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# TunaMud: a multi-purpose fertilizer from the canned tuna industry

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## Abstract

Tuna is the world's most commercially valuable fish. More than 60% of tuna fish caught worldwide is used by the global canned tuna industry. The latter industry had revenue exceeding \$20 billion in 2024. Before being packaged by canned tuna industrial companies, tuna's meat is cooked. The broths that derive from the tuna cooking process are fed to a wastewater treatment plant together with scraps that derive from the cutting phase of the raw tuna, the blood and the so-called sawdust composed of small fragments from the cutting are sent to the water treatment plant along with the aqueous exudate released by the tuna in the conditioning phase after cooking. Dubbed herein "TunaMud", we show in this study that the sewage sludge residue resulting from wastewater treatment at canned tuna industry factories is a multi-purpose fertilizer. Tested on arboreal (olive and pomegranate), herbaceous (pepper and lettuce), and horticultural (onion) plants, TunaMud improved the growth parameters of all plants grown, as well as the quality of fertilized soil. A biological resource so far disposed of as biowaste at substantial economic cost is thereby turned into a valued fertilizer. Along with recent outcomes concerning tuna industrial processing waste upgrade into valuable bioproducts via the LimoFish process, these findings close the material cycle through technically and economically viable circular economy processes for all by-products of the global canned tuna industry, including the mud-like residue from the wastewater treatment plant. While the use of food waste as fertilizer is not new, the innovation of this work lies in valorizing a previously unstudied industrial residue: the wastewater-treatment sludge generated specifically by the tuna-canning process. The composition of TunaMud, arising from a unique combination of cooking broths, blood, cutting scraps, and conditioning exudates, has no equivalent in the existing literature on organic fertilizers. The study further introduces a genuinely interdisciplinary approach that integrates green chemistry, wastewater engineering, agronomy, circular-economy analysis, and public-health considerations. Besides providing farming companies with a multi-purpose organic fertilizer free of antibiotics, these findings provide the canned tuna industry with the opportunity to save substantial waste disposal costs that in Europe approach €300/t. Benefits of TunaMud employment may be significant also for public health and the environment. Manure contaminated with antibiotics and resistant bacteria quickly increases the amount of antibiotics residues and antibiotic resistance

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genes, reducing the microbial diversity of the soil and driving widespread antimicrobial resistance. The approach to use fish biowaste as a source of organic fertilizers produced using innovative green chemistry (AnchoisFert) and green chemical engineering (TunaMud) technologies, provides an example of the required integration of disciplines working with industry to foster human health along with that of animals, plants and wider ecosystems. The use of locally produced waste marine biomass, naturally free from antibiotics, significantly reduces dependence on fertilizer markets, while lowering and stabilizing agricultural production costs. By transforming an abundant, renewable, and underutilized resource into a valuable agricultural input, this approach strengthens local economies and supports environmental sustainability. For public health systems, reducing the spread of antibiotics and antibiotic-resistance genes into agricultural soils directly addresses one of the key global priorities.

**Keywords** TunaMud, Tuna, Tuna processing waste, Organic fertilizer, Bioeconomy

## Introduction

A rich and abundant source of valuable proteins, omega-3 lipids, and essential vitamins, tuna is world's most commercially valuable fish [1]. Commercial tuna fisheries in 2018 reported \$40.8 billion revenues [1]. Chiefly commercialized as canned product, with skipjack tuna (*Katsuwonus pelamis*) accounting for 47.7 per cent revenue share followed by yellowfin tuna (*Thunnus albacares*), the global canned tuna market was valued at \$20.4 billion in 2024, and is expected to reach \$26.6 billion by 2030, at a compound annual growth rate of 4.5 per cent [2]. Out of 5.209 Gkg of tuna fish caught in 2018, the global canned tuna industry consumed about 3.215 Gkg [3].

About 70 per cent of the total weight of the live fish used in the canned tuna industry consists of by-products (head, viscera, skin, dark flesh, and bone), that are typically supplied to animal feed manufacturers who convert said biological resource into fishmeal [4]. Plentiful research has been devoted at upgrading tuna processing waste (TPW) into more valued bioproducts such as fish oil rich in omega-3 lipids, fish protein hydrolysates, minerals and bacterial peptones [5, 6]. Application of the LimoFish process based on treatment of milled fish processing waste (TPW, in this case) with *d*-limonene at room temperature affords a valued oil ("TunaOil") rich in tocopherol and omega-3 lipids and other valued lipids such as cetoleic and oleic acids in natural triglyceride form [7], whereas the de-oiled fish waste undergoes anaerobic co-digestion, affording plentiful recovery of biomethane and two fertilisers (struvite and digestate) [8].

