Journal of Soil Science and Plant Nutrition (2025) 25:8811–8827
https://doi.org/10.1007/s42729-025-02696-2

ORIGINAL PAPER

Assessing the Fertilizing Potential of Two Tannery Bio-Wastes Through Short-Term Soil Incubation and Plant Rhizosphere Bioassay

Andrea Ciurli[1] · Giampaolo Di Biase[1] · Martina Mazzon[1] · Claudio Ciavatta[1] · Luciano Cavani[1]

Received: 25 February 2025 / Accepted: 28 August 2025 / Published online: 15 September 2025

Abstract

The tannery industry is a significant global manufacturing sector, producing over 4 million tons of tannery bio-wastes (TBWs) annually, and to reintegrate TBWs into the productive cycle offers a significant alternative to landfill disposal. Despite TBWs are rich in organic matter, nitrogen (N), and carbon (C) they pose important environmental risks, such as the presence of heavy metals like chromium (Cr) that can be released into the environment. This study aimed at evaluating the fertilizing potential of two TBWs-based fertilizers through a complementary laboratory-scale approach by using a short-term soil incubation experiment and the Rhizotest (ISO16198: 2015) bioassay. Two TBWs-based fertilizers, Tannery Sludge (TS) and Integrated Leather Meal (ILM), were subjected to 42 days of soil incubation and to Rhizotest bioassay. Nitrogen release, chemical and biochemical indicators were assayed after the soil incubation. Tomato plants uptake of heavy metals and rhizosphere enzyme activities were assayed after Rhizotest. TS and ILM released 11% and 35% of their total N content respectively, with TS acting more as a slow-release fertilizer. Heavy metal contamination was negligible, except for Cr, which however remained in the soil in its trivalent form and was not absorbed by plants. Rhizotest allowed to highlight that TS and ILM stimulated broad and specific enzyme activities in the rhizosphere soil. This complementary approach enabled rapid, reproducible, and sensitive characterization of organic fertilizers produced from TBWs, which have the potential to be employed as organic fertilizers safely.

Keywords Organic fertilizers · Tannery bio wastes · Soil chemistry · Rhizotest · Rhizosphere · Enzymatic activity

1 Introduction

The accelerated worldwide industrialization that has occurred over the past few decades has posed severe challenges to global sustainability in the agri-food sector, which must contend with the doubling of global food demand – an expected increase of 59–98% by 2050 – (Bhat 2022). In association with the world meat chain, tannery industry for leather production is among the most important manufacturing industries all over the world (Moktadir et al. 2020). The global leather trade is valued at approximately US$100 billion per year, with 25% of production occurring in Europe (Thomasset and Benayoun 2024). However, the tannery industry is a significant contributor to environmental risks associated with leather production (Ozgunay et al. 2007). It is estimated that over 4 million tons of tannery bio-wastes (TBWs) are generated annually in conjunction with leather manufacturing (Mahmood Ali et al. 2023). TBWs result from processes such as fleshing, trimming, shaving, splitting, and wastewater treatment. These materials constitute 80% of the raw hide, amounting to 600,000 tons of TBWs generated globally each year (Mahmood Ali et al. 2023).

The tannery industry and leather production are experiencing sustained growth, particularly in the least-developed countries (Stefan et al. 2021). However, viable alternative disposal methods that do not involve landfill remain scarce (Stefan et al. 2021). The emergence of new industrial business models, particularly those based on circular economy principles, is crucial for facilitating the recovery of TBWs and their reintegration into productive system (Gatto 2023).

Tannery bio-wastes have been widely used as fertilizers due to their high organic nitrogen (N) content (Ciavatta et al. 2012; Feng et al. 2013; Rapisarda et al., 2022),

✉ Andrea Ciurli
andrea.ciurli3@unibo.it

[1] Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum - University of Bologna, Via G. Fanin 40, Bologna (BO) 40127, Italy

functioning as slow-release fertilizer (John Sundar et al. 2011). Additionally, they have a considerable organic matter and carbon (C) content (Rigueto et al. 2020). Studies have demonstrated that the application of tannery sludges to the soil positively impacts plant growth (Gonçalves Da Silva et al., 2010; Perdigão et al. 2022; Santos et al. 2020) and increases soil N and phosphorus (P) pools (Nabavinia et al. 2015), whereas the effects on chemical parameters such as soil pH and electrical conductivity (EC) have been less pronounced (Santos et al. 2020). However, critical aspects of using TBWs as fertilizers have been highlighted, including their content in chromium (Cr), formaldehyde, and other tanning or preservative agents, such as glutaraldehyde, nonylphenols, and phthalates depending on the kind of tannery process used (Khatun et al. 2024). TBWs management has been found to have considerable environmental and social impact (Koppiahraj et al. 2019; Tahiri and de la Guardia 2008). Furthermore, the amount and type of organic wastes vary depending on the manufacturing process (Ozgunay et al. 2007). Monitoring soil biochemical parameters such as microbial biomass C (MBC) and soil enzyme activity (Bakshi and Varma 2010) can help ascertain whether the reuse of TBWs as fertilizers enhances soil nutrient cycling and fertility without negatively affecting soil health (Maurya et al. 2020).

Soil fertility is defined as the capacity of soil to provide sufficient nutrients and moisture to sustain crop growth (Dick and Culman, 2017). Several factors influence soil fertility, including physical properties (structure, texture), chemical properties (nutrient forms and availability) and biological factors (e.g., microbial activity and plant roots) (Gregory and Nortcliff 2013). Rhizosphere was first defined by Hiltner in 1904 as the soil volume surrounding the root surface. The microbial community associated with rhizosphere soil is significantly stimulated by root activity, which involves the secretion of soluble exudates such as carbohydrates, amino acids, organic acids and other photosynthates (Nannipieri et al. 2003). Various soil enzymes essential for maintaining soil fertility are associated with plant roots and rhizosphere (Egamberdieva et al. 2010). Since plant nutrient uptake occurs through the rhizosphere, studying the enzyme activities of rhizosphere microbial communities as biochemical indicators of soil fertility can help identify key drivers of soil nutrient availability (Egamberdieva et al. 2010; Ren et al. 2021). Short-term soil incubation experiments (Tejada 2009) are widely used for this purpose, allowing the determination of nutrient release while monitoring soil fertility biochemical indicators, but they are limited because they do not account for processes occurring at the soil-root interface. Additionally, the wide variety of experimental conditions in studies using pot-grown plants in soil makes it difficult to obtain comparable and reproducible results (Luster 2006; Moran and McGrath 2021). Specifically, Rhizotest is a plant-based bioassay used to assess the bioavailability of micro- and trace-element in soil (Bravin et al. 2010). This method enables close contact between roots and soil while preventing root penetration into the soil layer through a 30-µm polyamide mesh. Notably, Rhizotest facilitates easy recovery of the rhizosphere soil, which can be considered the portion of soil directly affected by root activity (Beggio et al. 2024; Daly et al. 2015). This prevents the contamination of root tissue with soil particles and ensures accurate quantification of elements concentrations in soil or plant samples after the exposure. Nevertheless, the amount of rhizosphere soil collected in Rhizotest is very low (approximately 9 g of dry soil per pot) making it challenging to perform enzymatic activity assays and other biochemical analyses, such as the determination of microbial biomass C (MBC) content. To address this limitation, we performed microplate-scale fluorometric enzyme activity analyses (Giacometti et al. 2014). These assays, commonly used to measure the indicators of the biogeochemical cycling of key macro- and micronutrients (α- and β-glucosidases, β-xylosidases, chitinase and phosphomonoesterases, Schinner et al. 2012), require only small soil samples.

The aim of this study was to evaluate the fertilizing effect of two TBWs fertilizers through a short-term laboratory-scale experiment concerning: (i) the rate of N release in the soil and its effect on the main chemical/biochemical parameters of the soil fertility; (ii) the bioavailability of micro- and trace-elements to the plant and (iii) the effect of the fertilization on enzymatic activity as key drivers of soil nutrient availability in rhizosphere soil. To this aim, we employed a complementary approach by conducting a soil incubation experiment and the Rhizotest plant-based bioassay (ISO16198:2015). We hypothesized that, by means of this approach, it will be highlighted the fertilizing potential of TS and ILM as organic N fertilizers, as well as the stimulant effect on the main indicators of the nutritional processes in rhizosphere soil will be evidenced. Furthermore, the potential risks for Cr and other trace elements accumulation in the plant tissues would be addressed by measuring its translocation from soil to plant.

2 Materials & Methods

2.1 Characterization of Tannery Bio-Waste Fertilizers

The tested products were fertilizers converted from TBWs produced by the company Cuoiodepur SpA (https://www.cuoiodepur.it/). Tannery sludge (TS) was obtained from tannery wastewater treatment plant after solid removal phase

(i.e. sedimentation), sludge concentration by thickening and subsequent dewatering treatments by belt filterpress. After physical stabilization by heat drying, the dry sludge (i.e. protein sludge, viz. "*fanghi proteici*") is a stable material rich in organic matter and organic N, and potentially can be directly employed as fertilizer or blended with other tannery bio-waste, such as hydrolysed leather meal (Ciavatta and Sequi 1989) to constitute Integrated Leather Meal (ILM) (Caponi 2012), which is recognized as an organic N fertilizer, "*Pellicino integrato*" a "product obtained by mixing skin and meat meal and/or bone meal with stabilized protein sludge from the tanning cycle" (Italian Law n.75/2010).

Main physicochemical characteristics of the two fertilizers were determined according to the European method for fertilizers (https://eur-lex.europa.eu/eli/reg/2003/2003/oj) and are reported in Table 1. Humidity was determined by oven-drying at 105 °C after reaching a constant weight. Soil reaction (pH) was measured on extracts in 3:50 mass-to-H_2O ratio after 30 min of shaking at room temperature (RT). The electrical conductivity (EC) was measured on extracts in 1:10 mass-to-H_2O ratio after 30 min of shaking at RT. Total organic carbon (TOC) was determined by wet oxidation with potassium dichromate method (Ciavatta et al. 1989). Total N (TN) was determined after acid digestion with sulfuric acid and selenium-potassium persulfate as catalyser, using a Kjeldahl automatic instrument (KjelFlex K360, BUCHI Labortechnik AG, Flawil, Switzerland). The ammonium (NH_4^+-N) and nitrate(NO_3^--N) were determined after extraction with 1 M KCl (1:10 w: v) and steam distillation with Kjeldahl and added magnesium oxide for NH_4^+-N and Devarda's alloy for NO_3^--N (A.O.A.C 1990). The total organic N (TON) was calculated by the difference between total N and ammonium-N+ nitrate-N. Total phosphorus (P), total sulphur (S) and metals were determined by microwave wet acid digestion (Start-E, Milestone, U.S.A.) and by inductively coupled plasma optical emission spectrometer (Spectro Arcos ICP-OES Analyzer, Spectro Analytical Instrument GmbH, Kleve, Germany). ATR-FTIR spectra of TS and ILM were acquired using a Bruker TENSOR FTIR instrument (Bruker Optics, Ettlingen, Germany) equipped with an accessory for analysis in micro-attenuated total reflection. The microdiamond crystal constituting the sampling device had single reflection with an angle of incidence of 45° (Specac Quest ATR, Specac Ltd., Orpington, Kent, UK). Approximately 2 − 3 mg of fine-pulverized sample was placed on the surface of the crystal. Spectra were recorded from 4000 to 400 cm^{-1}, at a spectral resolution of 4 cm^{-1} and 64 scans. Before the analysis of each sample, background against air was done. The diamond was cleaned with ethanol after each analysed sample. Spectra were processed with GRAMS/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH, USA).

