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Abundance of Human Pathogenic Microorganisms in the Halophyte *Salicornia europaea* L.: Influence of the Chemical Composition of Shoots and Soils

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Abstract: *Salicornia europaea* L. is a halophilic plant species belonging to Chenopodiaceae, whose shoots are used as a vegetable. Since the shoots can be eaten raw, the objective of the present study was to investigate possible controls on the abundance of human pathogenic microorganisms (HPMOs) in the shoots as a health risk. For this reason, the molecular-chemical composition of shoots, site-specific soil organic matter (bulk and rhizosphere), and soil pH and salinity were analyzed. Plant and soil samples were taken from two test sites with differing salinity levels in France (a young and an old marsh). We hypothesized that the chemical traits of plants and soils could suppress or promote HPMOs and, thus, serve as risk indicators for food quality. The chemical traits of shoots and bulk and rhizosphere soil were measured through thermochemolysis using gas chromatography/mass spectrometry (GC/MS). The densities of cultivable HPMOs (*Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes*) were determined in plant shoots, rhizosphere soil, and bulk soil using selective media. Negative correlations between lignin content in the shoots and the abundance of *S. enterica*, as well as between lignin content in bulk soil and the abundance of *E. coli*, are explained by the lignin-based rigidity and its protective effect on the cell wall. In the shoot samples, the content of lipids was positively correlated with the abundance of *E. coli*. The abundance of *E. coli*, *S. enterica*, and *L. monocytogenes* in bulk soil decreased with increasing soil pH, which is linked to increased salinity. Therefore, soil salinity is proposed as a tool to decrease HPMO contamination in *S. europaea* and ensure its food safety.

Keywords: human pathogenic bacteria; lipids; lignin; gas chromatography-mass spectrometry; *Salicornia europaea*

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1. Introduction

Salicornia europaea L. (also known as glasswort or sea asparagus) is a halophilic plant species belonging to Chenopodiaceae [1,2]. It prefers temperate and subtropical climates [3,4]. This plant has an important economic value as a healthy food source [5] and is also used as forage and in pharmaceutics and cosmetics [6,7]. It is increasingly grown in open fields and greenhouses for edible or non-edible purposes, and can be irrigated with salt water, sea water, or wastewater with a moderate to high salinity, which cannot be used for other crop species [8]. Increasing soil salinity and scarcity of fresh water, which are the result of climate

change and anthropic activities, make halophytic species an alternative food source for which salt water is used [8]. The most important molecules commonly found in halophytes are phenolic compounds, polysaccharides, and lipids [9]. Lignin is a phenolic polymer that confers rigidity and protection to the cell wall [10,11]. It has been demonstrated that plants under attack by pathogens have a higher lignin content due to increased transcription of genes for lignin biosynthesis [12–14]. Lignin acts as a mechanical barrier that restricts the spread of pathogens, such as pathogenic bacteria, within the extracellular space of the plant tissues. When plants are under attack by pathogens, they induce lignification, a process that strengthens the cell wall's resistance to invasion [12]. Lipids have an important role in conferring resistance to plants against their pathogens [15,16] but are also produced in response to microbial colonization. During pathogen attack, the plant lipid profile is altered, resulting in modifications to plant membrane fluidity and lipid biosynthesis [17]. The genes that encode enzymes involved in lipid metabolism are often upregulated upon infection, leading to synthesis, modification, or re-allocation of lipid-derived molecules, which are crucial for establishing membrane integrity and function during the infection process [18]. The special properties of halophytes can also affect the level of infection by human pathogens of concern (HPMOs—human pathogenic microorganisms), which is especially important when they can be consumed in unprocessed forms, the preparation of which lacks steps to inactivate the pathogens [19]. Among the many enteric pathogens associated with ready-to-eat fresh produce (RTEFP) contamination, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus* spp. were the most commonly reported in previously published studies [19]. Certain HPMO bacteria, such as *Salmonella* sp. and *E coli*, use plants as vectors between animal hosts. These pathogens adhere to the life cycle patterns of plant-associated bacteria and can establish themselves in agricultural production areas, which may pose food safety risks, especially considering global warming [20]. HPMOs were found to be able to colonize many parts of different plant species including the epidermis, cortex, vascular tissue, pith, and apoplastic fluid in spinach and peanut [21–24]. Until now, no studies have shown the colonization of *S. europaea* by HPMOs. Furthermore, to the best of our knowledge, the relationships between the composition of plant and soil organic matter (SOM) and the abundance of HPMOs have not yet been investigated for *S. europaea*. Therefore, the aim of the present study is to investigate the chemical composition of plant and soil material and the abundance of HPMOs in order to deduce possible control tools for food safety in this regard. We hypothesize that lignin and lipids might be indicators of a suppressed abundance of HPMOs due to their role in plant protection and partly high resistance to decomposition.