Employed together, the LimoFish and anaerobic co-digestion processes close the material cycle for the world's most commercially valuable fish, whereas after the TunaOil extraction biobased limonene is readily recovered by evaporation under reduced pressure and reused in subsequent extraction runs.

Before being canned by canned tuna industrial companies, tuna's meat is cooked. Today's state of the art tuna cooking plants employ cooking with steam under vacuum with substantial water savings and improvement in

the quality of the canned meat over the old cooking conducted in boiling water. In brief, tuna-laden trolleys are rolled into the precooker, and the doors are closed. After closing, the air is removed by either venting with saturated steam (atmospheric precoolers) or with a vacuum pump (vacuum precoolers) [9].

Cooking under vacuum avoids nutrient leaching as it happens in immersion cooking, while the meat remains compact without becoming stringy and humid as it happens during conventional cooking in boiling water. Furthermore, the reduced amount of oxygen prevents oxidation of the meat. The broths that derive from the tuna cooking process are fed to a wastewater treatment plant together with scraps that derive from the cutting phase of the raw tuna, the blood and the so-called sawdust composed of small fragments from the cutting. Finally, also the aqueous exudate released by the tuna in the conditioning phase after cooking, is sent to the water treatment plant.

Though increasingly used as organic fertilizer, sewage sludge from urban wastewater treatment plants in farming presents serious heavy metal and microbial soil and plant contamination risks, requiring continued assessment of environmental and health risks [10].

This study answers two research questions: is the sewage sludge mud-like residue resulting from wastewater treatment at canned tuna industrial plants suitable as organic fertilizer? And, if yes, can it be applied as a multi-purpose fertilizer suitable to fertilize soils cultivated with arboreal, herbaceous, and horticultural plants?

To answer the aforementioned questions, "TunaMud" was tested on tree (olive and pomegranate), herbaceous (pepper and lettuce), and horticultural (onion) plants, investigating the growth parameters of all plants grown, as well as the quality of fertilized soil.

Taking into account that for sustainable technology to be viable, said technology, at the environmental-economic nexus, must demonstrate not only environmental but also economic sustainability [11], the conclusions highlight the economic, health and environmental benefits of TunaMud uptake as broad scope organic fertilizer.

## Experimental

### TunaMud and soil analysis

Dried tuna sewage sludge was kindly donated by a canned tuna industry factory (Giacinto Callipo Conserve Alimentari, Pizzo, VV, Italy). Anchovy fillet leftovers comprising the FISH fertilizer were obtained by filleting fresh anchovies purchased in Reggio Calabria, Italy.

Two sludge samples from different batches were analyzed: TunaMud 1 (ground once), and TunaMud 2 (ground twice).

The dried mud aggregates of the TunaMud samples were subjected to mild mechanical grinding to reduce particle size without altering their chemical composition. The grinding process produced particles with an approximate diameter of 0.5 mm, as verified by sieving. These standardized particles were subsequently used directly in the agronomic experiments to ensure uniformity in application and reproducibility of results.

A 0.5 g sample of each ground dried sludge was thus dispersed in 10 mL of distilled water and analyzed.

Soil samples were collected from each plot or open field and analyzed for the following parameters: electric conductivity (EC) was determined in distilled water by using 1:5 residue/water suspension, mechanically shaken at 15 rpm for 1 h to dissolve soluble salts and then detected by Hanna instrument conductivity meter; pH was measured in distilled water (soil/pad: solution ratio 1:2.5) with a glass electrode.

Organic carbon was assessed via the Walkley-Black dichromate oxidation method [12]. Total nitrogen (TN) was measured with Kjeldahl method [13]. Water soluble phenols were extracted according to the Kaminsky-Muller method [14]. Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin-Ciocalteu reagent. Tannic acid was used as a standard and the concentration of water-soluble phenolic compounds was expressed as gallic acid equivalents ( $\mu\text{g GAE g}^{-1}$  D.W.). Fluorescein diacetate hydrolase (FDA) was determined according to the method of Adam and Duncan [15]. Dehydrogenase (DHA) activity was determined by the method of von Mersi and Schinner [16].

Cations and anions were detected by ion chromatography (DIONEX ICS-1100). For anions, 0.5 g of dried material was extracted using 50 mL of anion solution ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  3.5 mM) stirring for 20 min. The extracts were filtered and chromatographic analysis carried out. For cations, 1 g of dry material was reduced to ash at 550 °C for 5–6 h in a porcelain capsule. The ash was then mineralized for 30 min at 100 °C using 1 M HCl solution. The solution was subsequently filtered and analysed by ion chromatograph (eluent meta-sulfonic acid 20 mM). All analyses were performed following standardized protocols as outlined in standards ISO 10390:2005 “Soil quality — Determination of pH” and

ISO 14240-2:1997 “Soil quality — Determination of soil microbial biomass”.