Table 1 Main properties of tannery sludge (TS) and integrated leather meal (ILM). Values represent average data and are expressed on dry weight basis, excluding humidity that is expressed on fresh weight (fw) basis

Parameter	Unit	TS	ILM
Description		Tannery sludge	Integrated Leather Meal
Humidity	%fw	26±2.8	12±1.6
Reaction (pH) in H_2O	-	8.5±0.01	7.5±0.01
Electrical conductivity	dS/m	2.8±0.04	3.5±0.1
Total organic C	C, %	20.4±0.3	27.8±0.7
Total N	N, %	2.2±0.01	4.1±0.41
Ammonium N	N, %	0.28±0.04	0.08±5e-3
Nitrate N	N, %	0.02±1e-4	0.02±4e-4
Organic N	Total N, %	86	98
C/N ratio	-	9.3	6.8
Total P	P, %	0.1±1e-3	0.2±1e-3
Total K	K, %	0.04±2e-4	0.07±1e-4
Total S	S, %	2.0±0.2	2.3±1.1
Total Fe	g kg^{-1}	18±1.1	19±0.9
Total Cu	mg kg^{-1}	22±1.2	32±3.1
Total Zn	mg kg^{-1}	181±9.8	198±22
Total Cr	g kg^{-1}	3.1±0.1	5.1±0.3
Total Cr(VI)	mg kg^{-1}	<0.2	<0.2
Total Cd	mg kg^{-1}	0.6±0.02	0.5±0.06
Total Co	mg kg^{-1}	5.2±0.2	3.8±0.4
Total Pb	mg kg^{-1}	5.0±0.2	3.0±0.3
Total Na	g kg^{-1}	6.2±0.4	8.4±0.5
Total Cl	g kg^{-1}	0.1±0.02	1.2±0.06

2.2 Soil Incubation Experiment and Analyses

The soil used in the experiments was a Fluvic Cambisol (WRB 2014) and it was collected from the experimental farm of the University of Bologna, located in Cadriano (BO) in the southern part of the Po Valley, Italy (45.53° N, 11.38° E, 28 m a.s.l.). The area is characterized by a mean annual temperature of 14.7 °C and annual precipitation of 621 mm. A plot that had remained untreated for the past two years was selected within the experimental fields. Soil was then collected from 5 different spots using a clean spade, taken from the top 25 cm of soil. Afterwards, collected soil was homogenised, air-dried and sieved at 2 mm, and the main characteristics were determined (Table 2). The soil was pre-incubated in 200 g pots for two weeks at 20 °C maintaining a constant humidity (30% of soil WHC). The fertilizers tested (TS and ILM) were then added on the basis of 100 $mg_N\ kg_{dry\ soil}^{-1}$, corresponding to a dose of about 240 kg ha^{-1} (Ciurli et al. 2024). The incubation lasted 42 days and samples for inorganic-N determination were taken after 0, 7, 14, 21, 28, 35, and 42 days to define the trend of N-release. Conversely, with the aim to determine the residual effect of the fertilization after most of the inorganic N was released, biochemical indicators of the soil fertility were measured on fresh samples on the last day of incubation. Following the incubation period, chemical indicators were measured on dried soil samples.

2.2.1 Nitrogen Release in the Soil

Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were analysed following the ISO 14256-2 protocol, extracted in 1 M KCl (2:10, w/v) shaking for 1 h at RT and determined colorimetrically using a flow autoanalyzer (AA3, Bran Lubbe, Germany).

2.2.2 Chemical and Biochemical Indicators

Soil pH, electrical conductivity (EC) and extractable metals in diethylenetriaminepentaacetic acid (DTPA) were determined in agreement with SSSA methods (Sparks et al. 1996). Soil pH was measured on H_2O extracts 1:2.5 (w/v) whereas EC was measured on H_2O extracts 1:5 (w/v), DTPA-extractable metals were extracted from approximately 10 g of soil with 20 mL of extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine adjusted to pH 7.3).

Biochemical indicators were determined and expressed on dry soil base (ds). Dehydrogenase activity was determined according to von Mersi and Schinner (1991) and the activity was expressed as mmol INTF $kg_{ds}^{-1}\ h^{-1}$. Soil microbial biomass C (MBC) was determined by the fumigation-extraction method (Vance et al. 1987). Soil samples were fumigated for 24 h and then extracted with 0.5 M K_2SO_4 in a 1:4 ratio with fresh soil for 30 min. Equivalent amounts of soil samples were treated in the same way without fumigation to measure dissolved organic C (DOC) and total dissolved N (TDN). Carbon and N in fumigated and non-fumigated samples were determined by OC-VCPH/CPN (Shimadzu, Japan).

2.3 Soil-Plant Interaction Experiment: Rhizotest Bioassay

2.3.1 Experimental Set-Up and Plant Growth Conditions

A parallel incubation of soil with each product (TS, ILM) and without as a control (CTR) was performed in 200 g pots for 2 weeks at 20 °C and kept at the same humidity level (30% WHC). The products tested were added to each pot at the same dose used for the soil incubation experiment (par. 2.2). Rhizotest was performed according to ISO 16198:2015 (Bravin et al. 2010) in a growth chamber with 200–400 µE photosynthetically active radiation (PAR), 12:12 light:day

Table 2 Main physico-chemical properties of the soil used in the experiments. Data are expressed on dry weight basis

Properties	Unit	Value
WRB Classification	-	Fluvic Cambisol
Texture (USDA)	-	sandy-loam
Sand	$g\ kg^{-1}$	600
Silt	$g\ kg^{-1}$	260
Clay	$g\ kg^{-1}$	140
Reaction (pH) in H_2O	-	7.8 ± 0.02
Total carbonates	$CaCO_3$, $g\ kg^{-1}$	65 ± 2.3
Total organic C (TOC)	C, $g\ kg^{-1}$	21.7 ± 2.4
Total N (TN)	N, $g\ kg^{-1}$	2.15 ± 0.2
C/N ratio	-	10.1
Cation exchange capacity (CEC)	$cmol_{(+)}\ kg^{-1}$	23.4 ± 1.5
Available P	P, $mg\ kg^{-1}$	64 ± 0.8
Water holding capacity (WHC)	%	62.1 ± 4.1

photoperiod, and a temperature of 25 ± 4 °C. Seeds of tomato (*Solanum lycopersicum* L.) cv. Marmande were hydroponically germinated for 7 days in nutrient solution (A) composed of 600 µmol L^{-1} $CaCl_2$ and 2 µmol L^{-1} H_3BO_3 for 7, covered with aluminium foil for the first 3 days. The seedlings were then grown for 7 days in solution (B) with the following composition: 500 µmol L-^{1} KH_2PO_4; 2000 µmol L^{-1} KNO_3; 2000 µmol L^{-1} $Ca(NO_3)_2$; 1000 µmol L^{-1} $MgSO_4$; 0,2 µmol L^{-1} $CuCl_2$; 10 µmol L^{-1} H_3BO_3; 2 µmol L^{-1} $MnCl_2$; 1 µmol L^{-1} $ZnSO_4$; 0,05 µmol L^{-1} Na_2MoO_4 L^{-1}; and 100 µmol L^{-1} NaFe(III)EDTA; solution B) was replaced every third day. Plants were then transferred in the soil test apparatus with root mat physically separated from the soil by a nylon mesh with 30 µm pores diameters. An aliquot of the incubated fresh soil corresponding to 9 g of dry soil was placed in the Rhizotest apparatus after the incubation with the tested products. Soil was continuously supplied by capillarity through a filter paper with solution (C) composed of 50 µmol L^{-1} KH_2PO_4; 2000 µmol L^{-1} KNO_3; 2000 µmol L^{-1} $Ca(NO_3)_2$; and 1000 µmol L^{-1} $MgSO_4$, to avoid nutrient limitations during plant growth. The test with soil and solution C) lasted 8 days. A schematic representation of the Rhizotest flowchart is reported in Fig. S1. The plant material was then rinsed with milliQ water and shoot and root tissues collected together. Plant samples were then frozen in liquid N_2 and immediately lyophilized (or stored at -80 °C prior to lyophilization) for 3 days. Soil was removed from the apparatus and stored at 4 °C for 2 weeks until all the enzymatic activities were completed.

2.3.2 Soil Enzymatic Activities Assay

Five soil extracellular hydrolytic enzymatic activities (α- and β-glucosidases, β-xylosidase, N-acetyl β-D glucosaminidase, and phosphomonoesterase) were determined using 4-methylumbelliferone (MUF) conjugates at different concentrations (Table 3) according to the procedure reported by Giacometti et al. (2014). Rates of fluorescence increase were converted to enzyme activity (nmol g^{-1} h^{-1}) according to German et al. (2011). Dehydrogenase activity was analysed spectrophotometrically by the same method used on bare soil after the incubation (von Mersi and Schinner 1991). All the values were corrected for the actual humidity reached after the last phase of the Rhizotest for each sample, which was about 20–25%.

2.3.3 ICP-OES Elemental Analysis

Soil and plant samples were prepared for elemental analyses: soil samples were oven-dried at 105 °C; lyophilized plant samples were finely ground with mortar. Approximately 200 mg of sample were subjected to microwave wet acid digestion (Start-E, Milestone, U.S.A.) in 65% HNO_3 + 37% HCl + ~ 1.5 mL H_2O_2 for soil samples and in 65% HNO_3 + 1.5 mL H_2O_2 for plant samples. Finally, the total micronutrients and trace elements were determined by ICP-OES (Inductively Coupled Plasma-OES, Spectro Arcos, Ametek).

2.3.4 Statistical Analysis

Net mineral-N was calculated by subtracting the control (CTR) at each sampling time and the curve was fitted to a first order kinetic model:

$N_{min} = N_0 \cdot (1 - e^{-kt})$.

Where Nmin is net mineral N, N_0 is the potentially mineralizable organic N, k is the first order kinetic constant, and t is the time.

All the obtained data followed a completely randomised experimental design and were analysed in the R environment using R studio 4.3.1 (Core Team 2022), which barplots and curve fitting were drawn. Data were analysed using one-way ANOVA model after assessing the normality of distributions using Shapiro–Wilk's test and the homogeneity of variances using Bartlett's test. The significance of the tests was set at p-value ≤ 0.05. Post-hoc HSD Tukey's test was used to examine differences between treatments when ANOVA yielded significant p-values.