2. Materials and Methods

2.1. Test Sites Description, Environmental Conditions and Sampling

The plant and soil samples were collected at two test sites in France in October 2022, which were selected based on the differing salinity levels between a young and an old marsh. Three plots (1 m × 1 m) were delineated at each site, from which five plants were taken, together with the soil adjacent to their roots (a total of 15 plants per site). The individual plots were located at a distance of 5–10 m from each other. In addition, a bulk soil sample (10 cm × 10 cm × 10 cm; approximately 200 g soil per sample) was taken at each site (a total of five samples per plot). Each sample was stored in a plastic bag and sent immediately by courier at 4 °C to the Department of Microbiology at Nicolaus Copernicus University in Torun (Poland) for further analyses. All root samples were collected in accordance with institutional, national, and international guidelines and legislation with the permission of the General Director for Environmental Protection (DZP-WG.6400.13.2022.EP.1). One test site (F1) was located in the estuary of L'Ilet in the municipality of Plurien (48.634379, −2.415823), and the second site (F2) was located in a salt marsh in the municipality of Beaussais-sur-Mer (48.582263, −2.159779). Both sites have a temperate oceanic climate (sub-type Cfb in the Köppen classification) but differ in their history. F1 is an old, mature salt marsh that developed from a sandy substrate (which is rather unusual for this type of

habitat). It is dominated by *Atriplex portulacoides*, and *Salicornia* stands that occur along the (natural) drainage creeks [25]. F2 is a recently (2020) created young salt marsh, developing on a classical muddy substrate, where pioneer stands of *Salicornia* were found all over the area.

2.2. Thermochemolysis and Pyrolysis-Gas Chromatography/Mass Spectrometry

For thermochemolysis, about 10 mg of plant material and 100 mg of soil, respectively, were inserted into Pasteur pipettes (ISO 7712, 150 mm, [26]) with the tips broken off. Tetramethylammonium hydroxide (TMAH) in water (25%) was added to the sample using a microsyringe (30 µL for plant material and 60 µL for soil). Afterwards, the pipette was connected to a second pipette filled with activated coarse charcoal and a small amount of glass wool at the tip. The whole system was flushed with a nitrogen stream; 5 min after adding TMAH, the sample was heated to 220 °C for 6 min using a hot air gun. After allowing it to cool for 5 min, the sample was scratched into a 4 mL vial and both pipettes were rinsed with 1.5 mL each of a dichlormethane/methanol mixture (4:1). The vial was placed in an ultrasonic water bath of 35 °C for 5 min, after which the suspension was allowed to settle for 55 min. For GC/MS, 1 µL from the upper part of the solution was injected into a Thermo Scientific Trace 1310-GC (Thermo Fisher Scientific, Waltham, MA USA 02451) equipped with a 60 m BP5 column (0.25 mm i.d., 0.25 µm coating) at an injector temperature of 300 °C. The carrier gas, helium 5.0, was set up with a constant flow of 1 mL min^{-1}. Following split injection for up to 45 s (splitless), the split ratio was 1:100 from 45 s up to 90 s and 1:5 from 90 s onward. The temperature program was set as 5 min at 100 °C, and was subsequently heated at a rate of 5 K min^{-1} to 280 °C for a total measurement time of 120 min. The GC was connected to a Thermo Scientific DFS magnetic sector MS (Thermo Fisher Scientific, Waltham, MA, USA, 02451). The conditions for mass spectrometric detection in the electron impact mode were as follows: 4.7 kV accelerating voltage, 70 eV electron energy, 1.2 kV multiplier voltage, m/z 48–600 mass range, 0.5 s $(\text{mass decade})^{-1}$ scan rate, and 0.6 s interscan time. The identified single compounds were summed into the compound classes of carbohydrates, lignin, and lipids. The peak areas of each compound class to the total peak area of the carbohydrates, lignin, and lipids are given in %. Peaks were assigned by comparing spectra with the NIST 2017 database using Thermo Xcalibur version 2.2.