### Experiments with herbaceous and horticultural plants

The agronomical experiments were conducted inside a greenhouse, using 30 cm diameter pots, filled with soil previously characterized in the laboratory. The aim was to evaluate how the sludge, coming from tuna processing waste, affects the vegetative development of the plants and the quality of the final product, compared to plants grown with the aid of commercial fertilizers. The experiment began on June 8, 2021 with the planting of 15 lettuce plants (var. *Canasta*) and 15 pepper plants (var. *Topepo*).

The experiment was carried out in a 30 cm pot on Red Onion (var. *Tropeana*), in order to evaluate the effects of the sludge from the canned tuna industry plant on the growth of the onion compared to conventional inorganic (NPK 20.10.10, namely 20% N, 10% P and 10% K) and organic (horse manure) commercial fertilizers (purchased from Iuzzolini Fortunato, Cirò Marina, KR, Italy).

The trial began on September 7, 2022 with the planting of the seedlings and ended with the harvest of the onions on February 6, 2023. Before planting the onion seedlings, the soil was analyzed for physical, chemical and biochemical properties to evaluate the effect of fertilizers on soil characteristics. Growth of the onion seedlings was evaluated through the following growth parameters:

- plant height,
- number of leaves,
- length of the leaves,
- average diameter of the bulb.

The substrate used for planting onions is a soil belonging to the sandy-loam textural class with 88% sand, 9% silt, 3% clay.

Soil was analyzed with respect to selected physical, chemical and biological characteristics at time zero before planting the seedlings: U% (% moisture content), pH, chloride concentration, electrical conductivity, total phenols, organic carbon, organic matter, total nitrogen, carbon/nitrogen ratio, DHA, FDA, and catalase activity.

### Experiments with arboreal plants

Pomegranate and olive trees were planted and fertilized with tuna sewage sludge and conventional manure and NPK commercial fertilizers. Data relating to the growth parameters of pomegranate plants were recorded from February 2, 2022 to January 20, 2023.

Parameters taken into consideration were:

- plant height (m);
- diameter at the base (mm);

**Table 1** Selected physical and chemical parameters for dried TunaMud and anchovy sludge (FISH) samples dispersed in water. Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same column indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Sample	pH	Electrical conductivity ( $\text{mS cm}^{-1}$ )	Total soluble phenols ( $\mu\text{g GAE g}^{-1}$ )	C (%)	N (%)	C/N
TunaMud 1	$7.00^a \pm 0.09$	$8.89^a \pm 9$	$1.12^b \pm 0.09$	$0.37^c \pm 0.05$	$0.082^c \pm 0.07$	$4.6^c \pm 0.5$
TunaMud 2	$7.02^a \pm 0.07$	$8.61^a \pm 11$	$0.92^b \pm 0.07$	$2.18^b \pm 0.09$	$0.123^b \pm 0.04$	$17.7^a \pm 1.1$
FISH	$6.27^b \pm 0.08$	$5.94^b \pm 0.9$	$8.51^a \pm 0.9$	$40^a \pm 9$	$12^a \pm 0.3$	$3.3^b \pm 0.3$

\*0.5 g sample of dried sludge ground once (TunaMud 1) and dispersed in 10 mL of distilled water, or ground twice (TunaMud 2) and dispersed in 10 mL of distilled water; FISH consists of European anchovy fillet leftovers derived from the industrial filleting of anchovies

- diameter at 0.6 m from the collar (mm).

The soil was used for planting 18 trees: 9 pomegranate trees (cv. *Wonderful*), and 9 olive trees (cv. *Carolea*). The following physical, chemical and biochemical analyses were conducted to characterize the selected soil: % of skeleton; granulometric analysis (determination of the textural class); pH; chloride concentration; electrical conductivity (EC); cation exchange capacity; determination of enzymatic activities (fluorescein diacetate hydrolytic, betaglucosidase and catalase activity).

Measured morphological characteristics of the plants were the plant height, and the diameter of the stem (at two different heights). These parameters will be used to evaluate the influence of the different fertilizations on the growth of the different species selected in the experiment. In addition, the chlorophyll content of the leaves was detected using the SPAD instrument.

