




Use of Slaughterhouse Sludge in the Bioremediation of an Oxyfluorfen-Polluted Soil

Paloma Ávila-Pozo¹ · Juan Parrado² · Pablo Caballero² · Marta Díaz-López³ · Felipe Bastida³ · Manuel Tejada¹ 

Received: 5 January 2021 / Revised: 10 May 2021 / Accepted: 17 June 2021 / Published online: 25 June 2021
© The Author(s) 2021, corrected publication 2021

Abstract

The use of organic matter is a highly accepted environmental practice among scientists for the bioremediation of polluted soils. In this manuscript we study under laboratory conditions the bioremediation capacity of a new biostimulant obtained from slaughterhouse sludge in a soil polluted by the oxyfluorfen at a rate of 4 l ha⁻¹ (manufacturer's rate recommended) over a 90-day period. We determined its effects on dehydrogenase, urease, β-glucosidase and phosphatase activities, the soil microbial community structure and the evolution of the herbicide in soil. Possibly due to the high content of low molecular weight proteins in the biostimulant, the enzymatic activities were stimulated mainly at the beginning of the experiment. Soil biological parameters were inhibited in oxyfluorfen-polluted soil. At the end of the experiment and compared with the control soil, dehydrogenase, urease, β-glucosidase, and phosphatase activities significantly decreased by 47.8%, 50.5%, 36.4%, and 45.5% in the oxyfluorfen-polluted soil. At 5 days into the experiment, the use of the biostimulant in oxyfluorfen-polluted soils decreased soil enzymatic activities and microbial community inhibition. At the end of the incubation period the oxyfluorfen concentration had decreased by 60% in the polluted soil and amended with biostimulants. These results suggested that the use of this biostimulant with higher amounts of low molecular weight proteins and peptides had a positive effect on the remediating oxyfluorfen-polluted soils. Therefore, this study provides the use of a new biostimulant obtained from slaughterhouse sludge by enzymatic hydrolysis processes used in the bioremediation of a soil polluted by the oxyfluorfen herbicide.

Article Highlights

- Oxyfluorfen herbicide caused a negative effect on soil biological properties
- The application of biostimulants obtained by enzymatic hydrolysis from slaughterhouse sludge decreased the toxic action of oxyfluorfen
- The low molecular weight protein of biostimulants increased the degradation of herbicide

The original online version of this article was revised due to add funding note.

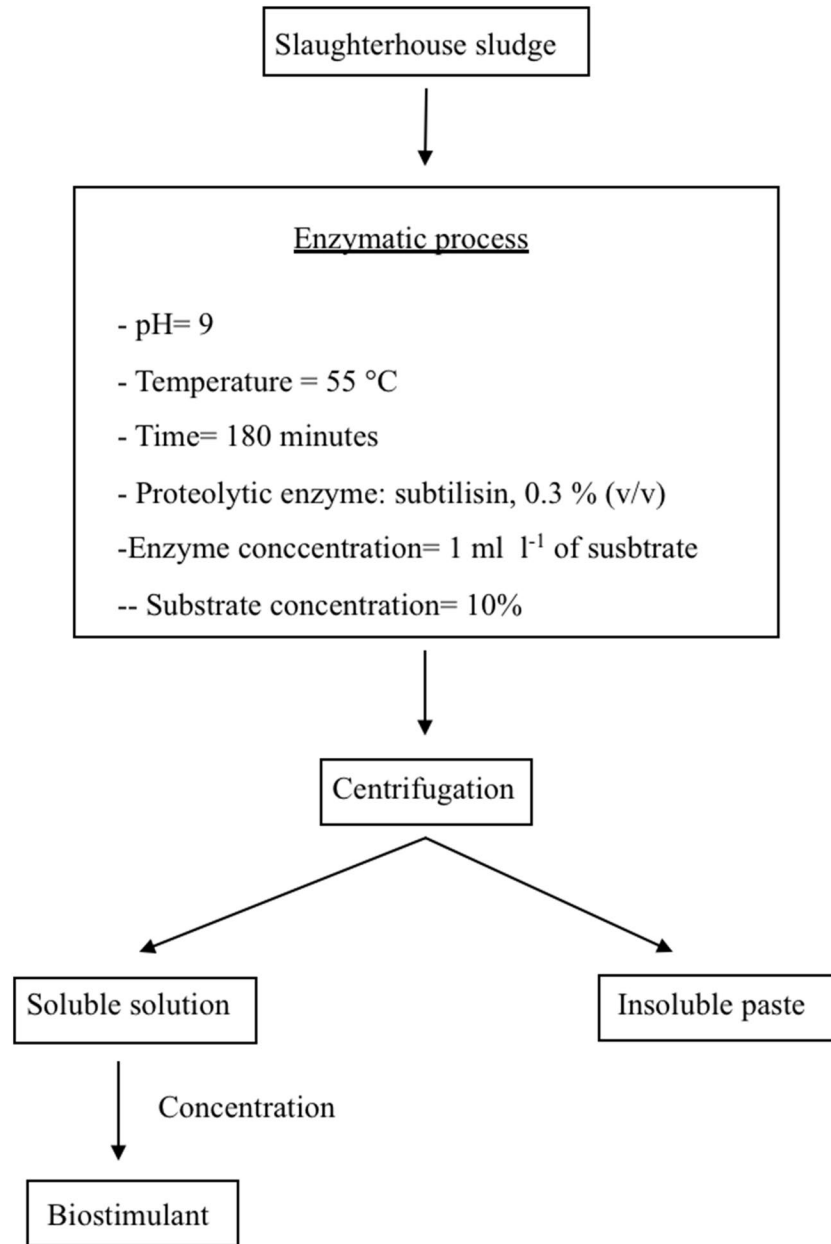
✉ Manuel Tejada
mtmoral@us.es

¹ Grupo de Investigación Edafología Ambiental,
Departamento de Cristalografía, Mineralogía y Química
Agrícola, E.T.S.I.A. Universidad de Sevilla, Crta de Utrera
km. 1, 41013 Seville, Spain