Table 3 List of enzymatic activities and their acronym, substrates, enzyme commission number (EC) and concentrations used to determine enzymatic activity in the soil after rhizotest

Enzyme	Substrate	EC	Concentration (µM)
β-1,4-Glucosidase (β-GLU)	4-MUF α-D-glucoside	3.2.1.20	400
α-1,4-Glucosidase (α-GLU)	4-MUF β-D-glucoside	3.2.1.21	1000
β-1,4-Xylosidase (β -XYL)	4-MUF β-D-xyloside	3.2.1.37	1000
N-acetyl-β-Glucosaminidase (NAG)	4-MUF-N-acetyl β-D-glucosamide	3.2.1.30	400
Phosphomonoesterase (PME)	4-MUF-phosphate	3.1.3.2	400

3 Results

3.1 Products Characterization

3.1.1 Physico-Chemical Characterization

The main characteristics of the Tannery Sludge (TS) and Integrated Leather Meal (ILM) are listed in Table 1. The samples presented most of the parameters in the same order of magnitude, although there were some differences in moisture and pH which were higher in TS. Electric conductivity (EC) instead was higher in ILM. Total organic C (TOC) and total N (TN) were higher in ILM. However, ILM exhibited ammonium-N more than 3 times higher than TS, whereas they exhibited the same amount of nitrate-N. Almost all the TN was in the organic form in ILM (98%). Carbon to nitrogen ratio (C/N) was about 30% lower in ILM than TS. Other macronutrients (K, P, S) and most of the micronutrients (Fe, Cu, Zn) were slightly higher (6, 33 and 10%, respectively) in ILM than TS. Non-essential nutrients (sodium, Na and chlorine, Cl) were higher in ILM, especially Cl was ten times higher in ILM than in TS. Total Cr was 3.1 and 5.1 g/kg in TS and ILM, respectively, however, hexavalent Cr [Cr(VI)] was below 0.2 mg/kg (the detection limit of the method) for both the fertilizers. Extractable Cr in DTPA (DTPA Cr) was 5 times higher in ILM than in TS, which represents 1.3% and 0.4% on the total Cr respectively.

3.1.2 ATR- FTIR Spectroscopy

Figure 1 shows the ATR-FTIR spectra of TS and ILM. The presence of similar functional groups between the two fertilizers is evident. A broad peak observed in the region around 3300 cm^{-1} is assigned to -OH stretching, and it is typically associated with the water content (Islam et al. 2024), which is present in both TS and ILM but with higher intensity in TS. The small peak observed at 2965 cm^{-1} can be ascribed to asymmetrical -CH_3 stretching (Bellamy 1954). Two distinct peaks are observed in both products at 2926 cm^{-1} and at 2851 cm^{-1}, which can be attributed to two characteristic peaks of CH_2 groups, in-phase and out-of-phase vibration of H atoms respectively (Bellamy 1954). The 1635 and 1539 cm^{-1} peaks can be assigned to amide II (Smidt et al. 2002). In addition, the 1578 cm^{-1} peak can be associated with NH- in-plane bond of amides II (Moisés et al. 2022). The region around 1260 cm^{-1} present some peaks that can be associated to amide III (Bellamy 1954). The peak at 1065 could be attributed to C-O reported in protein and collagen (Nandiyanto et al. 2019). The sharp and intense peaks at 1406 and 871 cm^{-1} appeared in both TS and ILM and can be related to carbonate (CO_3^{2-}) group (Bellamy 1954; Smidt et al. 2002).

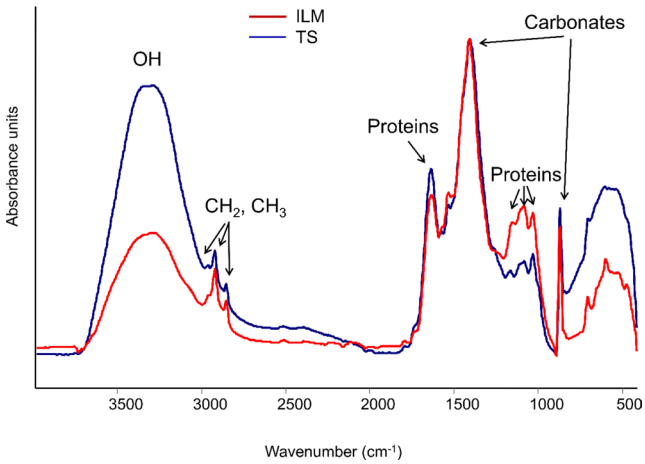

Fig. 1 ATR-FTIR spectra (4000–400 cm^{-1}) of ILM (red line) and TS (blue line)

3.2 Soil Incubation

3.2.1 Nitrogen Release into the Soil

Figure 2 shows the results of inorganic-N released in the soil samples treated with TS and ILM, compared to CTR, during the incubation period of 42 days. Specifically, ammonium-N release occurred within the first week of incubation (Fig. 2A). At t = 0 days, TS released on average 40% more ammonium-N than ILM ($p = 0.005$), even though both treatments did not show significant differences with CTR. Conversely at t = 7 days ILM released about 33% more ammonium-N than TS, whereas no differences occurred between TS and CTR ($p < 0.001$). During the following incubation period, differences became negligible. Nitrate-N (Fig. 2B) was mostly released during the first two weeks of incubation, with an opposite trend in respect to ammonium-N release. At the end of the incubation (42 days) ILM released about 40% more nitrate-than TS ($p < 0.001$). Figure 2C reports the net mineral N, calculated subtracting CTR means in each time point of the incubation and fitted to a first order kinetic model. The fitted curves revealed that the net N released tended to plateau after two weeks of incubation for ILM, whereas it continued to increase for TS showing slower kinetics. However, estimated N_0 and k values were only significant for ILM (Table S1).

3.2.2 Chemical Indicators

Figure 3 shows soil chemical indicators analysed after 42 days of soil incubation on dried soil samples. pH (Fig. 3A) was not affected by the treatment with values ranging from 7.88 to 8.01, whereas electrical conductivity (EC, Fig. 3B) showed increasing values with ILM (+21%) and TS (+31%) treatments ($p < 0.001$) with respect to the control.

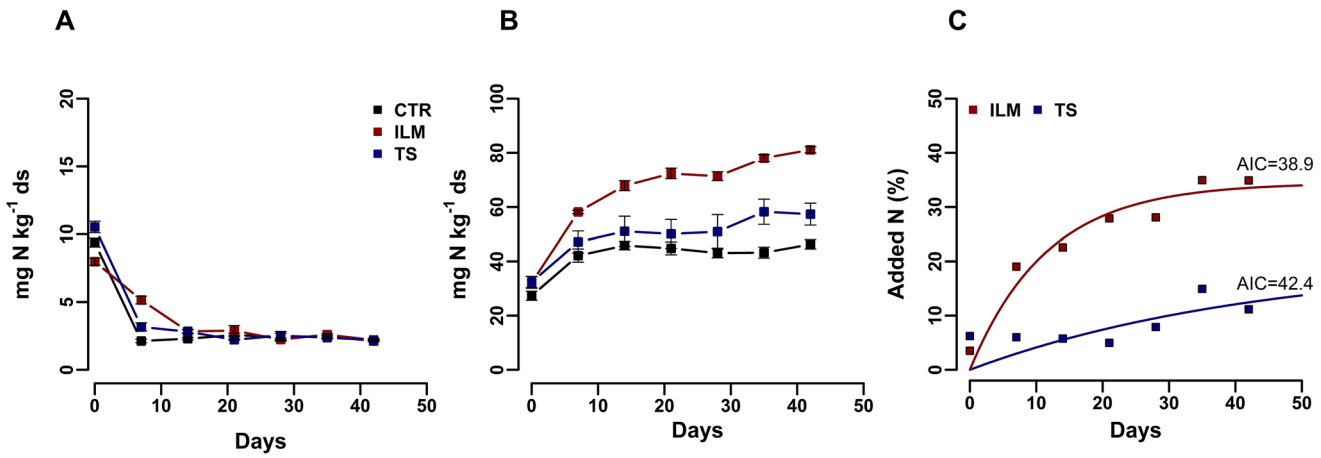

Fig. 2 Inorganic N release as ammonium-N (**A**), nitrate-N (**B**) and net mineral N (**C**) during the incubation period of 42 days. Data are expressed in mg of N per kg of dry soil. CTR: Control; ILM: Integrated Leather Meal; TS: Tannery Sludge. Data are means ± standard error

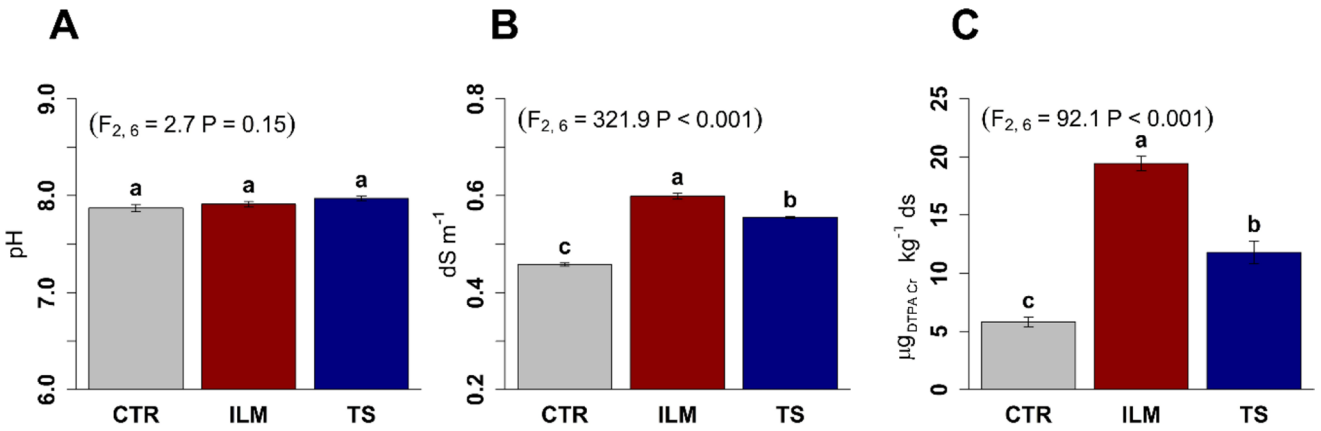

Fig. 3 Soil chemical parameters measured at the end of the incubation period (42 days). pH (**A**); Electric conductivity (EC, **B**); DTPA extractable Cr (**C**). CTR: Control; ILM: Integrated Leather Meal; TS: Tannery Sludge. Data are means ± standard error. Different lowercase letters indicate significance at one-way ANOVA after Tukey test ($p < 0.05$)

Extractable Cr in DTPA (Fig. 3C) presented the highest increase after ILM treatment (+230%), followed by TS (+102%) with respect to the control ($p < 0.001$).

3.2.3 Biochemical Indicators

Figure 4 shows soil biochemical indicators analysed after 42 days of soil incubation. Dissolved organic C (DOC, Fig. 4A) and total dissolved N (TDN, Fig. 4B) were increased of 12% and 60% respectively by ILM in respect to CTR ($p = 0.02$ and $p < 0.001$ respectively for DOC and TDN); conversely, TS exhibited intermediate values between CTR and ILM in DOC and comparable values with CTR in TDN. The ratio between DOC and TDN (Fig. 4C) was decreased of about 30% by ILM with respect to CTR ($p = 0.009$). Microbial biomass C (MBC, Fig. 4D) did not show significant differences among the treatments ($p = 0.88$), as well as dehydrogenase enzyme activity (Fig. 4E, $p = 0.06$).

3.3 Rhizotest

3.3.1 Soil and Plant ICP-OES Elemental Analysis

Table 4 reports the total micro- and trace-element analysed in plant tissue and rhizosphere soil samples after Rhizotest on tomato plants. In plants, the concentrations of total Cd, Co, Cr, Ni and Pb were below the limit of detection (LOD). Cl was increased of 53% by ILM with respect to CTR ($p = 0.04$) while TS showed intermediate values. Sodium was significantly increased of 66% and 63% respectively by both TS and ILM ($p < 0.001$). In rhizosphere soil samples, Mn was only higher in ILM than in CTR but at the limit of significance ($p = 0.05$). Conversely, Cr was significantly ($p < 0.001$) increased by both TS (+22%) and ILM (+47%).

