

Several authors consider that the humic substances obtained in the alkaline extraction do not represent the nature of most of the organic material that exists in nature, and that SOM characterization should be performed by direct investigation of organic matter chemistry *in situ*, while it is still associated with the mineral phase (Weil & Brady, 2017; Kleber & Lehmann, 2019). Nevertheless, when the edaphoclimatic conditions are similar, variations on these soil alkali-extracted fractions are still used by many authors to evaluate the influence of different soil management options on SOM properties (Kou *et al.*, 2022).

Taking all these into consideration, the aim of this study was to evaluate the effects on SOM fractions, extracted by alkali, and on soil biochemical properties (nitrification potential, dehydrogenase activity, and soil exoenzymes activity), of the use of three different biowaste-based amendments (sewage sludge and compost produced from urban or agricultural wastes).

MATERIALS AND METHODS

Field experiment

The field experiment was established in a Vertisol (WRB, 2022), in Beja (Portugal; 38°01'42.08"N, 7°52'11.70"W), sown with *Lolium multiflorum* Lam. The soil amendments used were: (1) dewatered sewage sludge (SS; 6, 12 and 24 t dry matter (dm) ha⁻¹), (2) mixed municipal solid waste compost (MSWC), and (3) agricultural wastes compost (AWC), applied in rates calculated to deliver the same amount of organic matter (OM) per unit area of soil, in two consecutive years (2014-2015). Briefly, the SS was obtained in a small municipal wastewater treatment plant (serving ~6000 inhabitants), after activated-sludge treatment, with high aeration rate, nitrification-denitrification, and mechanical dewatering by centrifugation (~15% dry matter content). The MSWC was obtained from unsorted municipal solid waste, mechanically segregated, and biologically treated, in a composting plant serving ~113000 inhabitants. The AWC was produced from the wastes of the cleaning of olive groves, and of harvesting and processing of olives to produce olive oil, and from manure generated in a biological farm (61% sheep manure, 21% olive mill waste, 10% olive leaves, and 8% meat flour) (Alvarenga *et al.*, 2015). Two control treatments were considered: one ploughed and sown, as the others, but without organic amendment application (zero (0) ton ha⁻¹ application rate), and a control with intact soil (Control). The thorough description of the experimental set-up and of the results obtained for the physicochemical properties of soil, and for the effects on plant and on soil PTEs' accumulation, were already reported and discussed (Alvarenga *et al.*, 2017).

Soil organic matter fractionation

SOM was separated in operationally defined fractions (humins, HA and FA), extracted following the simplified methodology described by Benites *et al.* (2003). A soil sample with, approximately 30 mg total organic carbon, was extracted with NaOH 0.1 M, shaken for 24 h (200 rpm) at 25 °C in the absence of light, and after centrifuged at 5,000 g for 30 min. This extraction procedure was repeated twice, and allowed the separation of the humin fraction, which is retained, insoluble, in the precipitate,

from the extract with soluble HA and FA fractions. The extract was then acidified to pH = 1.0, with H₂SO₄ 20% (w/w), to allow their separation: HA, with higher molecular weight, which precipitates in acid solutions, from FA, with lower molecular weight, which remains in the supernatant. The separation of both fractions was performed by filtration, through a 0.45 µm filter, under vacuum. The HA precipitate, retained in the filter, was further solubilized, by washing it with NaOH 0.1 M. Organic carbon in the three fractions (humins, in the precipitate, and HA and FA, in each extract), was quantified by the Walkley-Black method: titration with Mohr's salt (ammonium iron (II) sulfate, (NH₄)₂Fe(SO₄)₂(H₂O)₆), with ferroin indicator, after oxidation of each fraction with K₂Cr₂O₇ (0.1667 M for humins and 0.0125 M for HA and FA extracts), with concentrated H₂SO₄, at 150 °C for 30 min. All samples were measured in triplicate.