² Departamento de Bioquímica y Biología Molecular,
Facultad de Farmacia, Universidad de Sevilla, C/Prof. García
González 2, 41012 Seville, Spain

³ CEBAS-CSIC, Campus Universitario de Espinardo,
30100 Murcia, Spain

Graphic abstract



Keywords Slaughterhouse sludge · Biostimulant · Soil oxyfluorfen pollution · Soil biological properties

Introduction

In the last decades there has been a notable increase in the amount of sludge resulting from slaughterhouse wastewater treatment, a consequence of the increase in meat production. Bouwman et al. (2013) suggest that by 2050 meat production will have doubled. Consequently, it is very likely that the increase in the number of slaughterhouse facilities to

lead to an increase in the volume of slaughterhouse sludge (Bustillo-Lecompte and Mehrvar 2015).

Slaughterhouse wastewater has been classified by the United States Environmental Protection Agency as wastewater harmful to the environment (Alfonso-Muniozguren et al. 2021) mainly due to its high content of organic matter, suspended solids, oil and fats and nutrients (Aziz et al. 2019; Menegassi et al. 2020).

Currently, one of the most widely used bioremediation techniques among the scientific community is the application of organic matter to polluted soil, because it is a very cheap, effective technique that can be performed in situ (Davin et al. 2018). In these cases, organic matter reduces the concentration of contaminant in the soil via two different pathways. Firstly, this organic matter has the capacity to adsorb the toxins, decreasing their concentration in the soil solution and consequently reducing their toxicity. Second, the addition of this organic matter stimulates the growth of those microorganisms that are tolerant to the toxins presents in the soil. In doing so, the microorganisms increase the rate of pollutant degradation and so decrease their soil concentration (Gómez et al. 2014; Lipczynska-Kochany 2018; Ortiz-Botella et al. 2021). However, for this organic matter to be able to stimulate these soil microorganisms, there is a delay, while it is mineralized into simpler forms that are more easily available to the said microorganisms (Ortiz-Botella et al. 2021).

Using this slaughterhouse sludge to manufacture new edaphic biostimulant (BS) obtained by enzymatic hydrolysis processes would, therefore, have important socioeconomic and environmental impacts. On the one hand, there would be a solution to the accumulation of this organic waste and, on the other, the sludges could be of great use for bioremediating pesticide-polluted soils.

This study was prompted to obtain a new BS obtained from slaughterhouse sludge to bioremediate a soil contaminated with the oxyfluorfen herbicide, which exerts a persistent toxic effect on soil biological properties (Gómez et al. 2014; Rodríguez-Morgado et al. 2014; Franco-Andreu et al. 2016; Campos et al. 2019). Our hypothesis is supported by the existence of other BS obtained by enzymatic hydrolysis process from different organic wastes such as sewage sludge, chicken feathers, okara, rice bran, etc. used in the bioremediation of soils polluted by various pesticides (Gómez et al. 2014; Tejada et al. 2010, 2011a, b, 2014; Rodríguez-Morgado et al. 2014, 2015a; Orts et al. 2017). These BS are characterized by presenting a high number of low molecular weight peptides, amino acids, etc. that are easily absorbed by soil microorganisms, thus accelerating the degradation of the pollutant in the contaminated soil.

There is an abundant bibliography that suggests understanding the behaviour of any xenobiotic in soil requires the study of biological parameters (Campos et al. 2019; Wołejko et al. 2020). This is because these biological parameters react much faster than physical and chemical ones (Kadian et al. 2012; Franco-Andreu et al. 2016; Orts et al. 2017). Therefore, the study of soil enzymatic activities could be very useful to understand the effect of slaughterhouse sludge on the bioremediation of oxyfluorfen-polluted soils.

Within the frame work of this hypothesis, the aims were: (1) to obtain a new biostimulant created from slaughterhouse

sludge via enzymatic hydrolysis processes, and (2) to study the effectiveness of slaughterhouse sludge as a BS in the bioremediation of a soil polluted by oxyfluorfen, principally studying its repercussion on the target soil's biochemical properties.

Material and Methods

Characteristics of Organic Wastes, Soil and Herbicide

The slaughterhouse sludge was supplied by the “Matadero del Sur” company, located in Salteras (Seville, Spain). This sludge was subjected to two different treatments to obtain two different organic products.

The first of the treatments consisted of concentrating the slaughterhouse sludge until reaching a dry matter value of 15%, since at this value, an easily manageable product is obtained. This concentration was carried out at 75 °C with a rotary evaporator. This first organic compound is called concentrated slaughterhouse sludge (SS).

The second organic compound was obtained by subjecting the slaughterhouse sludge to an enzymatic hydrolysis process. The hydrolysis process was performed in a bioreactor according to the pH-stat methodology (Adler-Nissen 1977). Figure 1 shows the conditions under which this biochemical process was performed. Once the soluble product was obtained, it was also concentrated to 15% at 75 °C with a rotary evaporator. This second organic compound is called BS obtained from slaughterhouse sludge.

Table 1 shows the chemical composition of both organic compounds. The methodology measuring each chemical parameter is detailed in Rodríguez-Morgado et al. (2015b).

The experimental soil used was a Calcaric Regosol (FAO 1989). The soil characteristics are shown in Table 1. The analytical methods used in determining these soil parameters are detailed in Tejada et al. (2014).

Oxyfluorfen was used as experimental herbicide at rate of 4 l ha⁻¹ (recommended application rate). The commercial formulation Fenfen (24% p v⁻¹, 240 g l⁻¹) was purchased from Lainco, S.A (Spain).