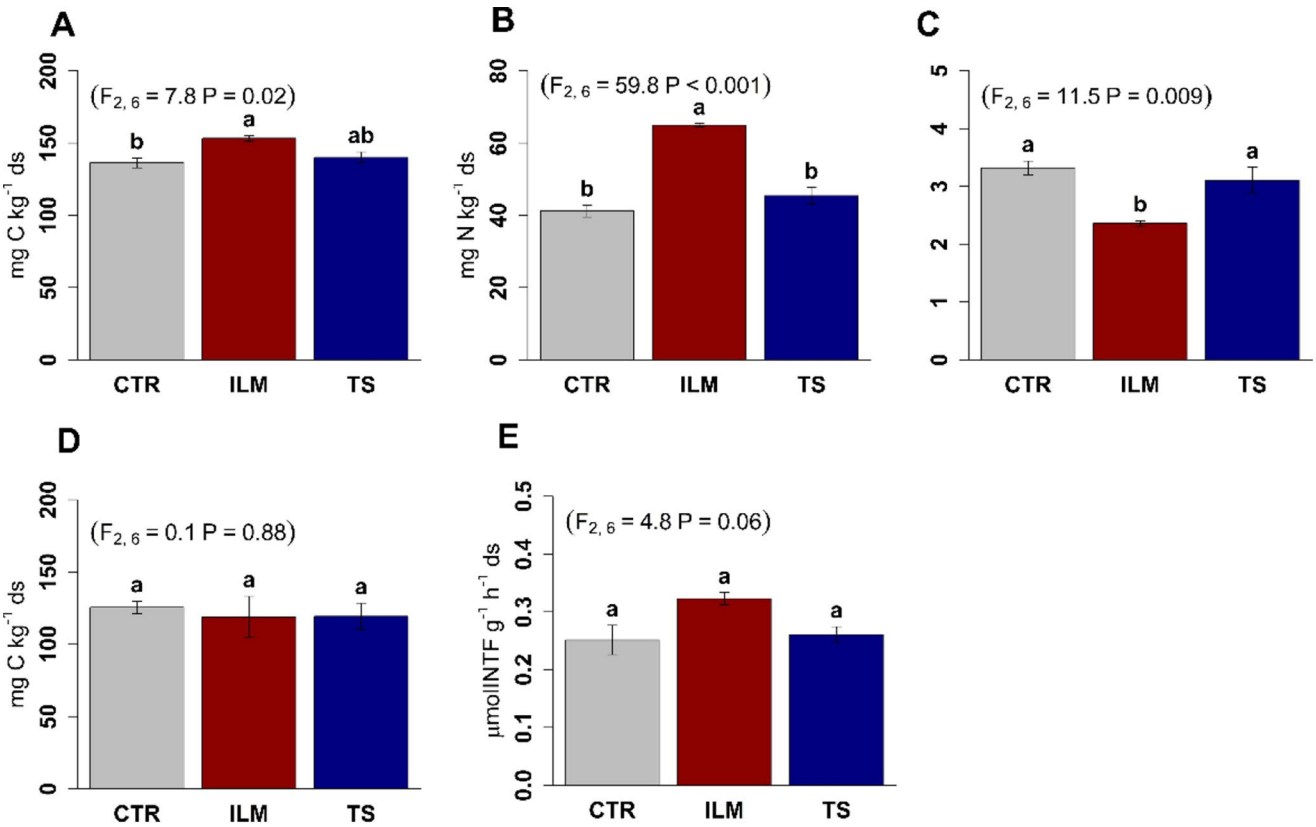

Fig. 4 Soil biochemical parameters of soil fertility measured at the end of the incubation period (42 days). Dissolved organic C (DOC, **A**), total dissolved N (TDN, **B**), DOC/TDN ratio (**C**), microbial biomass C (MBC, **D**), dehydrogenase activity (**E**). CTR: Control; ILM: Integrated Leather Meal; TS: Tannery Sludge. Data are means ± standard error. Different lowercase letters indicate significance at one-way ANOVA after Tukey test ($p < 0.05$)

Table 4 Elemental analysis through ICP-OES for micro- and trace elements in plant tissues and in soil samples after the exposure in the rhizotest. CTR: control; ILM: integrated leather meal; TS: tannery sludge. Data are means ± standard error. Different lowercase letters indicate significance at one-way ANOVA after Tukey test ($p < 0.05$)

	Cd	Co	Cr	Cl	Cu	Fe	Mn	Na	Ni	Pb	Zn
Plant	µg g^{-1}	µg g^{-1}	µg g^{-1}	mg g^{-1}	µg g^{-1}	mg g^{-1}	µg g^{-1}	mg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}
CTR	< LOD	< LOD	< LOD	**9.45 b**	2.22	0.18	22.16	**0.38 b**	< LOD	< LOD	17.02
TS	< LOD	< LOD	< LOD	**11.98 ab**	3.04	0.16	21.06	**0.63 a**	< LOD	< LOD	17.44
ILM	< LOD	< LOD	< LOD	**14.54 a**	6.88	0.21	24.87	**0.62 a**	< LOD	< LOD	17.85
LOD	*0.008*	*1.3e-4*	*0.029*	*0.003*	*3.3e-4*	*6.3e-6*	*0.1*	*0.007*	*0.046*	*0.084*	*0.073*
Soil											
CTR	< LOD	5.44	**37.50 c**	n.d.	38.13	13.02	**458.07 b**	0.27	23.74	9.57	69.68
TS	< LOD	5.48	**45.75 b**	n.d.	38.13	12.96	**461.46 ab**	0.24	24.18	10.58	69.88
ILM	< LOD	5.66	**55.13 a**	n.d.	37.24	13.14	**472.18 a**	0.28	24.47	9.26	70.79
LOD	*0.002*	*0.002*	*0.002*	-	*0.002*	*1.2e-6*	*3.8e-3*	*3.3e-6*	*0.003*	*0.004*	*2.7e-3*

3.3.2 Enzymatic Activity in Soil After Rhizotest

Enzymatic activities in rhizosphere soil samples after Rhizotest are shown in Fig. 5. Dehydrogenase activity (Fig. 5A) was significantly increased ($p < 0.001$) in both soils treated with TS (+41%) and ILM (+33%), α-glucosydase was increased of 23% by TS and of 30% by ILM (Fig. 5C, $p = 0.003$) and phosphomonoesterases was increased of 22% by TS and of 28% by ILM (Fig. 5F, $p = 0.006$). β-glucosidase (Fig. 5B) was significantly increased ($p = 0.008$) only by TS treatment with respect to CTR (+29%). Chitinase (Fig. 5E) was significantly increased ($p < 0.001$) by both treatments (+49% by TS and +27% by ILM) with significant highest values in TS, whereas β-xylosidase (Fig. 5D) was significantly increased only by TS (+27%, $p = 0.002$).

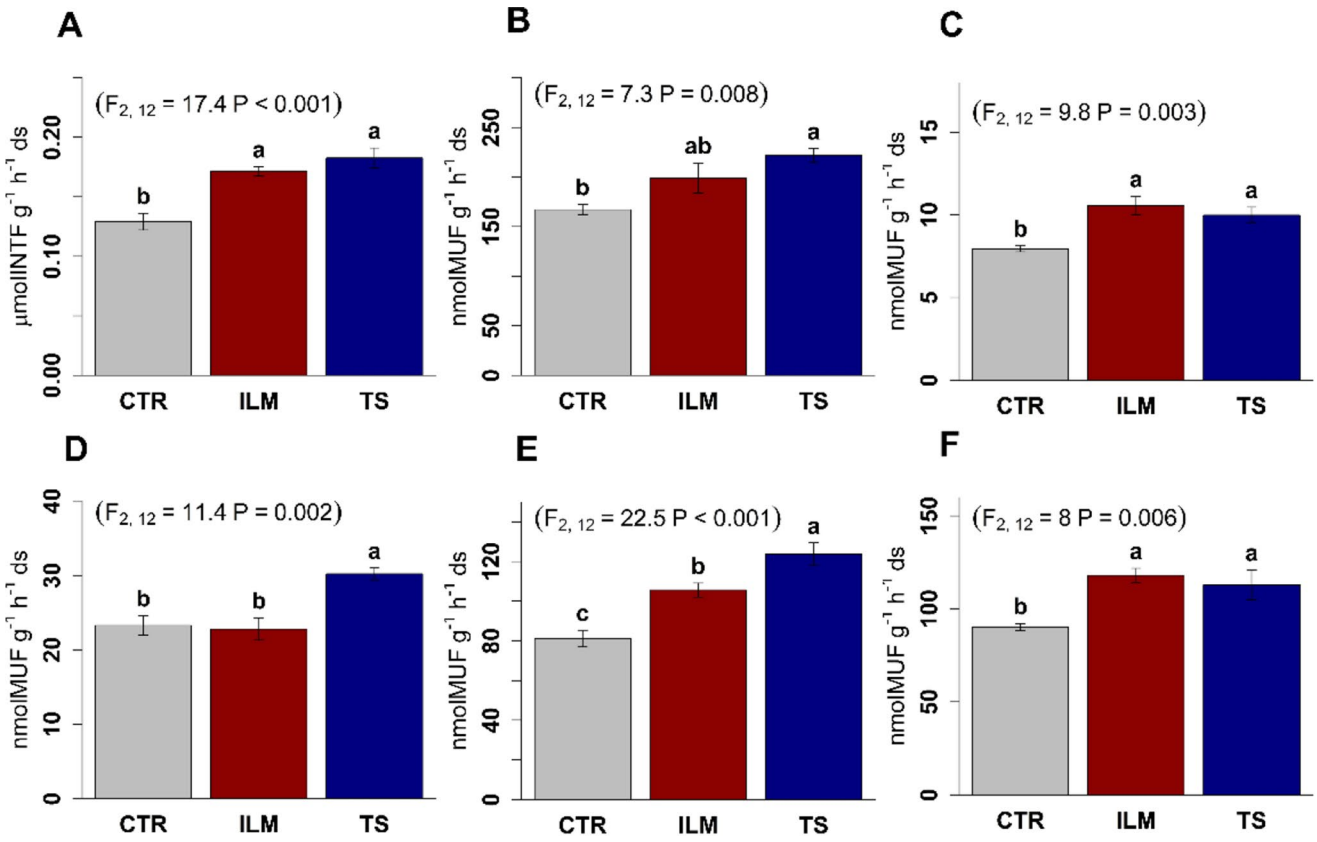

Fig. 5 Enzymatic activities performed on soil samples collected after the exposure in Rhizotest. Dehydrogenase (**A**), β-glucosidase (**B**), α-glucosidase (**C**), β-xylosidase (**D**), chitinase (**E**), phosphomonoesterase (**F**). CTR: Control; ILM: Integrated Leather Meal; TS: Tannery Sludge). Data are means ± standard error. Different letters indicate significance at one-way ANOVA after Tukey test ($p < 0.05$)

4 Discussion

The two TBWs fertilizers, TS and ILM, prior to be provided to the soil, were characterized for their main physico-chemical characteristics. Afterwards, the two fertilizers were incubated into the soil for 42 days to monitor mineral N-release kinetics. The effects of the fertilization with TS and ILM on the main chemical and biochemical indicators of the soil fertility – once most of the inorganic N was released – were analysed at the end of the incubation. Thereafter, we complemented the soil incubation experiment with Rhizotest plant-based bioassay to investigate about the influence of the fertilization with TS and ILM on the plant uptake of micro- and trace elements or on the rhizosphere processes. Rhizotest was implemented to study the bioavailability of micro- and trace elements in a device that is able to separate plant roots from soil with a 30 µm polyamide mesh and facilitate the collection of roots and rhizosphere (Bravin et al. 2010). The use of a root mat physically separated from the rhizoplane was already reported as a good system to study rhizosphere processes (Zoysa et al. 1997). Therefore, on the so collected rhizosphere soil, enzymatic activities were analysed to complement the previous findings.