Soil enzymatic activities

At the end of the experiment, soils were collected and maintained at field moisture content before analysis. Enzymatic activities analytical protocols were thoroughly described by Alvarenga *et al.* (2009), all using 2-mm sieved samples, and with the activities expressed on oven-dried weight basis (105 °C, 48 h). Dehydrogenase activity (Tabatabai, 1994) was measured as soon as possible after soil sampling (< 48 h), while for β-glucosidase (Alef & Nannipieri, 1995a), acid phosphatase (Alef *et al.*, 1995), cellulase (Hope & Burns, 1987), and protease (Alef & Nannipieri, 1995b), soil samples were kept refrigerated at 4 °C, and let to equilibrate for 1 h, at room temperature, before analysis. All analytical measurements were carried out in triplicate.

Potential nitrification

Potential nitrification was measured according to Berg & Rosswall (1985), with some modifications. Field-moist soil samples were incubated for 5 h at 25°C using ammonium sulphate 1 mM as substrate (2 g soil: 8 mL solution), in the presence of sodium chlorate has a nitrite oxidation inhibitor. Nitrite released during incubation was extracted with potassium chloride and determined colorimetrically at 540 nm.

Data analysis and statistical treatment of data

To avoid biased results related with soil sampling in different years, all results were expressed as a percentage, relative to the result obtained for the same variable in the control plot (Control) in that same year. Results were analysed using descriptive statistics (mean, and standard deviation), and were subjected to a two-way ANOVA to evaluate statistical differences between the results, and the interaction of the tested independent variables (treatment × year). Whenever significant differences were found ($p \leq 0.05$), a post hoc Tukey Honest Significant Difference (HSD) test was used to further elucidate differences among means ($p \leq 0.05$). All statistical analyses were performed with the STATISTICA 7.0 (Software™ Inc., PA, USA, 2004).

RESULTS AND DISCUSSION

Soil organic matter fractionation

SOM alkali extracted fractions (humins, FA and HA), increased with the treatment doses, and, as a general trend, from the first to the second year of the study, evidencing the benefit of amending the soil with exogenous sources of organic matter.

Humins (Figure 1A), is the most persistent and stable fraction of SOM, which plays a very important role in C sequestration in soil. Humins were dominant over the other fractions (between 80-91% of the total C in humins, for all treatments), as was expected in an agricultural soil (and in a Vertisol). For HA (Figure 1B), more stable than FA, their content in the soil increased more markedly following the composts application, with statistically significant differences after the AWC higher application dose ($p < 0.05$), reached in the second year of the study. The effects of SS application were more evident for the FA increase (Figure 1C), especially in the first year of their application. FA correspond to more active and soluble compounds (Benites *et al.*, 2003; Kou *et al.*, 2022), and it is expected that SS, an organic material less stabilized and with a higher rate of mineralization compared with both composts (Alvarenga *et al.*, 2016), would have a more marked effect on the FA fraction of SOM. In fact, in the second year of the study, the higher application dose of SS led to a slight decrease in humins, and to an increase in FA.