Experimental Design

Three hundred grams of dried soil was mixed with oxyfluorfen and treated with SS at a random rate of 3% (9 g of product) and BS at a rate of 3.9% (11.7 g of product) to apply the same amount of organic matter to the soil (5.84 g organic matter per kg of soil). A non-amended and non-polluted soil was used as control. The incubation treatments were detailed in Table 2.

Both organic compounds were liquid and were solubilized in distilled water before applying.

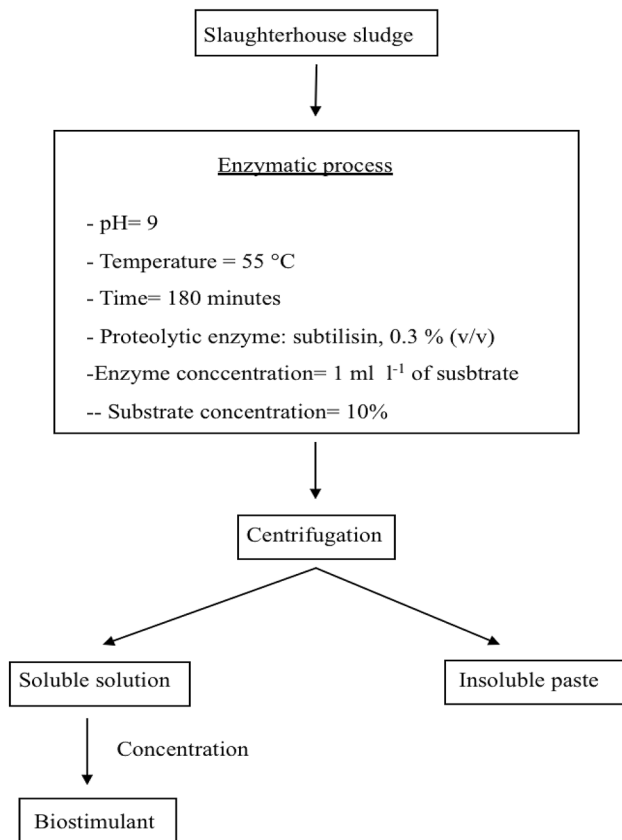


Fig. 1 Enzymatic hydrolysis process used for obtaining biostimulant from slaughterhouse sludge

Triplicate treatments were kept in microcosms at 25 ± 1 °C for 90 days. Distilled water was added to each soil to reach 30–40% of its water-holding capacity and the moisture losses were compensated by adding distilled water.

Soil Analysis

Dehydrogenase, urease, β -glucosidase and phosphatase activities for each treatment were determined in triplicate at days 5, 10, 20, 35, 55 and 90 using the methods described by García et al. (1994), Kandeler and Gerber (1988), Eivazi and Tabatabai (1988) and Tabatabai and Bremner (1969).

For each experimental treatment, microbial fatty acids were determined in triplicate at days 5 and 90 of the experiment. Ester-linked microbial fatty acids were extracted and quantified according to the methodology described by Montes de Oca-Vásques et al. (2020). The fatty acids i15:0, a15:0, i16:0, and i17:0 were representative of the Gram+ (G+) bacterial biomass, whereas the fatty acids 18:1 ω c, 18:1 ω 9t, cy17:0, and cy19:0 were representative of the Gram- (G-) bacterial biomass (Bardgett et al. 1996; Dungait et al. 2010). The fatty acid 18:2 ω 6 were representative of the fungal biomass (Zelles et al. 1992; Bååth 2003).

Table 1 Chemical characteristics and protein molecular weight distribution (mean \pm standard error, $n=3$) of soil, slaughterhouse sludge and biostimulant obtained by slaughterhouse sludge by enzymatic hydrolysis process

Parameters	Soil	SS	BS
pH (H ₂ O)	7.6 \pm 0.2		
Sand (g kg ⁻¹)	554 \pm 28		
Silt (g kg ⁻¹)	134 \pm 21		
Clay (g kg ⁻¹)	312 \pm 31		
Dry matter (%)		15.2a \pm 2.1	15.8a \pm 1.7
Organic matter (g kg ⁻¹)	18.3b \pm 1.8	789a \pm 37	649a \pm 22
N (g kg ⁻¹)	0.9b \pm 0.2	3.5a \pm 1.4	3.0a \pm 1.1
P (g kg ⁻¹)		5.6a \pm 2.1	5.4a \pm 1.8
K (g kg ⁻¹)		9.1a \pm 2.6	8.9a \pm 2.7
S (g kg ⁻¹)		12.8a \pm 3.3	11.7a \pm 2.1
Ca (g kg ⁻¹)		29.4b \pm 5.8	15.3a \pm 1.6
Mg (g kg ⁻¹)		1.6a \pm 0.4	1.5a \pm 0.7
Fe (g kg ⁻¹)		8.4b \pm 1.7	3.0a \pm 0.9
Cu (mg kg ⁻¹)		139a \pm 28	85.7a \pm 14.3
Mn (mg kg ⁻¹)		148b \pm 14	44.3a \pm 13.5
Zn (mg kg ⁻¹)		510b \pm 21	333a \pm 18
Pb (mg kg ⁻¹)		10.6b \pm 2.3	5.7a \pm 2.0
Ni (g kg ⁻¹)		10.4a \pm 1.7	7.8a \pm 1.1
Protein molecular weight distribution (Da)			
> 10,000		77.4b \pm 3.4	50.0a \pm 2.7
10,000–5000		3.4a \pm 1.6	4.8a \pm 1.7
5000–3000		2.1a \pm 0.5	2.9a \pm 1.1
3000–1000		3.8a \pm 1.2	5.9a \pm 1.3
1000–300		3.3a \pm 1.7	6.8a \pm 1.6
< 300		10.0a \pm 1.9	29.6b \pm 2.4

Files followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$)

SS slaughterhouse sludge, BS biostimulant obtained by slaughterhouse sludge

Table 2 Scheme of the incubation treatments performed

- (1) C, control soil, non-amended and without herbicide
- (2) C + SS, soil amended with SS and without herbicide
- (3) C + BS, soil amended with BS and without herbicide
- (4) C + Ox, soil non-organically amended and with herbicide
- (5) C + Ox + SS, soil amended with SS and with herbicide
- (6) C + Ox + BS, soil amended with BS and with herbicide

At days 5, 10, 20, 35, 55 and 90, the soil oxyfluorfen content was determined. Soil oxyfluorfen was extracted according to the Anastassiades et al. (2003) method. The methodology measuring the herbicide is described in Rodríguez-Morgado et al. (2014). The limit of detection (LOD) was 0.006 mg kg⁻¹ and the limit of quantification (LOQ) 0.01 mg kg⁻¹.