Physico-chemical characterization of the two TBWs revealed that, apart from some variations on important parameters such as a lower pH, a higher TOC and TN and a lower C/N in ILM than in TS (Table 1), their properties were very similar. In particular, pH was higher than other TBWs reported, which was often below 7 (Rapisarda et al., 2022; Wystalska and Sobik-Szołtysek 2019). After the incubation, soil pH was not affected by TS or ILM fertilization, and this is probably because soil pH was close to the pH of the products (7.8 vs. 8.5 and 7.5 in TS and ILM, respectively). Furthermore, considering their N content, limited quantities of products were provided to the soil to obtain the dose of 100 mg kg^{-1} of N. Otherwise, the soil EC was affected by fertilization (Fig. 3B), increasing from 0.46 dS/m in soil control to 0.55 dS/m in TS and 0.59 dS/m in ILM. However, values of EC below the range of 0.75–3.5 dS/m are considered non-critical for plant growth (Angelova et al. 2013; Carmo et al. 2016). Soil EC is associated to the total concentration of soil soluble salts and is a direct measurement of salinity. Tannery processes involve the use of sulfates, sodium, chloride salts etc., and many of them remain in the final products, therefore an increase in EC was expected in soil after the application of TBWs. The highest increase of

EC after ILM fertilization could be associated also with the soluble forms of N (Fig. 3B) due to soluble organic N forms contained in fertilizers (Kabdaşlı et al. 2003).

Generally, values for macronutrients in the two TBWs remained in the range of most commercial organic fertilizers (Möller and Schultheiß 2015). Total N in both TS and ILM was in the range of other organic fertilizers produced from tannery residues (0.7–15%) and other commercial organic fertilizers reported in literature (0.9-6%) (Islam et al. 2024; Rapisarda et al., 2022; Wystalska and Sobik-Szołtysek 2019), for this reason they can both be considered suitable as N sources. The higher TN observed in ILM than in TS reflects its lower C/N (Table 1) and is a consequence of the increased N content due to the addition of leather meal which has a great protein content (Ciavatta et al. 2012; Dell'Abate et al. 2003). The analysis of ATR-FTIR spectra of TS and ILM confirm their overlapping characteristics, even though the addition to TS of a protein part as leather meal to constitute ILM can be observed as a slight increase of peaks intensity related to protein amides and NH- bonds (Fig. 1), coherently with the higher organic N in ILM (Table 1). A strong prevalence of amides II groups in the spectra in association with C = O groups is often associated with high protein content, which is typical for leather meal (Oliveira et al. 2007) and was evident for both the two products. During the incubation, already at the first day of incubation, the higher ammonium-N released by TS with respect to ILM (Fig. 2A) reflects its composition in ammonium-N, which was the highest (12,7 and 1,9% of TN in TS and ILM respectively, Table 1). The release of nitrate-N was specular to the ammonium-N, indicating that ammonium-N was subjected to nitrification processes during the incubation. At the end of the incubation, ILM released around 35% of its TN content (estimated $N_0 = 34.4 \pm 2.7\%$, Table S1), in respect to 11% of TS (estimated $N_0 = 20.2 \pm 29.4\%$, Table S1). In similar experiments, different types of organic fertilizers released different amounts of their total-N depending on their characteristics (Cassity-Duffey et al. 2020), for instance poultry manure and its relative compost released 39.6% and 32.7%, respectively (Geisseler et al. 2021). Leather meals can release up to 65–75% of their total N (Dell'Abate et al. 2003) but other fertilizers from TBWs were reported to have a similar release than organic fertilizers (Rapisarda et al., 2022), probably depending on the limited prevalence of leather meals. The not significant estimated N_0 value in TS suggests that the first order kinetic model is not the most appropriate to predict the release of mineral N by this fertilizer (Lohr et al. 2023), which in 42 days could still be in its linear phase (Cassity-Duffey et al. 2020). The slower nitrate release kinetics exhibited by TS and the overall lower mineral N release compared to ILM and other organic fertilizers (Cassity-Duffey et al. 2020; Dell'Abate et al. 2003; Geisseler et al. 2021; Rapisarda et al., 2022) make it more similar to a slow N-release fertilizer (Dell'Abate et al. 2003; Rakhmad et al., 2019). The mineral N released at the end of the incubation reflected the C/N ratio of the fertilizers, which is often negatively related to released mineral N and is known to represents a good predictor of available N in organic fertilizers (Mooshammer et al. 2014; Rapisarda et al., 2022). The effect of the addition of organic N fertilizers on biochemical indicators of the soil fertility can be different on dissolved organic C (DOC) depending on the fertilizer origin and on the soil properties (Galvez et al. 2012; Mao et al. 2017). However, effects on DOC depend on the applied soluble C forms and are more evident after organic amendments application, especially in short term experiments (Chantigny 2003). Fertilizers doses of TS and ILM were based on TN content, therefore the amount of C applied was different between the two fertilizers. This produced a slight increase of DOC in respect to CTR after ILM treatment (Fig. 4A), even though with little statistical significance ($p = 0.02$). Broad microbial community functions such as MBC and overall activity (i.e. dehydrogenase, Beyer et al. 1993) are commonly associated to a strong increase in DOC after fertilization (Bei et al. 2022), especially in short-term experiments (Chantigny 2003). Therefore, the modest effect on DOC of the fertilization with TS and ILM is coherent with no significant effect on MBC and dehydrogenase activity (Ciurli et al. 2024; Van Midden et al. 2023). TDN was only increased after ILM treatment (Fig. 4B) and it is coherent with the higher mineral N released and the faster N-release kinetics (Fig. 2C). Consequently, the ratio of DOC to TDN in ILM resulted the lowest (Fig. 4C), which for this reason seems to act more as a mineral N-fertilizer rather than an organic fertilizer (Rakhmad et al., 2019; Valentinuzzi et al. 2020).

Tannery sludge and residues are often rich in heavy metals; the most common contaminants in these products are Cd (0.2–125 mg kg^{-1}), Zn (126–1300 mg kg^{-1}), Pb (20-1600 mg kg^{-1}) and, in particular, Cr (0.05–474 g kg^{-1}) (Juel et al. 2016; Wystalska and Sobik-Szołtysek 2019). TS and ILM showed very limited amount of most of these contaminants (Table 1), with values that were comparable or smaller than the lowest ranges found in literature (Wystalska and Sobik-Szołtysek 2019) and with other organic fertilizers (Liu et al. 2023; Möller and Schultheiß 2015). However, total Cr content was around 100 times higher than most organic fertilizers (Liu et al. 2023; Möller and Schultheiß 2015). Total Cr in ILM was 2.5 times higher than TS, due to its accumulation after the addition of leather meal obtained from chromium tanned leather (Ciavatta and Sequi 1989). Trivalent Cr [Cr(III)] is ubiquitous in biological systems of higher organisms, though its functional essentiality is not demonstrated according to the criteria required for essential

inorganic elements (EFSA 2014). In the environment, Cr is not mobile and insoluble, as it can be mainly found bound to organic matter in soil and can present relatively low environmental risks (Prasad et al. 2021). Conversely, hexavalent Cr [Cr(VI)] is mobile, soluble and highly toxic for living organisms and carcinogenic agent for humans (Prasad et al. 2021; Wystalska and Sobik-Szołtysek 2019). Hexavalent Cr in TS and ILM was always under the detection limits (0.2 mg kg^{-1}, Table 1), so we can assume that Cr was present almost exclusively in trivalent form. However, Cr(III) can be oxidized to Cr(VI) in the soil as a consequence of several factors such as the presence of Mn oxides (Apte et al. 2005), pH or redox potential changes (Gupta and Sinha 2007). For this reason, it is important to monitor the release of Cr after the application of TBWs fertilizers to the soil. Extractable Cr in DTPA represents a good estimate of the phytoavailable Cr in soil and it is extensively used to address Cr contamination from organic products (Quantin et al. 2008). Significant differences in DTPA-extractable Cr were found (Fig. 3C), which was increased by both TS and ILM, as expected using Cr-containing TBWs (Ozgunay et al. 2007). However, it was evident after Rhizotest that Cr was not translocated into the plant tissues from the soil (Table 4), even though in rhizosphere soil total Cr was found to be increased by TS and ILM treatments (Table 4). This finding is coherent with studies that demonstrates that the low solubility and mobility of Cr(III) in the soil (Fendorf 1995; Ma and Hooda 2010) lead to its exclusion from the plant roots transport, or in some cases to very small concentrations of Cr in plants (Kapoor et al. 2022; Wickliff et al. 1984). Nevertheless, Cr in plant tissues is poorly translocated and largely retained in roots, independently from the Cr form (Ciavatta et al. 2012; Shahid et al. 2017). In concentrations exceeding 500 mg kg^{-1} in soil, crop yield might still be decreased particularly in acidic soil with pH < 6 (Wickliff et al. 1982). Continuous application of TBWs fertilizers could eventually lead to soil Cr accumulation in the medium-long period. Moreover, successive application of Cr-rich TBWs for more than 10 years can decrease the soil microbial biomass (Araujo et al. 2022), particularly with higher rates of applications such as 10 and 20 ton ha^{-1} (Araujo et al. 2020). Therefore, containing the application rates is necessary to limit negative effects of TBWs application. Concerning the other contaminants of TBWs (Cd, Cu, Zn, Pb, Co and Ni), they were not released in the soil by the treatments with TS and ILM nor translocated in the plant's tissues (Table 4). TBWs normally contain 1-2.5% of NaCl (Ozgunay et al. 2007) as sodium chloride (NaCl) is commonly added to leather as preservative. However, we observed lower basal values for TS and ILM, even though ILM presented Na content about 10 times higher than TS. It is largely known that plants can translocate Na and Cl and store them in the leaves (Marschner 2012) so their toxicity might depend on the translocation of Na and Cl in the plant tissues. Toxicity threshold for Na in leaves can range around 2.5-5 mg g^{-1} (Maas and Grattan 2015) which is around 10 times higher than our findings (Table 4). Conversely, in tomato the toxicity range of Cl in plant leaves is around 4–7 mg g^{-1} (Maas and Grattan 2015) and was exceeded even in CTR plants in Rhizotest (Table 4), probably due to the continue supply of the rhizosphere soil with nutrient solution C). In fact, the treatment with ILM caused significant increase in Cl level in plant tissues of about 5 mg g^{-1} in respect to CTR, which was in the range of the toxicity limit. It was already reported that fertilization with organic fertilizers can actually cause soil salinization (Eneji et al. 2001), as we slightly observed after ILM treatment, even with no risks for plant growth. Moreover, the plant supplied with solution C) in presence of a good source of nutrient as ILM in the soil layer could increase evapotranspiration and water consumption, which can cause stress symptoms (Wanniarachchi and Sarukkalige 2022). Nevertheless, in our experiment tomato plants did not show significant physiological differences among the treatments (data not shown), indicating that Rhizotest was carried out without stressful conditions. These findings suggest that in Rhizotest it is possible to accurately evaluate plant uptake of the most important elements and contaminants. Nonetheless, attention should be posed in evaluating the salinization effect of fertilizers.