### Statistical analysis

All measurements were conducted in triplicate. Results are presented as the mean  $\pm$  standard deviation. One-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test was used to analyze the effects of fertilizer and crop rotation on each measured parameter. ANOVA and t-tests were performed using IBM SPSS Statistics, version 29.0, statistica software (IBM, Armonk NY, USA).

### Results and discussion

Table 1 summarizes selected physical and chemical parameters resulting from the analyses conducted by an independent analytical chemistry laboratory (GEO LAB, Rende, Italy).

The fish-based fertilizer (FISH in Table 1) consists of European anchovy (*Engraulis encrasicolus*) fillet leftovers. The waste typically comprises a heterogeneous mixture of non-edible anatomical parts, including heads, viscera, scales, bones, and skin. Rich in organic matter, this biomass is notable for its high protein and lipid content, as well as the presence of essential micronutrients and marine-derived bioactive compounds [17].

Besides results of metal concentrations showing ultralow values of Cd ( $< 0.1$  mg/kg dry weight), Ni ( $<$

**Table 2** Anion and cation content in dried TunaMud and anchovy sludge (FISH). Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same row indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Cations ( $\text{mg g}^{-1}$ )	TunaMud 1	TunaMud 2	Fish sludge
Sodium	$21.33^a \pm 1$	$20.14^a \pm 0.9$	$21.66^a \pm 1.1$
Ammonium	$49.63^b \pm 0.9$	$54.74^a \pm 1.9$	$51.33^a \pm 1.5$
Potassium	$5.44^b \pm 0.5$	$13.39^a \pm 0.9$	$5.5^b \pm 0.4$
Magnesium	$6.58^b \pm 0.7$	$8.09^a \pm 0.8$	$6.7^b \pm 0.9$
Calcium	$35.87^b \pm 3$	$107.98^a \pm 8$	$35.2^b \pm 4$
Anions ( $\text{mg/g}$ )			
Fluoride	$1.91^b \pm 0.2$	$4.54^a \pm 0.4$	$2.33^b \pm 0.3$
Chloride	$28.04^b \pm 0.9$	$75.05^a \pm 2$	$27.5^b \pm 0.5$
Bromide	$1.37^a \pm 0.09$	$1.42^a \pm 0.08$	$1.45^a \pm 0.09$
Nitrate	$53.94^a \pm 2$	$1.85^c \pm 0.09$	$25.36^b \pm 1$
Phosphate	$5.66^b \pm 0.1$	$9.47^a \pm 0.2$	$5.61^b \pm 0.1$
Sulphate	$15.37^a \pm 0.6$	$10.25^b \pm 0.3$	$16.21^a \pm 0.8$

\*0.5 g sample of dried sludge ground once (TunaMud 1) or ground twice (TunaMud 2) and fish sludge

**Table 3** Growth parameters for lettuce (var. Canasta) fertilized with NPK, horse manure (HM), anchovy sludge (FISH), and TunaMud (SLUD, canned tuna sewage sludge). Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same column indicate significant differences (Tukey's test,  $p \leq 0.05$ )

15 days after planting		30 days after planting		
Lettuce	Plant height (cm)	Lettuce	Plant height (cm)	Lettuce
CTR	$13.0^c \pm 0.11$	$5.3^d \pm 0.16$	$13.0^c \pm 0.15$	$11.7^d \pm 0.39$
NPK	$14.0^b \pm 0.22$	$5.7^c \pm 0.11$	$14.0^b \pm 0.16$	$11.3^d \pm 0.46$
HM	$15.0^a \pm 0.43$	$6.3^b \pm 0.12$	$16.0^a \pm 0.18$	$12.3^c \pm 0.29$
FISH	$15.3^a \pm 0.36$	$6.7^a \pm 0.26$	$16.2^a \pm 0.12$	$13.0^b \pm 0.16$
SLUD	$15.7^a \pm 0.21$	$7.0^a \pm 0.15$	$16.0^a \pm 0.17$	$13.7^a \pm 0.18$

6.6 mg/kg dry weight), Pb ( $< 0.6$  mg/kg dry weight) and Hg ( $< 0.1$  mg/kg dry weight), results in Table 2 show evidence that the sewage sludge from the canned tuna industry wastewater treatment plant shows that it possesses chemical and microbiological characteristics that permit its agronomic use in compliance with Italy's legislation [18], which implements in Italy the European Council Directive 86/278/EEC of 12 June 1986 on the protection of the soil when sewage sludge is used in agriculture. Table 3 summarizes the main anions and cations found in the two TunaMud samples.