Statistical Analysis

To determine the differences between the results obtained for each parameter analysed, the data were submitted to two-way analysis of variance (ANOVA) using two factors (treatments and sampling time). This was then followed by the Tukey post-hoc test (HSD, $p < 0.05$). The ANOVA was performed using the Statgraphics Plus 2.1 software package. For the statistical analysis, triplicate data were used for each treatment and each day of incubation.

Results and Discussion

After the enzymatic hydrolysis process and with respect to the SS, the BS showed a significant ($p < 0.05$) decrease in the Ca and micronutrients concentration (Table 1). It was also observed that significant changes occurred in the protein molecular weight distributions. In this respect, and compared with the SS, the proteins with the highest molecular weight (> 1000 KDa) in the new BS decreased by 35.4%. Also, and with respect to the SS, the 1000–300 Da and < 300 Da molecular weight proteins in the new BS increased significantly by 51.5% and 66.2%.

The application of SS and BS significantly ($p < 0.05$) stimulated the soil enzymatic activities studied (Tables 3 and 4). Compared with the C treatment, the application of SS to the soil progressively increased the dehydrogenase, urease, β -glucosidase and phosphatase activities by 65.7%, 70.3%, 78.8% and 68%, respectively. However, the behavior of the enzymatic activities studied after applying the BS to the soils was very different. Dehydrogenase, β -glucosidase and

phosphatase activities reached maximum stimulation 5 days after the start of the experiment, then gradually decreasing until 35 days after the experiment. From this date, the dehydrogenase, β -glucosidase and phosphatase activity began to increase again until the end of the incubation period, reaching a higher value than that obtained in the control treatment. In this sense, the stimulation of dehydrogenase, β -glucosidase and phosphatase activities was 54%, 68.6% and 59.8.

Compared with the C + SS treatment, after the application of the BS to the soil, the behavior of the urease activity was very similar throughout the incubation period (Table 3).

We think that the cause of the different behaviour in the two experimental organic wastes can be due to the difference in the molecular protein weight distribution percentage. Our results highlight that in both organic wastes the protein size distribution differs mainly in sizes of > 1000 Da and < 300 Da. The SS has a higher percentage of high molecular weight proteins, while BS showed a higher percentage of low molecular weight proteins. Since the microorganisms in the soil cannot directly absorb the high molecular weight proteins, to obtain energy these microorganisms need to degrade them. This mineralisation process is usually slow, and therefore, microbial stimulation in the SS-amended soil increased progressively throughout the experimental period. According to Rodríguez-Morgado et al. (2015b), when applying BS to the soil, microorganisms directly absorb these low molecular weight proteins, favouring their stimulation. The subsequent decrease in their enzymatic activities to values similar to the control at day 35 suggest that by then soil microorganisms had already consumed these low

Table 3 Evolution of dehydrogenase and urease activities (mean \pm standard error, $n = 3$) in soils amended with slaughterhouse sludge (SS) and hydrolysate slaughterhouse sludge (BS) and with oxyfluorfen during the experimental period

	Incubation days					
	5	10	20	35	55	90
Dehydrogenase activity ($\mu\text{g INTF g}^{-1} \text{h}^{-1}$)						
C	2.7b \pm 0.6	2.8b \pm 0.4	2.5b \pm 0.3	2.3b \pm 0.2	2.4b \pm 0.3	2.3b \pm 0.3
C + SS	4.1c \pm 0.9	4.9c \pm 1.0	5.1c \pm 1.2	5.4c \pm 1.1	6.1c \pm 1.4	6.7c \pm 1.5
C + BS	15.2d \pm 2.6	6.8c \pm 1.7	5.7c \pm 0.8	4.2c \pm 0.5	4.5c \pm 0.8	5.0c \pm 1.1
C + Ox	1.3a \pm 0.2	1.4a \pm 0.3	1.1a \pm 0.1	1.4a \pm 0.3	1.5a \pm 0.2	1.2a \pm 0.3
C + Ox + SS	2.7b \pm 0.5	3.3b \pm 0.8	4.4c \pm 1.0	4.7c \pm 0.8	4.9c \pm 1.1	5.4c \pm 0.8
C + Ox + BS	10.3d \pm 1.9	5.5c \pm 0.7	4.0c \pm 0.7	3.7bc \pm 0.6	3.7bc \pm 0.7	4.1c \pm 1.0
Urease activity ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{h}^{-1}$)						
C	1.7b \pm 0.2	1.7b \pm 0.2	1.8b \pm 0.1	1.7b \pm 0.2	1.8b \pm 0.3	1.9b \pm 0.3
C + SS	2.7b \pm 0.9	3.2bc \pm 0.6	3.7c \pm 0.8	4.8c \pm 1.0	5.2c \pm 0.9	6.4c \pm 1.7
C + BS	2.9b \pm 1.1	3.4bc \pm 0.8	4.0c \pm 1.0	4.7c \pm 0.8	5.8c \pm 0.6	6.9c \pm 0.8
C + Ox	0.94a \pm 0.13	0.98a \pm 0.12	0.91a \pm 0.13	0.83a \pm 0.17	0.90a \pm 0.09	0.94a \pm 0.11
C + Ox + SS	1.7b \pm 0.6	2.2b \pm 0.6	2.9b \pm 0.7	3.0b \pm 0.8	3.8c \pm 0.6	4.5c \pm 1.0
C + Ox + BS	2.2b \pm 0.4	2.5b \pm 0.4	2.7b \pm 0.5	2.7b \pm 0.6	3.1bc \pm 0.8	3.4c \pm 0.7