2.3. Abundance of HPMOs in Bulk Soil, Rhizosphere, Shoot and Root of *S. europaea*

Three plots (A, B, C) with five replicates per plot were considered for each site. Rhizosphere and/or bulk soil (1 g) was transferred into falcon tubes for the next part of the experiment. Separated plant organs (roots and shoots) were surface sterilised with 70% ethanol (3 min), rinsed three times with 2% NaCl (1 min each time), treated with 7.5% H_2O_2 (3 min), and washed three times with 2% NaCl (1 min each time). Then, dried shoots and roots (1 g of fresh biomass) were homogenized in 2% NaCl (1:9 ratio) using a sterile mortar and pestle. Serial dilutions for plant (10^{-1}–10^{-2}) and soil (10^{-1}–10^{-3}) samples were prepared and 100 µL of dilutions were plated in triplicate on selective media for the abundance of HPMOs: *L. monocytogenes* (Chromogenic *Listeria* acc. to Ottaviani and Agostii LAB-AGAR™ Base and Chromogenic *Listeria* Supplement acc. to ISO 11290-1:2017 [27], Biomaxima, Lublin, Poland), *E. coli* (*E. coli* Chromogenic Medium, Biomaxima, Lublin, Poland), *S. enterica* (Chromogenic, *Salmonella* LAB-AGAR™ and *Salmonella* Chromogenic Supplement, EN-ISO 6579 [28], Biomaxima, Lublin, Poland), and *B. cereus* (*B. cereus* Selective LAB—AGAR™ Base and *B. cereus* Supplement, EN ISO 7932:2004 [29], Biomaxima, Lublin, Poland). The plates were incubated for seven (for *S. enterica*, *L. monocytogenes*, *E. coli*) and two days (for *B. cereus*) at 37 °C. The colony forming units (CFU) were counted and calculated per 1 g of dry weight. The CFU g^{-1} was transformed into $\log_{10}$.