### Comparative effects of different fertilizers on herbaceous and horticultural plants

Tables 3 and 4 show the data of the growth parameters of the lettuce plants (var. *Canasta*) and bell pepper plants (var. *Topepo*) fertilized with inorganic fertilizer (NPK 20.10.10), and organic fertilizers: (horse manure), fish sludge and TunaMud from the canned tuna company's water treatment plant.

Figures S1 and S2 display photographs of the plants cultivated in pots with no fertilizer (CTR), or fertilized with NPK, horse manure (HM), anchovy sludge (FISH) and TunaMud (SLUD).

The growth parameters were measured 15 and 30 days after planting. Data evidenced a better growth in terms of plant height and leaf number over time of both lettuce and pepper.

To investigate the effects of soil fertilization with TunaMud on the quality of onions (var. *Tropeana*) grown in pot, we investigated the effects of the new fertilizer both on the fruits and soils. The amount of soil contained in each pot was about 9 kg. Each batch was composed of three pots differently fertilized. Batch of soil called NPK was fertilized with 1.2 g of NPK (20.10.10). Batch of soil named HM was added with 13 g of horse manure. A batch of pots called SLUD was added with 8.5 g of TunaMud.

Results in Table 5 show that fertilization with TunaMud systematically afforded enhanced onions in terms of bulb weight, that was substantially higher than onions fertilized with both HM and NPK inorganic and organic conventional fertilizers. Similarly, the weight of roots, the length of leaves, and the overall height of plants growing in soil fertilized with TunaMud were in each case investigated the highest.

The main chemical and physical properties of soils fertilized with different fertilizers were thus compared prior (Table S1) and after (Table S2) onion harvesting.

Clearly, growing onion, bell pepper and lettuce plants in soil fertilized with TunaMud eventually reduced catalase activity of soils. Results in Table S1 concerning the activity of catalase, a key enzyme reflecting microbial activity and soil aeration, show lower values compared to the previous year in soils treated with TunaMud, as with

**Table 4** Growth parameters for pepper (var. *Topepo*) grown in unfertilized soil (CTR, control) and soil fertilized with NPK, horse manure (HM), anchovy sludge (FISH), and TunaMud (SLUD, canned tuna sewage sludge). Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same column indicate significant differences (Tukey's test,  $p \leq 0.05$ )

	15 days after planting		30 days after planting	
	Plant height (cm)	Leaf number	Plant height (cm)	Leaf number
Pepper				
CTR	18.0 <sup>b</sup> $\pm$ 0.19	4.3 <sup>c</sup> $\pm$ 0.31	13.5 <sup>c</sup> $\pm$ 0.11	11.7 <sup>c</sup> $\pm$ 0.18
NPK	17.2 <sup>c</sup> $\pm$ 0.23	4.7 <sup>c</sup> $\pm$ 0.43	15.0 <sup>b</sup> $\pm$ 0.19	11.3 <sup>c</sup> $\pm$ 0.43
HM	17.7 <sup>c</sup> $\pm$ 0.27	5.3 <sup>b</sup> $\pm$ 0.31	16.2 <sup>a</sup> $\pm$ 0.13	12.3 <sup>b</sup> $\pm$ 0.18
FISH	18.2 <sup>b</sup> $\pm$ 0.31	5.3 <sup>b</sup> $\pm$ 0.29	16.2 <sup>a</sup> $\pm$ 0.12	13.0 <sup>a</sup> $\pm$ 0.39
SLUD	19.3 <sup>a</sup> $\pm$ 0.21	5.7 <sup>a</sup> $\pm$ 0.34	16.3 <sup>a</sup> $\pm$ 0.21	13.7 <sup>a</sup> $\pm$ 0.27

other treatments. Referring to the previous year's results, data indicate good soil health.

Catalase indeed acts as an efficient scavenger of reactive oxygen species by mediating the decomposition of  $H_2O_2$  formed during aerobic respiration as a by-product in a number of cellular systems, to water and molecular oxygen, thereby preventing oxidation of protein SH-groups. On the other hand, excessive reduction in catalase activity is an indicator of soil stress [19].

On the other hand, the dehydrogenase activity (DHA) went from 31.0 to 38.7  $\mu$ g TPF  $g^{-1}$  in soil under lettuce, from 28.4 to 38.37  $\mu$ g TPF  $g^{-1}$  in soil under bell pepper, while no change was observed in soil under onion. The fluorescein diacetate hydrolase activity (FDA) increased in soil under all the horticultural crops.

We briefly remind that DHA is a direct measure of microbial oxidative activities in soil and thus of overall soil microbial activity as dehydrogenases mediate the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors [20]. On the other hand, the FDA is a direct measure of hydrolytic microbial activity, and even better biological indicator than dehydrogenase activity [21].