Columns followed by the same letter(s) are not significantly different ($p > 0.05$)

INTF 2-*p*-iodo-3-nitrophenyl formazan

Table 4 Evolution of β -glucosidase and phosphatase activities (mean \pm standard error, $n=3$) in soils amended with slaughterhouse sludge (SS) and hydrolyzate slaughterhouse sludge (BS) and with oxyfluorfen during the experimental period

	Incubation days					
	5	10	20	35	55	90
β -glucosidase activity (mmol PNP g ⁻¹ h ⁻¹)						
C	1.4b \pm 0.2	1.3b \pm 0.2	1.3b \pm 0.1	1.3b \pm 0.2	1.2b \pm 0.3	1.1b \pm 0.1
C + SS	2.7c \pm 0.8	2.9c \pm 0.9	3.2c \pm 1.2	3.8c \pm 0.8	4.4cd \pm 1.0	5.2d \pm 1.2
C + BS	8.4d \pm 1.8	5.9c \pm 1.1	3.1c \pm 0.6	2.4c \pm 0.4	3.0c \pm 0.5	3.5c \pm 0.8
C + Ox	0.72a \pm 0.09	0.79a \pm 0.11	0.75a \pm 0.08	0.76a \pm 0.08	0.74a \pm 0.12	0.70a \pm 0.14
C + Ox + SS	2.3c \pm 0.8	2.2c \pm 0.4	2.5c \pm 0.2	2.6c \pm 0.7	2.9c \pm 0.3	3.1c \pm 0.8
C + Ox + BS	5.8c \pm 0.4	4.1c \pm 1.3	2.7c \pm 0.3	1.7b \pm 0.3	1.9bc \pm 0.7	2.1c \pm 0.5
Phosphatase activity (μ mol PNP g ⁻¹ h ⁻¹)						
C	3.0b \pm 1.0	3.3b \pm 0.9	3.4b \pm 1.2	3.4b \pm 1.2	3.2b \pm 1.1	3.3b \pm 0.9
C + SS	4.9b \pm 1.4	5.4b \pm 1.7	6.2c \pm 1.0	7.5c \pm 1.8	8.6c \pm 1.4	10.3d \pm 2.1
C + BS	20.4d \pm 3.1	11.5d \pm 2.2	12.7d \pm 1.4	7.2c \pm 1.8	7.8c \pm 1.1	8.2c \pm 1.5
C + Ox	1.7a \pm 0.4	1.9a \pm 0.3	1.8a \pm 0.4	1.9a \pm 0.2	1.8a \pm 0.3	1.8a \pm 0.3
C + Ox + SS	4.4b \pm 1.1	4.7b \pm 1.5	5.2b \pm 1.3	6.1c \pm 1.0	6.8c \pm 1.2	7.2c \pm 1.8
C + Ox + BS	15.8d \pm 2.0	10.4d \pm 2.4	6.4c \pm 1.8	4.2b \pm 1.4	4.2b \pm 1.2	4.6b \pm 1.0

Columns followed by the same letter(s) are not significantly different ($p > 0.05$)

PNP *p*-nitrophenol

molecular weight proteins. The progressive increase from day 35 on the soil biochemical activity is a consequence of the fact that the BS still showed a high number of high molecular weight proteins, implying that, as of this day, soil microorganisms began to excrete extracellular enzymes to obtain energy. This means that there was a progressive increase in the soil biochemical activity until the end of the incubation period.

The soil behaviour of this new BS obtained from slaughterhouse sludge differs greatly from that obtained from other BSs also by enzymatic hydrolysis using the same enzyme from sewage sludge, chicken feathers, rice brain, condensed distillates of soluble wheat, and okara, which, after application, caused an increase in the soil biological activity during the first days of incubation. Subsequently this activity decreased and showed a biochemical activity similar to that of a non-polluted soil (Tejada et al. 2010, 2011b, 2014; Rodríguez-Morgado et al. 2015b; Orts et al. 2017). This is a consequence of the fact that in this type of BS, the amount of low molecular weight proteins (< 300 Da) was greater than that obtained with the BS of slaughterhouse sludge. Therefore, when the BS obtained from the slaughterhouse sludge was applied to the soil, the microorganisms quickly assimilated the low molecular weight peptides. Once these had been absorbed, the microorganisms had to obtain their energy from the high molecular weight proteins, excreting extracellular enzymes to degrade said proteins.

Tejada and Benítez (2020) also observed that the biochemical activity was higher in soils amended with organic matter with a higher percentage of low molecular weight peptides. These authors also concluded that the higher soil microbial stimulation was possibly a consequence of a

higher absorption of low molecular weight peptides by the microorganisms.