2.4. Bulk and Rhizosphere Soil Analysis

The soil samples were air-dried at room temperature and passed through a 2 mm mesh sieve to remove debris. Total carbon (C_t) and total nitrogen (N_t) were determined using Elementar Vario CNS analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). The calcium carbonate ($CaCO_3$) content of the soil was determined by the volumetric method using a Scheibler apparatus. Total inorganic carbon (TIC) was calculated from the calcium carbonate content. Total organic carbon (TOC) was calculated as the difference between TC and TIC. Bio-available phosphorus (P_{ca}) was determined colorimetrically by the citrate method using a Rayleigh UV-1601 spectrophotometer (Beifen-Ruili Analytical Instrument Co. Ltd. Beijing, China). The saturated paste extracts were prepared according to the methodology of van Reeuwijk. The pH ($CaCl_2$) was measured potentiometrically using a CP-551 Elmetron pH meter. Electrical conductivity (EC) was determined at 25 °C using the conductometric method with a CPC-401 conductivity meter (Elmetron Ltd., Zabrze, Poland). The concentrations of Mg and Ca were determined by atomic absorption spectrometry (AAS), while the concentrations of K and Na were determined by optical emission spectrometry (OES) using a SOLAAR Unicam 969 (Unicam Ltd., Cambridge, UK) flame spectrometer. The concentration of bicarbonates (HCO_3^-) was determined by titration with 0.1 M HCl. The concentration of sulphate (SO_4^{2-}), chloride (Cl^-), and bromide (Br^-) was determined by ion chromatography using a Thermo Scientific Dionex Aquion system (Thermo Fisher Scientific Inc., Waltham, MA, USA, 02451). Soil texture was measured using 10 g of bulk soil from each test site as follows: Carbonate was dissolved in the soil sample taken from the young marsh by incubation in a 1:3 dilution with 10% HCl for 60 min. This suspension was filtrated, and the filtrate was collected in a tube, to which NH_4^+ oxalate was added. For the organic matter destruction, 10 mL bi-distilled water (H_2O_{bidest}) and about 300 to 500 mL H_2O_2 (30%) were added to the remaining soil under heat (ca 95 °C) for approximately 2 weeks (until no reaction was visible). The resulting dry samples were then transferred to new vessels. For sedimentation preparation, the remaining soil was weighed, and 25 mL pyrophosphate (0.1 M) was added to the samples, mixing the solution thoroughly. Disaggregation was carried out in a supersonic bath (15 min in H_2O_{bidest}). For the final step, sedimentation, the samples were transferred to a 1 L cylinder, with H_2O_{bidest} added, and placed in a Sedimat instrument (Sedimentation device; Sedimat 4–12 Umwelt-Geräte-Technik GmbH (Hallbergmoos, Germany). Sedimentation took place in a 25 °C water bath for one night. The instrument automatically separated the silt and clay fractions and transferred them to vessels of known mass, where the samples were dried and weighed. The remaining soil in the cylinder was transferred to a sieve (0.063 mm mesh size) to determine the sand fraction. Finally, the total texture fraction composition was calculated.

2.5. Statistical Analyses

The results showing the abundance of HPMOs in the selective media, the % peak area of lipids and lignin from GC/MS, and the log pH values of bulk soil and rhizosphere soil samples were analysed by evaluating the Pearson coefficient of correlation (r) and the significance of correlation ($p < 0.05$) in R 5.2-0 (RStudio, Hmisc package). Statistical analyses of carbohydrate, lignin, and lipid content in shoots, bulk soil, and rhizosphere soil were performed using R (RStudio, agricolae package) in order to mark the significance: the raw data were normally distributed in all variants (Shapiro–Wilk test, $p > 0.05$); the equality of variance was performed according to Levene test ($p > 0.05$); the significance was evaluated using one-way ANOVA analyses and the Tukey test ($p < 0.05$). Correlation graphs were calculated in R version 4.4.0 with package corrr 0.4.4.

3. Results

At the old marsh site, the bulk soil and the rhizosphere soil were slightly alkaline (pH 7.4–7.8) with the exception of rhizosphere soil at plot A (neutral, pH 6.6–7.3). At the young marsh site, the bulk soil was slightly alkaline (pH 7.4–7.8) while the rhizosphere soil

was moderately alkaline (pH 7.9–8.4) (Table 1). With regard to electrical conductivity at the old marsh site, the bulk soil was moderately saline (8 < EC < 16 mS cm^{-1}) and the rhizosphere soil was strongly saline (EC ≥ 16), with the exception of plot C (moderately saline, 8 < EC < 16). At the young marsh site, the bulk soil was strongly saline (EC ≥ 16) and the rhizosphere soil was moderately saline (8 < EC < 16).