To connect the results of the soil incubation experiment with the main indicators of rhizosphere soil nutritional processes, we determined enzymatic activities on rhizosphere soil collected after Rhizotest. Enzymatic activities were determined as proxies of a soil broad function (dehydrogenase activity) and of more specific soil processes: β-glucosidase (cellulose degradation), α-glucosidase (starch degradation), β-xylosidase (xylan degradation), N-acetyl-β-glucosaminidase (chitin degradation), and phosphomonoesterase (phytin degradation). A schematic overview of the outcome of these enzymatic activities in rhizosphere soil is resumed in Fig. 6. Dehydrogenase can represent an indicator of the overall biological activity of soil (Beyer et al. 1993) and was increased by both TS and ILM in rhizosphere soil (Figs. 5A and 6) while it was not increased in bare soil after the incubation (Fig. 3E). This finding indicates that the plant activity was determinant in activating the broad microbial community to produce soil dehydrogenases (Jat et al. 2021) in response to the presence of a source of nutrients as TS and ILM. Carbon sources in rhizosphere soil can be various depending on the plant activity and, to monitor the impact of N-fertilizers on the different C-sources, diverse enzyme activities should be monitored (German et al. 2011; Sun et al. 2021). Among the extracellular enzymes, glucosidases play a major role in degradation

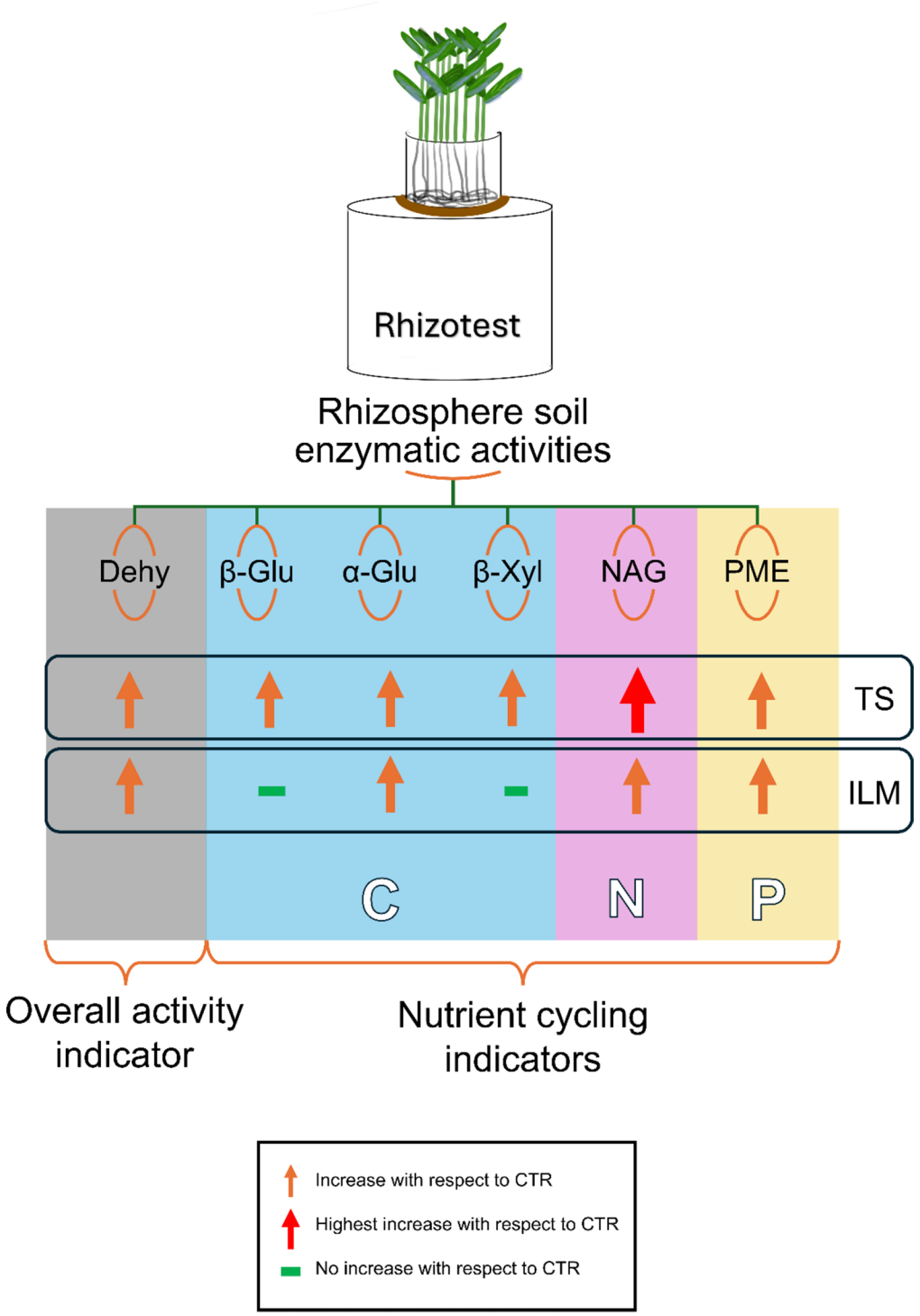

Fig. 6 Schematic representation of the results obtained from the enzymatic activity analyses on rhizosphere soil samples after Rhizotest. TS: Tannery Sludge; ILM: Integrated Leather Meal; Dehy: Dehydrogenase; β-Glu: β-glucosidase; α-Glu: α-glucosidase; β-Xyl: β-xylosidase; NAG: N-Acetil- β-D glucosaminidase; PME: Phosphomonoesterase

of carbohydrates in soils. β-Glucosidase and α-glucosidase catalyses the hydrolysis of β- and α-D-glucopyranosides and are involved in the hydrolysis of cellobiose and maltose respectively (Eivazi and Tabatabai 1988). Normally, these enzymes are increased when applying organic matter to the soil for instance through manure-based fertilization (Zhang et al. 2015). Furthermore, β-glucosidases are more prominent in soil than α-glucosidase. β-xylosidases are involved

in the hydrolysis of hemicelluloses, in particular xylans, and the resulting monosaccharides can be further used by microorganisms for growth (Bosetto et al. 2016). We found that TS was able to stimulate all the three enzymatic activity concerning C-cycling (Figs. 5B, C and D and 6), whereas ILM significantly increased only α-glucosidase and reported intermediate values for β-glucosidase, confirming that different C/N and C-forms of organic fertilizers can have different impact on soil enzymatic activity (Kracmarova et al. 2020). Massive N-fertilization can cause a reduction in the production of extracellular enzymes related to C-cycling (Jian et al. 2016), that in our experiment resulted in a less stimulation of glucosidases and xylosidase after ILM treatment (Fig. 6). N-acetyl-β-D-glucosaminidase (also referred as chitinase) is considered to play a significant role in carbon and nitrogen cycling in soil, particularly concerning N nutrition (Ueno et al. 1991). In this study we found that TS increased chitinase more than ILM in respect to CTR (Figs. 5E and 6). Our findings are coherent with the fact that chitinase activity is generally increased after the application of N-sources to the soil (Hu et al. 2023). However, studies revealed that the addition of mineral N fertilizer can reduce or not increase the activity of chitinase, because abundant N sources can inhibit the activity of N-related hydrolytic enzymes (Ma et al. 2021; Yuan et al. 2021) and this would explain the less stimulation of this enzyme activity by ILM. Phosphomonoesterase activity was analysed as an indicator of P-cycling in the rhizosphere soil (Utobo and Tewari 2015) and we found that it was increased by N-fertilization with both TS and ILM (Figs. 4F and 5). The supply of N to the soil increases the N-uptake and induces a higher growth rate of soil microorganism, leading to a greater production of P-mobilizing enzymes (Zuccarini et al. 2023). Therefore, an increase in P-related enzyme activity is expected after the application of organic N-fertilizers and is coherent with previous findings (Giacometti et al. 2014). Taken together, the obtained results highlight both a broad and a specific soil functionality stimulant effect of TS and ILM in the rhizosphere soil.

5 Conclusion

Two tannery bio-waste fertilizers—Integrated Leather Meal (ILM) and Tannery Sludge (TS)—were investigated for their fertilizing potential through a complementary approach using a 42-days soil incubation experiment and Rhizotest bioassay. Short term soil incubation allowed to assess that ILM released 35% of its total N versus 11% for TS and showed faster N-release kinetics, while standard soil chemical indicators remained mostly unchanged. Then, Rhizotest was used to assess the bioavailability of trace elements to tomato plants and revealed that trace-elements were not absorbed by plants. Then, the soil sampled after Rhizotest was analysed as rhizosphere soil. Compared with untreated samples, total Cr increased 5 and 4 times respectively consequently to the fertilizing treatments, as total Cr was 0.5 and 0.3% in ILM and TS respectively. Analyses of enzymatic activities were performed on rhizosphere soil. Both amendments stimulated overall enzymatic activities. However, ILM reported a reduced stimulatory effect on C-cycling enzymes in response to the higher mineral N supplied. This complementary short-term approach rapidly profiles fertilizing potential producing highly standardizable results. Further longer-term, mesocosm, and field trials across diverse soils will help to validate and apply these results on larger scale.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-025-02696-2.

Acknowledgements The technical support in the laboratory provided by Paola Gioacchini, Maurizio Quartieri, Salvatore Rapisarda and Andrea Simoni is acknowledged.

Author Contributions Andrea Ciurli: Conceptualization, Investigation; Formal Analysis; Data Curation; Writing - Original Draft. Giampaolo Di Biase: Investigation; Data Curation. Martina Mazzon: Conceptualization; Data Curation. Claudio Ciavatta: Conceptualization, Funding Acquisition. Luciano Cavani: Conceptualization; Investigation; Data Curation; Writing - Review & Editing; Funding Acquisition.

Funding Open access funding provided by Alma Mater Studiorum - Università di Bologna within the CRUI-CARE Agreement. This study was founded by joint project developed by Alma Mater Studiorum University of Bologna and Cuoiodepur SpA.

Data Availability Data will be made available by authors on reasonable request.