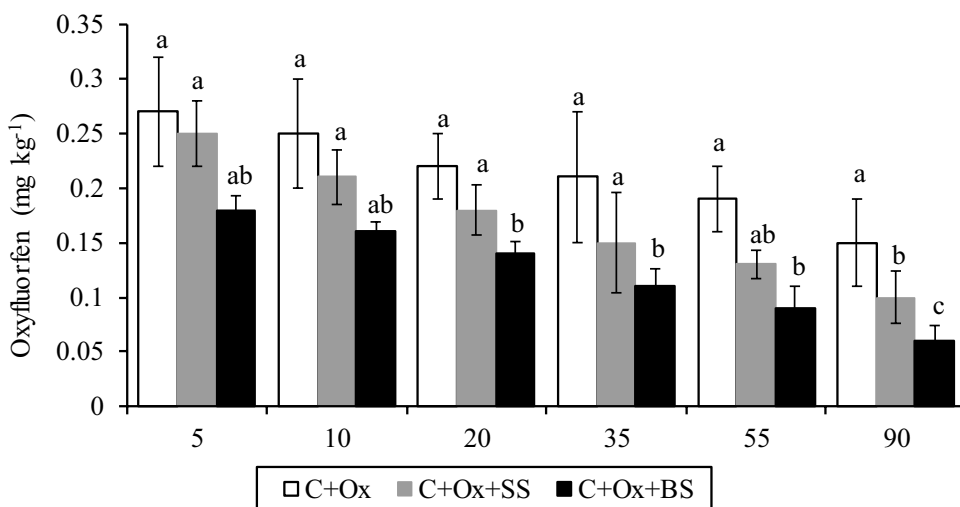
Applying oxyfluorfen to non-amended soil caused a significant ($p < 0.05$) inhibition in the soil enzymatic activities during the experiment (Tables 2 and 3). At the end of the experiment and compared with the C treatment, dehydrogenase, urease, β -glucosidase, and phosphatase activities significantly decreased by 47.8%, 50.5%, 36.4%, and 45.5%, respectively (Tables 3 and 4).

Applying oxyfluorfen to non-polluted soil caused an inhibitory effect on the soil biochemical activity and microbial population. These results are in accordance with Nadjjer et al. (2013), Gómez et al. (2014), Franco-Andreu et al. (2016), Campos et al. (2019) and Wołejko et al. (2020), who highlighted the toxic effect of oxyfluorfen on soil biochemical activity.

The application both organic compounds decreased the soil oxyfluorfen concentration (Fig. 2). At the end of the experiment, the soil oxyfluorfen had decreased 44.4% compared to the concentration at day 5. Applying both organic compounds to the polluted soil caused a significant decrease ($p < 0.05$) in the soil oxyfluorfen concentration. Compared with the C + Ox treatment, the oxyfluorfen concentration had decreased by 33.3% in the C + Ox + SS treatment and by 60% in the C + Ox + BS treatment.

Gómez et al. (2014) and Rodríguez-Morgado et al. (2014) observed a significant decrease in the concentration of oxyfluorfen after the application of various organic compounds to contaminated soil. These authors observed that this decrease was greater in soils amended with organic matter with a high content of low molecular weight proteins, possibly due to the fact that oxyfluorfen-tolerant soil

Fig. 2 Evolution of oxyfluorfen (mean ± standard error, *n* = 3) in soils during the experimental period. Columns followed by the same letter(s) are not significantly different (*p* > 0.05)



microorganisms absorb these peptides more easily, favoring their proliferation in the soil and increasing the degradation of said herbicide in soil.

This decrease in the soil oxyfluorfen concentration caused a decrease in the enzymatic activities inhibition shown in the C + Ox treatment (Tables 3 and 4). Also, the chemical composition of both organic wastes influenced this action differently. For example, at the end of the experimental period and compared with the C + Ox treatment, dehydrogenase activity had increased by 87.7% and 70.7% in the C + Ox + SS and C + Ox + BS treatments. Urease activity increased by 79.1% and 72.3% in the C + Ox + SS and C + Ox + BS treatments. β-glucosidase activity had increased by 77.4% and 66.7% in the C + Ox + SS and C + Ox + BS treatments, respectively. Phosphatase activity increased by 75% and 60.9% in the C + Ox + SS and C + Ox + BS treatments.

The oxyfluorfen degradation was higher in the BS-amended soil than with SS. The higher percentage of low

molecular weight proteins (< 300 Da) in the BS enabled the herbicide-tolerant microorganisms to the said peptides more quickly. This stimulated the microbial population and consequently led to a greater degradation of the herbicide. The high percentage of high molecular weight protein in SS results in a lower stimulation in the herbicide-tolerant microbial population. Consequently, over time oxyfluorfen degradation is slower than when SS is applied.

Applying the herbicide to non-organic amended soil did not change the microorganisms population during the experimental period (Table 5). Similar results were found by Rodríguez-Morgado et al. (2014) and Gómez et al. (2014) in a soil with similar physicochemical characteristics and contaminated with the same dose of oxyfluorfen during a period of 120 days.

The application of organic matter to the soil caused an increase in total bacterial PLFA and total fungal PLFA population. Tian et al. (2017) and Zheng et al. (2021) also

Table 5 Evolution of bacterial Gram⁺, bacterial Gram⁻, total bacterial and fungal PLFAs (nmol g⁻¹), during the experimental period

Treatment	Incubation days	bacterial Gram ⁺	bacterial Gram ⁻	Total bacterial PLFA	Fungal PLFA
C	5 days	28.1a ± 2.4	18.6a ± 1.8	47.7a ± 3.9	3.1a ± 1.1
	90 days	28.9a ± 2.0	19.5a ± 2.2	48.4a ± 4.4	2.8a ± 0.9
C + SS	5 days	31.4a ± 2.5	24.4a ± 2.0	55.8a ± 4.3	3.8a ± 1.1
	90 days	49.9b ± 3.1	50.2b ± 2.7	100.1b ± 5.5	5.2b ± 1.2
C + BS	5 days	50.2b ± 2.9	30.6ab ± 3.1	80.8b ± 6.3	3.7a ± 1.2
	90 days	50.0b ± 3.6	38.9b ± 2.2	88.9b ± 5.6	4.0a ± 1.7
C + Ox	5 days	29.9a ± 1.3	23.5a ± 1.6	53.4a ± 2.7	3.6a ± 1.1
	90 days	27.3a ± 2.6	22.9a ± 1.2	50.2a ± 3.6	3.2a ± 0.8
C + Ox + SS	5 days	29.8a ± 2.9	25.7a ± 2.6	55.5a ± 5.6	4.0a ± 1.7
	90 days	47.0b ± 1.6	49.1bc ± 3.3	96.4b ± 4.7	4.8ab ± 1.5
C + Ox + BS	5 days	49.3b ± 2.7	30.4ab ± 2.4	79.7ab ± 4.8	4.0a ± 1.0
	90 days	46.7b ± 2.9	33.8b ± 1.7	80.5b ± 4.5	3.8a ± 1.2

Data are the means of three samples. Columns (mean ± S.E.) followed by the same letter(s) are not significantly different (*p* > 0.05)

found an increase in microbial biodiversity in soils with a high content of organic matter. These authors suggest that the application of organic matter to the soil provides substrates that can increase the total abundance of PLFA.