Table 1. Physicochemical parameters of the bulk and rhizosphere soils (RS) at an old, mature salt marsh (OM) and a recently (2020) created salt marsh (YM) in France (mean values and standard deviations values are presented). Significant differences ($p < 0.05$, one-way ANOVA with Newman–Keuls post hoc comparisons) between sites (OM, YM)) are marked by different letters. The following parameters are included: C_{org}—organic carbon, N_t—total nitrogen, the C:N ratio, the calcium carbonate ($CaCO_3$) content, the P_{citr}—concentration, the pH values, the electroconductivity (EC), the chloride—(Cl^-), sulfate—(SO_4^{2-}), hydrogen carbonate—(HCO_3^-), bromide—(Br^-), calcium-, magnesium, sodium, and potassium concentrations.

	Old Marsh Soil	Old Marsh RS	Young Marsh Soil	Young Marsh RS
C_{org} (%)	1.8 ± 1.6 a	1.2 ± 2 a	2.8 ± 0.6 a	3.7 ± 0.2 a
N_t (%)	0.2 ± 0.1 ab	0.2 ± 0.1 b	0.4 ± 0 ab	0.4 ± 0 a
C:N	7.2 ± 3.1 a	4.3 ± 5.4 a	7.6 ± 0.7 a	8.5 ± 0.6 a
$CaCO_3$ (%)	6.9 ± 2.1 c	13.2 ± 3.8 c	27.5 ± 0.7 b	35.4 ± 2.8 a
P_{citr} (mg kg^{-1})	257 ± 72.8 b	147.4 ± 12.6 b	413.8 ± 79.5 a	285.8 ± 12.6 ab
pH	7.6 ± 0.1 ab	7.5 ± 0.3 ab	7.5 ± 0.1 b	8.1 ± 0.1 a
EC (mS cm^{-1})	10.4 ± 1.6 b	15.6 ± 6.3 ab	24.4 ± 2.3 a	14.1 ± 1.6 b
Cl^- (mg L^{-1})	2933.8 ± 488.8 b	4910.5 ± 2385.9 ab	7745.1 ± 1067.1 a	4435.5 ± 820.6 ab
SO_4^{2-} (mg L^{-1})	440.5 ± 74.1 b	638.6 ± 286.6 b	2409.4 ± 271.7 a	721.5 ± 116.3 b
HCO_3^- (mg L^{-1})	252.6 ± 58 b	462.6 ± 74.2 a	439.6 ± 42 a	427.4 ± 69 a
Br^- (mg L^{-1})	9.7 ± 2.5 b	18.5 ± 8.2 ab	22.9 ± 2.4 a	13 ± 2.2 ab
Ca (mg L^{-1})	63.8 ± 26.8 b	73.8 ± 33.5 b	547.7 ± 76.3 a	156 ± 20 b
Mg (mg L^{-1})	95.9 ± 36.6 b	131.2 ± 70.6 b	549.7 ± 61.6 a	233.9 ± 35.4 b
Na (mg L^{-1})	964.6 ± 351.1 b	1418.5 ± 681 b	3753.5 ± 359.7 a	1795.4 ± 293.8 b
K (mg L^{-1})	71.4 ± 65.6 a	74.9 ± 29.5 a	160.3 ± 29.2 a	154.8 ± 1.4 a

The soil texture at the OM site was loamy sand/clayey sand while that at the YM site was loamy silt/clayey silt (Table 2).

Table 2. Proportions of particle-size fractions (%) and classification of the bulk soil texture at an old, mature salt marsh (Old marsh) and a recently (2020) created salt marsh (Young marsh) in France.