Code Availability Not applicable.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

A.O.A.C (1990) Official Methods of Analysis (Volume 1)

Angelova VR, Akova VI, Artinova NS, Ivanov KI (2013) The effect of organic amendments on soil chemical characteristics

Apte A, Verma S, Tare V, Bose P (2005) Oxidation of Cr(III) in tannery sludge to Cr(VI): field observations and theoretical assessment. J Hazard Mater 121:215–222. https://doi.org/10.1016/j.jhazmat.2005.02.010

Araujo ASF, De Melo WJ, Araujo FF, Van Den Brink PJ (2020) Long-term effect of composted tannery sludge on soil chemical and biological parameters. Environ Sci Pollut Res 27:41885–41892. https://doi.org/10.1007/s11356-020-10173-9

Araujo ASF, De Araujo Pereira AP, Mendes LW (2022) Applications of Cr-rich composted tannery sludge in the soil decrease microbial biomass and select specific bacterial groups. Environ Sci Pollut Res 29:75113–75118. https://doi.org/10.1007/s11356-022-22933-w

Bakshi M, Varma A (2010) Soil enzyme: the State-of-Art. In: Shukla G, Varma A (eds) Soil enzymology, soil biology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1–23. https://doi.org/10.1007/978-3-642-14225-3_1

Beggio G, Bonato T, Marangoni S, Bravin MN, Fantinato E, Nigris S, Pivato A, Piazza R (2024) Uptake and translocation of brominated flame retardants in tomato plants (Solanum lycopersicum L.): results from a standard soil-based biotest. Chemosphere 353:141594. https://doi.org/10.1016/j.chemosphere.2024.141594

Bei S, Li X, Kuyper TW, Chadwick DR, Zhang J (2022) Nitrogen availability mediates the priming effect of soil organic matter by preferentially altering the straw carbon-assimilating microbial community. Sci Total Environ 815:152882. https://doi.org/10.1016/j.scitotenv.2021.152882

Bellamy LJ (1954) The Infra-red Spectra of Complex Molecules, 1975th ed

Beyer L, Wachendorf C, Elsner DC, Knabe R (1993) Suitability of dehydrogenase activity assay as an index of soil biological activity. Biol Fertil Soils 16:52–56. https://doi.org/10.1007/BF00336515

Bhat R (2022) Emerging trends and sustainability challenges in the global agri-food sector. Future foods. Elsevier, pp 1–21. https://doi.org/10.1016/B978-0-323-91001-9.00041-4

Bosetto A, Justo PI, Zanardi B, Venzon SS, Graciano L, Dos Santos EL, De Cássia Garcia Simão R (2016) Research progress concerning fungal and bacterial β-Xylosidases. Appl Biochem Biotechnol 178:766–795. https://doi.org/10.1007/s12010-015-1908-4

Bravin MN, Michaud AM, Larabi B, Hinsinger P (2010) RHIZOtest: A plant-based biotest to account for rhizosphere processes when assessing copper bioavailability. Environ Pollut 158:3330–3337. https://doi.org/10.1016/j.envpol.2010.07.029

Caponi E (2012) From tannery wastewater sludge and solid waste, to fertilizer: an eco-innovative and sustainable project. Conf. Proceeding Int. Symp. Sanit. Environ. Eng. Milano

Carmo DLD, Lima LBD, Silva CA (2016) Soil fertility and electrical conductivity affected by organic waste rates and nutrient inputs. Rev Bras Ciênc Solo 40. https://doi.org/10.1590/18069657rbcs20150152

Cassity-Duffey K, Cabrera M, Gaskin J, Franklin D, Kissel D, Saha U (2020) Nitrogen mineralization from organic materials and fertilizers: predicting N release. Soil Sci Soc Am J 84:522–533. https://doi.org/10.1002/saj2.20037

Chantigny MH (2003) Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113:357–380. https://doi.org/10.1016/S0016-7061(02)00370-1

Ciavatta C, Sequi P (1989) Evaluation of chromium release during the decomposition of leather meal fertilizers applied to the soil. Fertil Res 19:7–11. https://doi.org/10.1007/BF01080680

Ciavatta C, Antisari LV, Sequi P (1989) Determination of organic carbon in soils and fertilizers. Commun Soil Sci Plant Anal 20:759–773. https://doi.org/10.1080/00103628909368115

Ciavatta C, Manoli C, Cavani L, Franceschi C, Sequi P (2012) Chromium-Containing organic fertilizers from tanned hides and skins: A review on chemical, environmental, agronomical and legislative aspects. J Environ Prot 03:1532–1541. https://doi.org/10.4236/jep.2012.311169

Ciurli A, Di Biase G, Rossi M, Grigatti M, Ciavatta C, Cavani L (2024) Dried anaerobic digestate from slaughterhouse by-products: emerging cues for a bio-based fertilization. https://doi.org/10.1007/s12649-024-02737-4. Waste Biomass Valorization

Core Team R (2022) R: A language and environment for statistical computing

Daly KR, Mooney SJ, Bennett MJ, Crout NMJ, Roose T, Tracy SR (2015) Assessing the influence of the rhizosphere on soil hydraulic properties using X-ray computed tomography and numerical modelling. J Exp Bot 66:2305–2314. https://doi.org/10.1093/jxb/eru509

Dell'Abate MT, Benedetti A, Trinchera A, Galluzzo D (2003) Nitrogen and carbon mineralisation of leather meal in soil as affected by particle size of fertiliser and Microbiological activity of soil. Biol Fertil Soils 37:124–129. https://doi.org/10.1007/s00374-002-0568-z

Dick WA, Culman SW. (2017) Biological and biochemical tests for assessing soil fertility. In: Chatterjee A, Clay D, editors. Soil Fertility. Madison (WI): American Society of Agronomy and Soil Science Society of America. p. 134–147. https://doi.org/10.2134/soilfertility.2014.0007

EFSA (2014) Scientific opinion on dietary reference values for chromium. EFSA J. https://doi.org/10.2903/j.efsa.2014.3845

Egamberdieva D, Renella G, Wirth S, Islam R (2010) Enzyme activities in the rhizosphere of plants. In: Shukla G, Varma A (eds) Soil enzymology, soil biology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 149–166. https://doi.org/10.1007/978-3-642-14225-3_8

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

Eneji AE, Honna T, Yamamoto S, Manuring effect on rice grain yield and extractable trace elements in soils (2001) J Plant Nutr 24:967–977. https://doi.org/10.1081/PLN-100103797

Fendorf SE (1995) Surface reactions of chromium in soils and waters. Geoderma 67:55–71. https://doi.org/10.1016/0016-7061(94)00062-F

Feng G-T, Shan Z-H, Li S, Chen H (2013) Prod Org Fertilizer Using Tannery Sludge 108:189–196

Galvez A, Sinicco T, Cayuela ML, Mingorance MD, Fornasier F, Mondini C (2012) Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. Agric Ecosyst Environ 160:3–14. https://doi.org/10.1016/j.agee.2011.06.015

Gatto A (2023) Quantifying management efficiency of energy recovery from waste for the circular economy transition in Europe. J Clean Prod 414:136948. https://doi.org/10.1016/j.jclepro.2023.136948

Geisseler D, Smith R, Cahn M, Muramoto J (2021) Nitrogen mineralization from organic fertilizers and composts: literature survey and model fitting. J Environ Qual 50:1325–1338. https://doi.org/10.1002/jeq2.20295

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Giacometti C, Cavani L, Baldoni G, Ciavatta C, Marzadori C, Kandeler E (2014) Microplate-scale fluorometric soil enzyme assays as tools to assess soil quality in a long-term agricultural field experiment. Appl Soil Ecol 75:80–85. https://doi.org/10.1016/j.apsoil.2013.10.009

Gonçalves D, Silva MA, Roque SAT, Muniz AS, Marchetti ME, Da Matta JDDV, Pelisson N (2010) Efficiency of organic compost from Agri-Industrial wastes as fertilizer for corn and wheat. Commun Soil Sci Plant Anal 41:2517–2531. https://doi.org/10.1080/00103624.2010.514371

Gregory PJ, Nortcliff S (eds) (2013) Soil conditions and plant growth. Wiley-Blackwell, Chichester, West Sussex, UK; Ames, Iowa, USA

Gupta AK, Sinha S (2007) Assessment of single extraction methods for the prediction of bioavailability of metals to brassica juncea L. Czern. (var. Vaibhav) grown on tannery waste contaminated soil. J Hazard Mater 149:144–150. https://doi.org/10.1016/j.jhazmat.2007.03.062

Hu Q, Liu T, Ding H, Li C, Tan W, Yu M, Liu J, Cao C (2023) Effects of nitrogen fertilizer on soil microbial residues and their contribution to soil organic carbon and total nitrogen in a rice-wheat system. Appl Soil Ecol 181:104648. https://doi.org/10.1016/j.apsoil.2022.104648

Islam MM, Tujjohra F, Roy UK, Rahman MM (2024) A circular economy approach for utilization of tannery fleshing hydrolysate and kitchen wastes into organic fertilizer through enzymatic decomposition. Biochem Eng J 212:109519. https://doi.org/10.1016/j.bej.2024.109519

Jat HS, Datta A, Choudhary M, Sharma PC, Dixit B, Jat ML (2021) Soil enzymes activity: effect of climate smart agriculture on rhizosphere and bulk soil under cereal based systems of north-west India. Eur J Soil Biol 103:103292. https://doi.org/10.1016/j.ejsobi.2021.103292

Jian S, Li J, Chen J, Wang G, Mayes MA, Dzantor KE, Hui D, Luo Y (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biol Biochem 101:32–43. https://doi.org/10.1016/j.soilbio.2016.07.003

John Sundar V, Gnanamani A, Muralidharan C, Chandrababu NK, Mandal AB (2011) Recovery and utilization of proteinous wastes of leather making: a review. Rev Environ Sci Biotechnol 10:151–163. https://doi.org/10.1007/s11157-010-9223-6

Juel MAI, Chowdhury ZU. (2016 Dec) Heavy metal speciation and toxicity characteristics of tannery sludge. In: Proceedings of the 11th International Conference on Mechanical Engineering (ICME 2015). 19-21; Dhaka, Bangladesh. p. 060009. https://doi.org/10.1063/1.4958450.

Kabdaşlı I, Ölmez T, Tünay O (2003) Nitrogen removal from tannery wastewater by protein recovery. https://doi.org/10.2166/wst.2003.0058

Kapoor RT, Mfarrej B, Alam MF, Rinklebe P, Ahmad J, P (2022) Accumulation of chromium in plants and its repercussion in animals and humans. Environ Pollut 301:119044. https://doi.org/10.1016/j.envpol.2022.119044

Khatun J, Mukherjee A, Dhak D (2024) Emerging contaminants of tannery sludge and their environmental impact and health hazards. Springer Nature Switzerland, Cham. https://doi.org/10.1007/978-3-031-58441-1

Koppiahraj K, Bathrinath S, Saravanasankar S (2019) Leather waste management scenario in developed and developing nations: A comparative. Int J Eng Adv Technol 9:852–857. https://doi.org/10.35940/ijeat.A1056.1291S419

Kracmarova M, Kratochvilova H, Uhlik O, Strejcek M, Szakova J, Cerny J, Tlustos P, Balik J, Demnerova K, Stiborova H (2020) Response of soil microbes and soil enzymatic activity to 20 years of fertilization. Agronomy 10:1542. https://doi.org/10.3390/agronomy10101542

Liu Z, Bai Y, Gao J, Li J (2023) Driving factors on accumulation of cadmium, lead, copper, zinc in agricultural soil and products of the North China plain. Sci Rep 13:7429. https://doi.org/10.1038/s41598-023-34688-6

Lohr D, Gruda NS, Meinken E (2023) Estimating nitrogen release from organic fertilizers for soilless production by analysis of C and N pools. Horticulturae 9:767. https://doi.org/10.3390/horticulturae9070767

Luster J (ed) (2006) Handbook of methods used in rhizosphere research. Swiss Federal Research Inst. WSL, Birmensdorf

Ma Y, Hooda PS (2010) Chromium, nickel and cobalt. In: Hooda PS (ed) Trace elements in soils. Wiley, pp 461–479. https://doi.org/10.1002/9781444319477.ch19