When oxyfluorfen was applied to soils amended with both organic compounds, the bacteria and fungi population was not different from that observed for C + SS and C + BS treatments. This fact is possibly due to the fact that oxyfluorfen did not change the microbial biodiversity of the soil, as previously mentioned.

Conclusions

According to the authors' knowledge, the main novelty of this manuscript is the first study in which bioremediation tests have been carried out on soils polluted by the oxyfluorfen herbicide using slaughterhouse sludge, both concentrated and in the form of biostimulants after subjecting said sludge concentrated to an enzymatic hydrolysis process. The results obtained in this experiment indicated that slaughterhouse sludge decreased the inhibitory effect of the oxyfluorfen on the soil's biological properties. Consequently, slaughterhouse sludge could be considered as being very useful in bioremediating oxyfluorfen-polluted soils. However, the greatest bioremediation effects were obtained when the slaughterhouse sludge was transformed into a biostimulant by enzymatic processes rich in low molecular weight proteins. These proteins are easily assimilated by toxic-tolerant soil microorganisms which accelerated the degradation of the herbicide in soil.

However, the bioremediation effect of slaughterhouse sludge should be studied further. The type of contaminant in the soil, as well the soil properties, are characteristics to be considered for future studies to better understand the effect of these organic wastes on the bioremediation of polluted soils.

Acknowledgements This work was supported by the Ministerio de Ciencia, Innovación y Universidades (Spain), Plan Nacional I+D+I with reference RTI2018-097425-B-100, Junta de Andalucía (Consejería de Economía y Educación), Proyectos I+D+i FEDER Andalucía 2014-2020, with reference US-1263885 and Comisión Europea, Horizonte 2020 with reference 3052/0048.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source,

provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Adler-Nissen J (1977) Enzymatic hydrolysis of food protein. *Proc Biochem* 12:15–32
- Alfonso-Muniozguren P, Gomes AI, Saroj D, Vilar VJP, Lee J (2021) The role of ozone combined with UVC/H₂O₂ process for the tertiary treatment of a real slaughterhouse wastewater. *J Environ Manage* 289:112480. <https://doi.org/10.1016/j.jenvman.2021.112480>
- Anastassiades M, Lehotay SJ, Stajnbaher D, Shenck FJ (2003) Fast and easy multiresidue method employing extraction/partitioning and dispersive soil phase extraction for the determination of pesticide residues in produce. *J AOAC Intern* 86:412–431
- Aziz A, Basheer F, Sengar A, Irfanullah KSU, Farooqi IH (2019) Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. *Sci Total Environ* 686:681–708. <https://doi.org/10.1016/j.scitotenv.2019.05.295>
- Bååth E (2003) The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *MicrobEcol*. <https://doi.org/10.1007/s00248-003-2002-y>
- Bardgett RD, Hobbs PJ, Frostergard A (1996) Changes in soil fungal: bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biol Fertil Soils* 22:261–264
- Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E (2013) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proc Natl Acad Sci USA* 110(52):20882–20887
- Bustillo-Lecompte CF, Mehrvar M (2015) Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: a review on trends and advances. *J Environ Manage* 161:287–302. <https://doi.org/10.1016/j.jenvman.2015.07.008>
- Campos JA, Peco JD, García-Noguero E (2019) Antigerminative comparison between naturally occurring naphthoquinones and commercial pesticides. Soil dehydrogenase activity used as bioindicator to test soil. *Sci Total Environ* 694:133672. <https://doi.org/10.1016/j.scitotenv.2019.133672>
- Davin M, Starren A, Deleu M, Lognay G, Colinet G, Fauconnier ML (2018) Could saponins be used to enhance bioremediation of polycyclic aromatic hydrocarbons in aged-contaminated soils? *Chemosphere* 194:414–421. <https://doi.org/10.1016/j.chemosphere.2017.11.174>
- Dungait JAJ, Kemmitt SJ, Michallon M, Guo S, Wen Q, Brookes PC, Evershed RP (2010) Variable responses of the soil microbial biomass to trace concentrations of ¹³C-labelled glucose, using ¹³C-PLFA analysis. *Eur J Soil Sci* 62:117–126. <https://doi.org/10.1111/j.1365-2389.2010.01321.x>
- Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. *Soil Biol Biochem* 20:601–606. [https://doi.org/10.1016/0038-0717\(88\)90141-1](https://doi.org/10.1016/0038-0717(88)90141-1)
- FAO (1989) *Carte mondiale des sols. Légende revise*, Rome