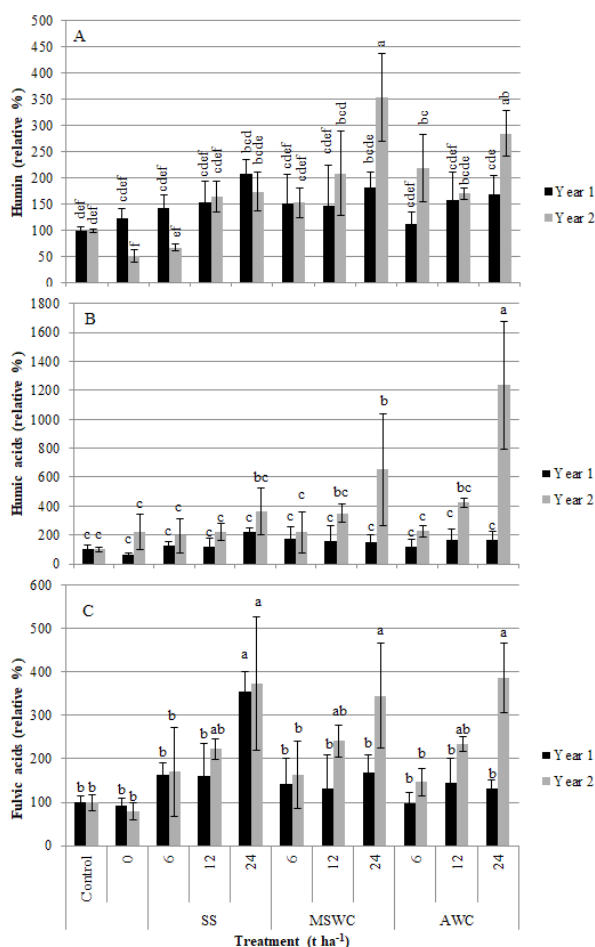


Figure 1 - Effects of the treatments on soil organic matter fractions in the two years of the study: (A) humin, (B) humic acids and (C) fulvic acids (mean ± standard deviation, n=4). Results are reported as percentage, relative to the result obtained in the Control. Columns marked with the same letter are not significantly different (Tukey test, $p > 0.05$).

Effects on soil biochemical indicators

Except for SS application in some cases or for some doses, overall, the biochemical indicators were positively affected by organic amendments application (Figure 2), with considerable increases in their values, relatively to the control or to the seeded non-amended plot.

Regarding dehydrogenase activity (DHA), an overall indicator of soil microbial activity (Figure 2A), while in the first year of the study the beneficial effect of SS application was higher than that

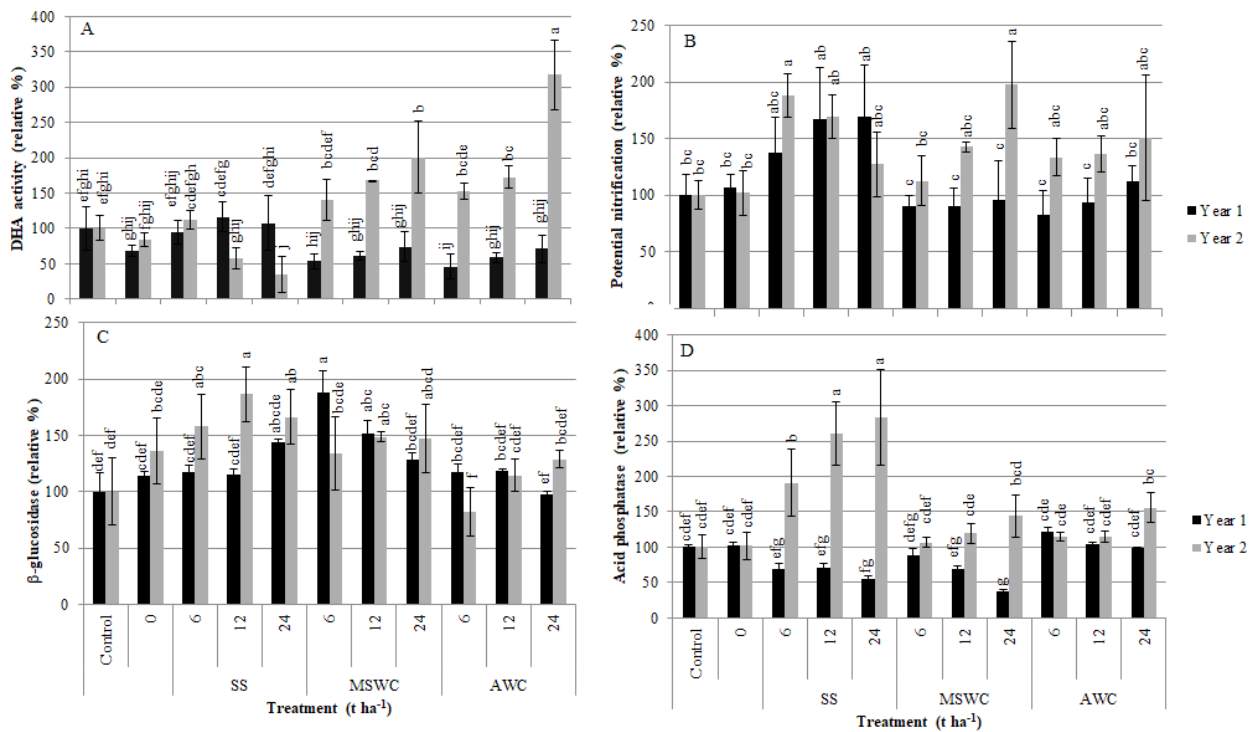


Figure 2 - Effects of the treatments on soil biochemical properties in the two years of the study: (A) dehydrogenase activity (DHA), (B) potential nitrification, (C) β -glucosidase activity, and (D) acid-phosphatase activity (mean \pm standard deviation, n=4). Results are reported as percentage, relative to the result obtained in the Control. Columns marked with the same letter are not significantly different (Tukey test, $p > 0.05$).