The largest increase was observed in soil under bell pepper with values going from to 1.9 to 4.26  $\mu$ g FDA  $g^{-1}$ . Similarly, the FDA noticeable increase prior and after harvesting onions in soil fertilized with TunaMud points to substantial increase in the soil microflora activity.

**Table 5** Parameters of onion plants (var. *Tropeana*) grown in pot with unfertilized soil (CTR, control) and soil fertilized with NPK, horse manure (HM), anchovy sludge (FISH), and TunaMud (SLUD, canned tuna sewage sludge). Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same column indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Fertilizer	Leaf number	Leaf Length (longest)	Leaf Length (shortest)	Root weight (g)	Leaf weight (g)	Bulb weight (g)	Plant height (cm)	bulb diameter (cm)
NPK	18.0 <sup>b</sup> $\pm$ 0.59	82.0 <sup>b</sup> $\pm$ 0.32	25.0 <sup>c</sup> $\pm$ 0.25	5.6 <sup>c</sup> $\pm$ 0.29	89.5 <sup>c</sup> $\pm$ 0.22	41.4 <sup>d</sup> $\pm$ 0.24	76.3 <sup>c</sup> $\pm$ 0.34	2.8 <sup>a</sup> $\pm$ 0.44
HM	21.0 <sup>a</sup> $\pm$ 0.34	83.7 <sup>b</sup> $\pm$ 0.29	22.3 <sup>d</sup> $\pm$ 0.34	7.2 <sup>b</sup> $\pm$ 0.31	118.0 <sup>a</sup> $\pm$ 0.13	51.3 <sup>b</sup> $\pm$ 0.27	82.0 <sup>b</sup> $\pm$ 0.28	3.1 <sup>a</sup> $\pm$ 0.37
FISH	17.0 <sup>b</sup> $\pm$ 0.51	71.0 <sup>c</sup> $\pm$ 0.31	27.7 <sup>b</sup> $\pm$ 0.63	7.0 <sup>b</sup> $\pm$ 0.21	107.6 <sup>b</sup> $\pm$ 0.23	44.8 <sup>c</sup> $\pm$ 0.51	69.7 <sup>d</sup> $\pm$ 0.35	2.7 <sup>a</sup> $\pm$ 0.41
SLUD	15.3 <sup>c</sup> $\pm$ 0.29	91.7 <sup>a</sup> $\pm$ 0.45	31.0 <sup>a</sup> $\pm$ 0.31	13.6 <sup>a</sup> $\pm$ 0.19	118.7 <sup>a</sup> $\pm$ 0.21	56.4 <sup>a</sup> $\pm$ 0.26	93.0 <sup>a</sup> $\pm$ 0.21	3.2 <sup>a</sup> $\pm$ 0.28

**Table 6** Physiological parameters of pomegranate tree for plants grown in soil with NPK, horse manure (HM), anchovy sludge (FISH), and TunaMud (SLUD, canned tuna sewage sludge) between 2023/2024. Data are the means of three replicates  $\pm$  standard deviation. Different letters in the same column indicate significant differences (Tukey's test,  $p \leq 0.05$ )

Fertilizer	Height (%)	Average (%)	Ø plant collar (%)	Average (mm, %)	Ø 0.6 m (%)	Average (%)
NPK	20.59	20.59 <sup>c</sup> $\pm$ 1	12.2	13.8 <sup>c</sup> $\pm$ 0.9	14.5	17.9 <sup>b</sup> $\pm$ 0.9
NPK	20.69		11.0		18.9	
NPK	20.65		18.4		20.5	
HM	34.15	23.8 <sup>b</sup> $\pm$ 0.7	26.0	17.5 <sup>b</sup> $\pm$ 0.7	22.0	21 <sup>a</sup> $\pm$ 0.9
HM	19.16		6.9		32.0	
HM	18.18		18.6		9.2	
SLUD	22.41	25.45 <sup>a</sup> $\pm$ 0.8	25.0	21.1 <sup>a</sup> $\pm$ 0.9	23.0	21 <sup>a</sup> $\pm$ 1
SLUD	25.00		23.3		19.9	
SLUD	28.95		14.9		18.9	

**Table 7** Physiological parameters of Olive tree for plants grown in soil with NPK, horse manure (HM), anchovy sludge (FISH), and TunaMud (SLUD, canned tuna sewage sludge) between 2023/2024