- Franco-Andreu L, Gómez I, Parrado J, García C, Hernández T, Tejada M (2016) Behavior of pesticides in a soil subjected to severe drought. Effects on soil biology. *Appl Soil Ecol* 105:17–24. <https://doi.org/10.1016/j.apsoil.2016.04.001>
- García C, Hernández T, Costa F (1994) Microbial activity in soils under mediterranean environmental conditions. *Soil Biol Biochem* 26:1185–1191. [https://doi.org/10.1016/0038-0717\(94\)90142-2](https://doi.org/10.1016/0038-0717(94)90142-2)
- Gómez I, Rodríguez-Morgado B, Parrado J, García C, Hernández T, Tejada M (2014) Behavior of oxyfluorfen in soils amended with different sources of organic matter. Effects on soil biology. *J Hazard Mater* 273:207–214. <https://doi.org/10.1016/j.jhazmat.2014.03.051>
- Kadian N, Malik A, Satya S, Dureja P (2012) Effect of organic amendments on microbial activity in chlorpyrifos contaminated soil. *J Environ Manage* 95:S199–S202. <https://doi.org/10.1016/j.jenvman.2010.10.023>
- Kandeler E, Gerber G (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6:68–72
- Lipczynska-Kochany E (2018) Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: a review. *Chemosphere* 202:420–437. <https://doi.org/10.1016/j.chemosphere.2018.03.104>
- Menegassi LC, Rossi F, Dominical LD, Tommaso G, Montes CR, Gomide CA, Gomes TM (2020) Reuse in the agro-industrial: Irrigation with treated slaughterhouse effluent in grass. *J Clean Prod* 251:119698. <https://doi.org/10.1016/j.jclepro.2019.119698>
- Montes de Oca-Vásquez G, Solano-Campos F, Vega-Baudrit JR, López-Mondéjar R, Odriozola I, Vera A, Moreno JL, Bastida F (2020) Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity. *J Hazard Mater* 391:122224. <https://doi.org/10.1016/j.jhazmat.2020.122224>
- Nadiger S, Babu R, Kunar BNA (2013) Bioefficacy of pre-emergence herbicides on weed management in maize. *Karnataka J Agric Sci* 26:17–19
- Ortiz-Botella M, Gómez I, Patricia Paneque P, Caballero P, Parrado J, Vera A, Bastida F, García C, Tejada M (2021) Use of biostimulants obtained from okara in the bioremediation of soils polluted by imazamox. *Bioremediat J*. <https://doi.org/10.1080/10889868.2021.1884531>
- Orts A, Cabrera S, Gómez I, Parrado J, Rodríguez-Morgado B, Tejada M (2017) Use of okara in the bioremediation of chlorpyrifos in soil: effects on soil biochemical properties. *Appl Soil Ecol* 121:172–176. <https://doi.org/10.1016/j.apsoil.2017.09.042>
- Rodríguez-Morgado B, Gómez I, Parrado J, Tejada M (2014) Behaviour of oxyfluorfen in soils amended with edaphic biostimulants/biofertilizers obtained from sewage sludge and chicken feathers. Effects on soil biological properties. *Environ Sci Pollut Res* 21:11027–11035. <https://doi.org/10.1007/s11356-014-3040-3>
- Rodríguez-Morgado B, Gómez I, Parrado J, García C, Hernández T, Tejada M (2015a) Accelerated degradation of PAHs using edaphic biostimulants obtained from sewage sludge and chicken feathers. *J Hazard Mater* 300:235–242. <https://doi.org/10.1016/j.jhazmat.2015.05.045>
- Rodríguez-Morgado B, Gómez I, Parrado J, García-Martínez AM, Aragón C, Tejada M (2015b) Obtaining edaphic biostimulants/biofertilizers from different sewage sludges. Effects on soil biological properties. *Environ Technol* 36:2217–2226. <https://doi.org/10.1080/09593330.2015.1024760>
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenol phosphate in assay of soil phosphatase activity. *Soil Biol Biochem* 1:301–307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
- Tejada M, Benítez C (2020) Effects of different organic wastes on soil biochemical properties and yield in an olive grove. *Appl Soil Ecol* 146:103371. <https://doi.org/10.1016/j.apsoil.2019.103371>
- Tejada M, García-Martínez AM, Gómez I, Parrado J (2010) Application of MCPA herbicide on soils amended with biostimulants: short-time effects on soil biological properties. *Chemosphere* 80:1088–1094. <https://doi.org/10.1016/j.chemosphere.2010.04.074>
- Tejada M, Benítez C, Parrado J (2011a) Application of biostimulants in benzo(a)pyrene polluted soils: short-time effects on soil biochemical properties. *Appl Soil Ecol* 50:21–26. <https://doi.org/10.1016/j.apsoil.2011.08.002>
- Tejada M, Gómez I, del Toro M (2011b) Use of organic amendments as a bioremediation strategy to reduce the bioavailability of chlorpyrifos insecticide in soils. Effects on soil biology. *Ecotoxicol Environ Saf* 74:2075–2081. <https://doi.org/10.1016/j.ecoenv.2011.07.005>
- Tejada M, Rodríguez-Morgado B, Gómez I, Parrado J (2014) Degradation of chlorpyrifos using different biostimulants/biofertilizers: effects on soil biochemical and microbial community. *Appl Soil Ecol* 84:158–165. <https://doi.org/10.1016/j.apsoil.2014.07.007>
- Tian J, Lou Y, Gao Y, Fang H, Liu S, Xu M, Blagodatskaya E, Kuz'yakov Y (2017) Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization. *Biol Fertil Soils* 53:523–532. <https://doi.org/10.1007/s00374-017-1189-x>
- Wolejko E, Jabłońska-Trypuć A, Wydro U, Butarewicz A, Bożena Ł (2020) Soil biological activity as an indicator of soil pollution with pesticides. A Review. *Appl Soil Ecol* 147:103356. <https://doi.org/10.1016/j.apsoil.2019.09.006>
- Zelles L, Bai QI, Beck T, Beese F (1992) Signature fatty-acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biol Biochem* 24:317–323. [https://doi.org/10.1016/0038-0717\(92\)90191-Y](https://doi.org/10.1016/0038-0717(92)90191-Y)
- Zheng T, Miltner A, Liang Ch, Nowak KM, Kästner T (2021) Turnover of gram-negative bacterial biomass-derived carbon through the microbial food web of an agricultural soil. *Soil Biol Biochem* 152:108070. <https://doi.org/10.1016/j.soilbio.2020.108070>