observed for both composts application, the second year of application of the same material evidenced an opposite tendency, with a decrease in DHA activities with increasing SS application rates. An opposite tendency was observed following composts application, with more stabilized OM, leading to an increase of DHA activity, with the increase in the application rates of both composts. As for potential nitrification (Figure 2B), the response of this biochemical indicator followed the same trend as the DHA, but with values, for SS, considerable higher than the controls in the second year of the study ($p < 0.05$).

As for these biochemical parameters, associated with microbial activity, there is an apparent benefit of applying stable high-quality organic matter, at least in the medium term, with a negative response to the consecutive application of non-stabilized organic matter, still in an active mineralization phase, as SS.

Generally, soil enzymatic activities (β -glucosidase (Figure 2C), acid-phosphatase (Figure 2D),

cellulase and protease), increased with the treatment doses, and markedly from the first to the second year of the study. In fact, following the first year of application of the amendments, the differences for the enzymatic activities of β -glucosidase and acid-phosphatase, between the amended and non-amended soil, were not significant ($p > 0.05$), but their activities increased markedly in the second year of application, especially for SS application, with statistically significant higher activities than with the compost application, MSWC and AWC ($p < 0.05$).

As for, cellulase and protease, their activities were below the quantification limit of the technique in the first year but increased their activities to quantifiable values following the second year of amendment applications (data not shown). Their activities were, however, still low and, because of that, with high standard deviation between replicates. Nevertheless, it was possible to observe that SS was the amendment which promoted the highest increase in their activities, possible because of the higher rate of mineralization of SS, compared

to the stabilized composts, already pointed by Alvarenga *et al.* (2017), when discussing the effects of the treatments on the soil physicochemical parameters.

Despite the rather higher response of the hydrolases to the SS application, when compared to the compost application, the application of 24 t ha⁻¹ of SS, in the second year of application, also induced a decrease in the enzymatic activity of β -glucosidase (Figure 2C) and cellulase (data not shown), both enzymes from the C-cycle.

CONCLUSIONS

These results emphasize the importance of applying biowaste-based amendments to boost microbial and biochemical properties of soil, which increased by their application doses (especially

with the 12 and 24 t ha⁻¹ application doses), and from the first to the second year of application. However, for some properties, mainly those related with microbial activity, like DHA activity and potential nitrification, the positive response decreased in the second year of application of less stabilized organic materials, like SS, while the same properties continued to increase with composts application.