Fertilizer	Height (%)	Average (%)	Ø plant collar (%)	Average (%)	Ø 0,6 m (%)	Average (%)
NPK	30.41	33.8 <sup>b</sup> $\pm$ 1	36.57	27.2 <sup>b</sup> $\pm$ 0.9	12.86	19.4 <sup>c</sup> $\pm$ 0.9
NPK	32.50		20.81		20.00	
NPK	38.64		24.44		25.33	
HM	30.41	33.6 <sup>b</sup> $\pm$ 0.9	19.21	21.5 <sup>c</sup> $\pm$ 0.7	38.75	25.9 <sup>b</sup> $\pm$ 1
HM	34.15		7.43		18.67	
HM	36.32		37.78		20.65	
SLUD	30.05	36.6 <sup>a</sup> $\pm$ 1	31.49	30 <sup>a</sup> $\pm$ 0.9	28.17	31.0 <sup>a</sup> $\pm$ 1.2
SLUD	39.46		28.51		35.56	
SLUD	40.00		28.50		28.75	

Together, these results suggest that TunaMud is able to increase soil biodiversity as demonstrated by the contemporary increase of DHA and FDA markers of the total hydrolytic and oxidative enzymatic soil activity.

Finally, the total phenolic content (TPC) substantially decreased after all horticultural crop cultivation most likely due to biophenol transfer from soil to plant and onions. Remarkably, we already measured such substantial rise in biophenol content in Tropea onions cultivated with AnchoisFert, namely the anchovy-based fertilizer obtained from anchovy fillet leftover following de-oiling with limonene [17].

The nearly doubling in electrical conductivity, in its turn, is due to both the substantially higher amount of humidity of soil fertilized with TunaMud that translates into more pronounced solubilization of the ions abundant in TunaMud (Table 2).

#### Comparative effects of different fertilizers on arboreal plants and fertilized soil

Table 6 displays the increase percentage of all pomegranate growth parameters data for plants grown in 2023 and 2024. Fig.S4 an Fig.S5 display, respectively, photographs of pomegranate and olive trees cultivated in pots whose soil was fertilized with NPK, horse manure (HM), fish sludge (FISH) and TunaMud (canned tuna sewage sludge, SLUD) at the beginning of the experiment, after 8 days, and after 7 months.

Results show once again that TunaMud was the best fertilizer when compared both to inorganic (NPK) and organic (manure) commercial fertilizers in promoting the plant development in terms of height, diameter at the base, and diameter at 0.6 m from the collar.

Only horse manure gave comparable results when considering the latter parameter (diameter at 0.6 m from the collar), but not when considering the height and the base diameter of the plant.

Similarly, results in Table 7 show that TunaMud was the best fertilizer also for olive trees grown during the same period (2023/2024), with even larger percentage increases with reference to each of the parameters investigated. TunaMud in this case was particularly effective in promoting the growth in height (nearly 37% vs. nearly 34% for NPK) and in that of diameter at 0.6 m from the collar (31% vs. 26% for manure).

Results in Table S3 show evidence that soil fertilized with TunaMud shows optimal data regarding microbial activity. Both DHA and FDA indeed showed substantial increase between 2023 and 2024 in soils fertilized with TunaMud as well as with the other fertilizers, showing evidence that the soil, after one year, had improved microbial vitality, which leads to a better ability to degrade the organic compounds present in the soil.

Outcomes summarized in Table S4, in their turn, show that in the case of pomegranate NPK was the best fertilizer in promoting increase in the total phenolics content,

followed by TunaMud. On the other hand, in the case of olive trees, neither TunaMud nor NPK were particularly effective in promoting the growth in TPC, that slightly increased by a small percentage between 2024 and 2023. Likewise to what happened with horticultural and herbaceous plants, also in the case of arboreal plants all tested fertilizers including TunaMud promoted a significant decrease of the catalase activity of soils, indicating again good soil health of fertilized soil [19].

Finally, all types of fertilization treatments led to an increase in the amount of organic substance after one year (Table S5). Invariably, for both pomegranate and olive trees, said increase was highest for soils fertilized with TunaMud. Interestingly, the amount of nitrogen in soils fertilized with TunaMud slightly decreased showing evidence of enhanced nitrogen absorption by plants planted in soil fertilized with tuna sewage sludge. Finally, TunaMud was nearly equally effective as NPK and horse manure in promoting chlorophyll formation in the leaves of both olive (Table S6) and pomegranate (Table S7) trees, with NPK being the most effective in promoting chlorophyll formation. Compared to the organic commercial fertilizer, TunaMud's activity was similar or slightly superior.

#### Implications for economy, health and the environment

In brief, a biological resource so far disposed of as bio-waste at substantial and increasing economic cost (€240/t by early 2025) [22] can now be turned into a valued fertilizer that can be used as such.