Ma S, Chen G, Du E, Tian D, Xing A, Shen H, Ji C, Zheng C, Zhu, Jianxiao, Zhu, Jiangling, Huang H, He H, Zhu B, Fang J (2021) Effects of nitrogen addition on microbial residues and their contribution to soil organic carbon in china's forests from tropical to boreal zone. Environ Pollut 268:115941. https://doi.org/10.1016/j.envpol.2020.115941

Maas EV, Grattan SR. (2015) Crop yields as affected by salinity. In: Skaggs RW, Van Schilfgaarde J, editors. Agronomy monographs. Madison (WI): American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 55–108. https://doi.org/10.2134/agronmonogr38.c3

Mahmood Ali A, Khan A, Shahbaz M, Imtiaz Rashid M, Imran M, Shahzad K, Binti Mahpudz A (2023) A renewable and sustainable framework for clean fuel towards circular economy for solid waste generation in leather tanneries. Fuel 351:128962. https://doi.org/10.1016/j.fuel.2023.128962

Mao R, Zhang X-H, Li S-Y, Song C-C (2017) Long-term phosphorus addition enhances the biodegradability of dissolved organic carbon in a nitrogen-limited temperate freshwater wetland. Sci Total Environ 605–606. https://doi.org/10.1016/j.scitotenv.2017.06.200

Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. ed. Academic, London Waltham, MA

Maurya S, Abraham JS, Somasundaram S, Toteja R, Gupta R, Makhija S (2020) Indicators for assessment of soil quality: a mini-review. Environ Monit Assess 192:604. https://doi.org/10.1007/s10661-020-08556-z

Moisés J, Martinez JM, Iocoli GA, Duval ME, Galantini JA (2022) Spectrometric evaluation of biotransformed agro-industrial residues and their humic substances by UV–visible and infrared spectroscopy and their effect on winter wheat productivity. Int. J. Recycl. Org. Waste Agric

Moktadir MA, Ahmadi HB, Sultana R, Zohra F-T-, Liou JJH, Rezaei J (2020) Circular economy practices in the leather industry: A practical step towards sustainable development. J Clean Prod 251:119737. https://doi.org/10.1016/j.jclepro.2019.119737

Möller K, Schultheiß U (2015) Chemical characterization of commercial organic fertilizers. Arch Agron Soil Sci 61:989–1012. https://doi.org/10.1080/03650340.2014.978763

Mooshammer M, Wanek W, Hämmerle I, Fuchslueger L, Hofhansl F, Knoltsch A, Schnecker J, Takriti M, Watzka M, Wild B, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2014) Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nat Commun 5:3694. https://doi.org/10.1038/ncomms4694

Moran J, McGrath C (2021) Comparison of methods for mapping rhizosphere processes in the context of their surrounding root and soil environments. Biotechniques 71:604–614. https://doi.org/10.2144/btn-2021-0021

Nabavinia F, Emami H, Astaraee A, Lakzian A (2015) Effect of tannery wastes and Biochar on soil chemical and physicochemical properties and growth traits of radish. Int Agrophysics 29:333–339. https://doi.org/10.1515/intag-2015-0040

Nandiyanto ABD, Oktiani R, Ragadhita R (2019) How to read and interpret FTIR spectroscope of organic material. Indones J Sci Technol 4:97. https://doi.org/10.17509/ijost.v4i1.15806

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670. https://doi.org/10.1046/j.1351-0754.2003.0556.x

Oliveira LCA, Gonçalves M, Oliveira DQL, Guerreiro MC, Guilherme LRG, Dallago RM (2007) Solid waste from leather industry as adsorbent of organic dyes in aqueous-medium. J Hazard Mater 141:344–347. https://doi.org/10.1016/j.jhazmat.2006.06.111

Ozgunay H, Colak S, Mutlu MM, Akyuz F (2007) Characterization of leather industry wastes

Perdigão A, Marques F, Pereira JLS (2022) Effect of different tannery sludge composts on the production of ryegrass: A pot experiment. Open Agric J 16:e187433152207270. https://doi.org/10.2174/18743315-v16-e2207270

Prasad S, Yadav KK, Kumar S, Gupta N, Cabral-Pinto MMS, Rezania S, Radwan N, Alam J (2021) Chromium contamination and effect on environmental health and its remediation: A sustainable approaches. J Environ Manage 285:112174. https://doi.org/10.1016/j.jenvman.2021.112174

Quantin C, Ettler V, Garnier J, Šebek O (2008) Sources and extractibility of chromium and nickel in soil profiles developed on Czech serpentinites. Comptes Rendus Géoscience 340:872–882. https://doi.org/10.1016/j.crte.2008.07.013

Rakhmad F, Suwardi, Dyah TS (2019) Release pattern of ammonium, nitrate, and potassium from Slow-Release fertilizer (SRF) in the soil. IOP Conf Ser Earth Environ Sci 383:012037. https://doi.org/10.1088/1755-1315/383/1/012037

Rapisarda S, Di Biase G, Mazzon M, Ciavatta C, Cavani L (2022) Nitrogen availability in organic fertilizers from tannery and slaughterhouse By-Products. Sustainability 14:12921. https://doi.org/10.3390/su141912921

Ren J, Liu X, Yang W, Yang X, Li W, Xia Q, Li J, Gao Z, Yang Z (2021) Rhizosphere soil properties, microbial community, and enzyme activities: Short-term responses to partial substitution of chemical fertilizer with organic manure. J Environ Manage 299:113650. https://doi.org/10.1016/j.jenvman.2021.113650

Rigueto CVT, Rosseto M, Krein DDC, Ostwald BEP, Massuda LA, Zanella BB, Dettmer A (2020) Alternative uses for tannery wastes: a review of environmental, sustainability, and science. J Leather Sci Eng 2:21. https://doi.org/10.1186/s42825-020-00034-z

Santos AJM, Backes C, Rodrigues LM, Teodoro AG, Godoy LJGD, Tomazello DA, Campos LFC, Ribon AA, Lopes TA, Boas RLV (2020) Chemical characteristics of soil after application of tannery sludge as fertilizer in the sugarcane plant crop. Aust J Crop Sci 641–648. https://doi.org/10.21475/ajcs.20.14.04.p2234

Schinner F, Öhlinger R, Kandeler E, Margesin R (2012) Methods in soil biology.

Shahid M, Shamshad S, Rafiq M, Khalid S, Bibi I, Niazi NK, Dumat C, Rashid MI (2017) Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. Chemosphere 178:513–533. https://doi.org/10.1016/j.chemosphere.2017.03.074

Smidt E, Lechner P, Schwanninger M, Haberhauer G, Gerzabek MH (2002) Characterization of waste organic matter by FT-IR spectroscopy: application in waste science. Appl Spectrosc 56:1170–1175. https://doi.org/10.1366/000370202760295412

Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnson CT, Sumner ME (1996) Methods of soil analysis. Part 3, Chemical Methods.

Stefan D, Bosomoiu M, Constantinescu R, Ignat M (2021) Composite polymers from leather waste to produce smart fertilizers. Polymers 13:4351. https://doi.org/10.3390/polym13244351

Sun X, Ye Y, Ma Q, Guan Q, Jones DL (2021) Variation in enzyme activities involved in carbon and nitrogen cycling in rhizosphere and bulk soil after organic mulching. Rhizosphere 19:100376. https://doi.org/10.1016/j.rhisph.2021.100376

Tahiri S, de la Guardia M (2008) Treatment and valorization of leather industry solid wastes: A review. 104:52–67

Tejada M (2009) Evolution of soil biological properties after addition of glyphosate, Diflufenican and glyphosate + Diflufenican herbicides. Chemosphere 76:365–373. https://doi.org/10.1016/j.chemosphere.2009.03.040

Thomasset A, Benayoun S (2024) Review: leather sustainability, an industrial ecology in process. J Ind Ecol 28:1842–1856. https://doi.org/10.1111/jiec.13547

Ueno H, Miyashita K, Sawada Y, Oba Y (1991) Assay of chitinase and N-acetylglucosaminidase activity in forest soils with 4-methylumbelliferyl derivatives. Z. Für Pflanzenernähr. Bodenkd 154:171–175. https://doi.org/10.1002/jpln.19911540304

Utobo EB, Tewari L (2015) Soil enzymes as bioindicators of soil ecosystem status. Appl Ecol Environ Res 13. https://doi.org/10.15666/aeer/1301_147169

Valentinuzzi F, Cavani L, Porfido C, Terzano R, Pii Y, Cesco S, Marzadori C, Mimmo T (2020) The fertilising potential of manure-based biogas fermentation residues: pelleted vs. liquid digestate. Heliyon 6:e03325. https://doi.org/10.1016/j.heliyon.2020.e03325

Van Midden C, Harris J, Shaw L, Sizmur T, Pawlett M (2023) The impact of anaerobic digestate on soil life: A review. Appl Soil Ecol 191:105066. https://doi.org/10.1016/j.apsoil.2023.105066

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. https://doi.org/10.1016/0038-0717(87)90052-6

von Mersi W, Schinner F (1991) An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol Fertil Soils 11:216–220. https://doi.org/10.1007/BF00335770

Wanniarachchi S, Sarukkalige R (2022) A review on evapotranspiration Estimation in agricultural water management: past, present, and future. Hydrology 9:123. https://doi.org/10.3390/hydrology9070123

Wickliff C, Volk VV, Tingey DT, Griffis WL, Trunk MY, Witherow JL (1982) Reactions of Chrome tannery sludge with organic and mineral soils. Water Air Soil Pollut 17:61–74. https://doi.org/10.1007/BF00164092

Wickliff C, Volk VV, Tingey DT, Griffis WL, Trunk MY, Witherow JL (1984) Response of tall fescue, Bush bean, and maize to Chrome tannery sludge in soils. Environ Pollut Ser Ecol Biol 33:353–377. https://doi.org/10.1016/0143-1471(84)90143-0

WRB (2014) World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. LCC MAKS Press. https://doi.org/10.29003/m4174.978-5-317-07235-3

Wystalska K, Sobik-Szołtysek J (2019) Sludge from tannery industries. Industrial and municipal sludge. Elsevier, pp 31–46. https://doi.org/10.1016/B978-0-12-815907-1.00002-7

Yuan Y, Li Y, Mou Z, Kuang L, Wu W, Zhang J, Wang F, Hui D, Peñuelas J, Sardans J, Lambers H, Wang J, Kuang Y, Li Z, Liu Z (2021) Phosphorus addition decreases microbial residual contribution to soil organic carbon pool in a tropical coastal forest. Glob Change Biol 27:454–466. https://doi.org/10.1111/gcb.15407

Zhang L, Chen W, Burger M, Yang L, Gong P, Wu Z (2015) Changes in soil carbon and enzyme activity as a result of different Long-Term fertilization regimes in a greenhouse field. PLoS ONE 10:e0118371. https://doi.org/10.1371/journal.pone.0118371

Zoysa AKN, Loganathan P, Hedley MJ (1997) A technique for studying rhizosphere processes in tree crops: soil phosphorus depletion around camellia (Camellia Japonica L.) roots

Zuccarini P, Asensio D, Sardans J, Ogaya R, Liu L, Peñuelas J (2023) Effects of nitrogen deposition on soil enzymatic activity and soil microbial community in a mediterranean Holm oak forest. Geoderma 430:116354. https://doi.org/10.1016/j.geoderma.2023.116354