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BIBLIOGRAPHIC REFERENCES

- Alef, K.; Nannipieri, P. & Trasar-Cepeda, C. (1995) - Phosphatase activity. *In: Alef, K. & Nannipieri, P. (Eds.) - Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, p. 335-344.
- Alef, K. & Nannipieri, P. (1995a) - β -Glucosidase activity. *In: Alef, K. & Nannipieri, P. (Eds.) - Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, p. 350-352.
- Alef, K. & Nannipieri, P. (1995b) - Protease activity. *In: Alef, K. & Nannipieri, P. (Eds.) - Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, p. 313-315.
- Alvarenga, P.; Palma, P.; Gonçalves, A.P.; Fernandes, R.M.; de Varennes, A.; Vallini, G.; Duarte, E. & Cunha-Queda, A.C. (2009) - Organic residues as immobilizing agents in aided phytostabilization: (II) Effects on soil biochemical and ecotoxicological characteristics. *Chemosphere*, vol. 74, p. 1301-1308.
<https://doi.org/10.1016/j.chemosphere.2008.11.006>
- Alvarenga, P.; Mourinha, C.; Farto, M.; Santos, T.; Palma, P.; Sengo, J.; Morais, M.-C. & Cunha-Queda, C. (2015) - Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors. *Waste Management*, vol. 40, p. 44–52. <https://doi.org/10.1016/j.wasman.2015.01.027>
- Alvarenga, P.; Mourinha, C.; Farto, M.; Palma, P.; Sengo, J.; Morais, M.-C. & Cunha-Queda, C. (2016) - Quality assessment of a battery of organic wastes and composts using maturity, stability and enzymatic parameters. *Waste and Biomass Valorization*, vol. 7, p. 455–465. <https://doi.org/10.1007/s12649-015-9468-y>
- Alvarenga, P.; Palma, P.; Mourinha, C.; Farto, M.; Dôres, J.; Patanita, M.; Cunha-Queda, C.; Natal-da-Luz, T. Renaud, M. & Sousa, J.P. (2017) - Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. *Waste Management*, vol. 61, p. 582–592.
<https://doi.org/10.1016/j.wasman.2017.01.004>
- Bhaduri, D.; Sihi, D.; Bhowmik, A.; Verma, B.C.; Munda, S. & Dari B. (2022) - A review on effective soil health bio-indicators for ecosystem restoration and sustainability. *Frontiers in Microbiology*, vol. 13, art. 938481.
<https://doi.org/10.3389/fmicb.2022.938481>
- Benites, V.M.; Madari, B. & Machado, P.L.O.A. (2003) - *Extração e Fracionamento Quantitativo de Substâncias Húmicas do Solo: um Procedimento Simplificado de Baixo Custo*. Comunicado Técnico N. 16. Ministério da Agricultura, Pecuária e Abastecimento. Rio de Janeiro.
- Berg, P. & Rosswall, T. (1985) - Ammonium oxidizer numbers, potential and actual oxidation rates in two Swedish arable soils. *Biology and Fertility of Soils*, vol. 1, p. 131-140. <https://doi.org/10.1007/BF00301780>
- Decree-Law No. 276/2009, 2 of October 2009, on the sewage sludge application to soil. *Diário da República*, 1.ª série – No. 192 – 2 de outubro 2009. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, pp. 7154-7165.
- Decree-Law No. 30/2022, 11 of April 2022, on fertilizing materials. *Diário da República*, 1.ª série - No.71 - 11de Abril de 2022. Presidência do Conselho de Ministros. pp. 2-22.
- Hope, C.F.A. & Burns, R.G. (1987) - Activity, origins and location of cellulase in a silt loam soil. *Biology and Fertility of Soils*, vol. 5, p. 164-170. <https://doi.org/10.1007/BF00257653>
- Kleber, M. & Lehmann, J. (2019) - Humic Substances Extracted by Alkali Are Invalid Proxies for the Dynamics and Functions of Organic Matter in Terrestrial and Aquatic Ecosystems. *Journal of Environmental Quality*, vol. 48, n. 2, p. 207-216. <https://doi.org/10.2134/jeq2019.01.0036>
- Kou, B.; Hui, K.; Miao, F.; He, Y.; Qu, C.; Yuan, Y. & Tan, W. (2022) - Differential responses of the properties of soil humic acid and fulvic acid to nitrogen addition in the North China Plain. *Environmental Research*, vol. 214, n. 4, p. 113980. <https://doi.org/10.1016/j.envres.2022.113980>
- Portaria No. 185/2022, 21 of July 2022, on fertilizing materials. *Diário da República*, 1.ª série - No.140 – 21 de julho de 2022. Ministérios da Economia e Mar, Ambiente e Ação Climática e Agricultura e Alimentação. pp. 5-44.
- Tabatabai, M.A. (1994) - Soil enzymes. *In: Mickelson, S.H. & Bigham, J.M. (Eds.) - Methods of Soil Analysis. Part 2 - Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, Wisconsin, p. 775-833.
- Weil, R.R. & Brady, N.C. (2017) - *The Nature and Properties of Soils*, fifteenth ed. Columbus, Pearson.
- WRB (2022) - *World Reference Base for Soil Resources*. International soil classification system for naming soils and creating legends for soil maps. 4th edition. International Union of Soil Sciences (IUSS), Vienna, Austria.