Rich in organic carbon, as well as valued nutrients in the form of minerals (Mg, Ca, K, P), nitrogen in readily absorbed ammonium and nitrate forms, as well as valued sulphates, the composition of TunaMud meets the EU requirements for approved use of sewage sludge as organic fertilizer [18].

Recent research on the valorization of tuna canned industry's processing waste has shown that the LimoFish process coupled to anaerobic co-digestion of the de-oiled tuna processing waste converts said biowaste into valuable bioproducts (fish oil, biomethane and fertilizers) [7, 8].

Canned tuna industry companies may now rely on technically viable circular economy processes to valorize not only the by-products of the canned tuna industry [7, 8], but also the sewage sludge formed upon compulsory treatment of the industry's wastewater. Should the economic assessment of TunaMud uptake based on energy costs associated with preparing fertilizer from sludge, savings resulting from avoiding waste disposal and potential economic benefits resulting from increased yields compared to the cost of purchasing commercial NPK fertilizer or manure, confirm that the process is

economically viable, then the canned tuna industry will become a regular supplier of this multi-purpose fertilizer.

Farming companies, in their turn, are provided with a multi-purpose organic fertilizer free of antibiotic residues that can be equally successfully employed to fertilize soils cultivated with arboreal, herbaceous, and horticultural plants.

Benefits of TunaMud employment, as well as of AnhoisFert, and other organic fertilizers sourced from fish waste using the LimoFish process [23], however, will be significant also for public health and the environment, well beyond economic benefits for the suppliers and users of these antibiotic-free organic fertilizers. Significant antibiotic contamination of manure residue of intensive cattle farming employed as organic fertilizer is leading to serious public health and environmental issues. Fertilizers contaminated with antibiotics quickly increase the amount of antibiotics residues as well as the relative abundance of antibiotic resistance genes in the fertilized soil [24]. Besides reducing the microbial diversity of the soil, residual antibiotics and resistant bacteria in manure contaminate soil, water, and the food chain, driving widespread rise of antimicrobial resistance [25]. Accordingly, researchers and environmental groups worldwide are advocating policy measures to establish that animal manure should be considered antimicrobial-containing waste and thus treated to reduce the amount of antibiotics residues prior to using it in agriculture ecosystems [26].

#### Conclusions

In summary, we have discovered that the sewage sludge mud-like residue resulting from wastewater treatment at canned tuna industry factories is a multi-purpose fertilizer. Dubbed "TunaMud", the sludge was tested on arboreal (olive and pomegranate), herbaceous (pepperoni and lettuce), and horticultural (onion) plants, improving in each case all growth parameters of the plants grown, as well as the quality of fertilized soil.

Besides advancing circular economy practices in the agri-food sector in line with agriculture and food security based on sustainable agriculture, the discovery of TunaMud (and of AnchoisFert) addresses the dual challenge of agro-industrial waste management and the needs of the "One Health" integrated approach to health based on recognition that human health and the health of the wider environment including plants and animals are closely inter-dependent. Rather than dealing with fish and fishery biowaste as an environmental and economic issue of relevance to fish processing companies, the approach uses the latter biowaste as source of organic fertilizers produced using green chemistry (in the case of AnchoisFert) and green chemical engineering (in the case of TunaMud) technologies. Discoveries in question

provide an example of the required integration of disciplines working together with the agri-food industry to foster human health and well-being along with that of animals, plants and wider ecosystems.

Likewise to any other study, the present investigation has its own limitations. Limitations indeed include pot experiment constraints, lack of safety analyses, interannual variability, and the need for long-term salinity and nutrient-cycling assessments. Further investigations following this proof-of-concept study will investigate all these aspects.

Future research, in conclusion, will also investigate the techno-economic feasibility of widespread TunaMud uptake in agriculture, as well as the use of artificial intelligence tools to optimize TunaMud utilization in agriculture, as well in its production and delivery to customers at farming companies.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40066-025-00592-7>.

Supplementary Material 1.

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### Author contributions

FM was involved in investigation, methodology, data curation, visualization, and software. RC contributed to conceptualization, methodology, writing-reviewing and editing. MP contributed to conceptualization, methodology, writing-original draft preparation, writing-reviewing and editing. FrM was involved in conceptualization, data curation, writing-reviewing and editing. MR contributed to conceptualization, methodology, writing-reviewing and editing. AM contributed to the conceptualization, data curation, visualization, methodology, writing-reviewing and editing. All the authors have read and approved the final manuscript.

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### Data availability

All data that support the findings are available upon reasonable request to the corresponding Authors.

### Declarations

### Competing interests

The authors declare no competing interests.

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