Particle-Size Fraction	Old Marsh	Young Marsh
Clay	13.9	18.7
Fine silt	2.9	3.2
Medium silt	4.6	12.6
Coarse silt	17.9	41.9
Fine sand	60.7	23.6
Texture class	Loamy sand/clayey sand	Loamy silt/clayey silt

Statistical analyses of carbohydrate, lipid, and lignin content in shoots, bulk soil, and rhizosphere soil for the two sites (old marsh and young marsh) (Table S4) in France revealed no statistically significant differences in carbohydrate content across all sample types and locations (Figure 1a). However, lignin content analysis (Figure 1b) yielded significant differences ($p < 0.05$) between shoots and rhizosphere soil at both test sites. Notably, the rhizosphere soil consistently exhibited the highest lignin content, while shoots had markedly lower levels. Furthermore, a significant difference ($p < 0.05$) in lipid content was observed between bulk soil samples from the two test sites (Figure 1c). Specifically, the bulk soil at the young marsh site contained a higher concentration of lipids compared to the old marsh site.

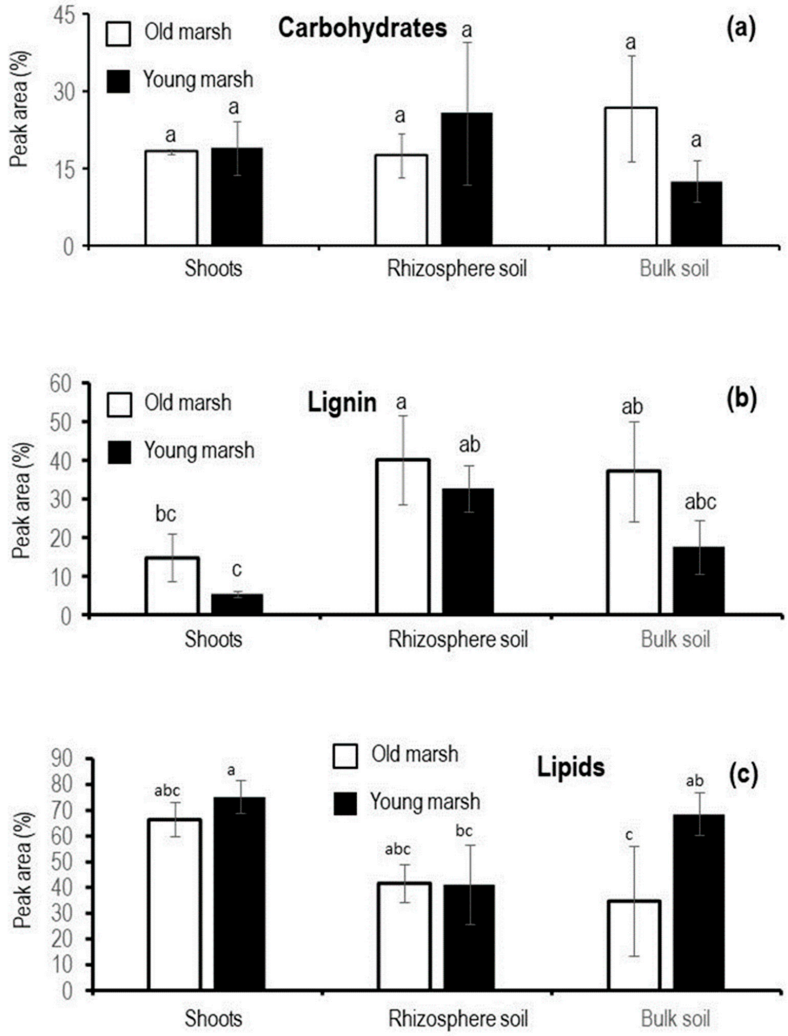


Figure 1. Relative abundance (% peak area) of (**a**) carbohydrates, (**b**) lignin, and (**c**) lipids in shoots, rhizosphere soil, and bulk soil as mean values with standard deviation at an old, mature salt marsh (Old marsh) and a recently (2020) created salt marsh (Young marsh) in France. Different letters indicate significance of differences between samples.

In shoots (Table S1), the abundance of *S. enterica* was negatively correlated with the content of lignin (p = 0.0130; r = −0.82) (Figure 2a), while the abundance of *E. coli* was positively correlated with the content of lipids (p = 0.0304; r = 0.75) (Figure 2b).

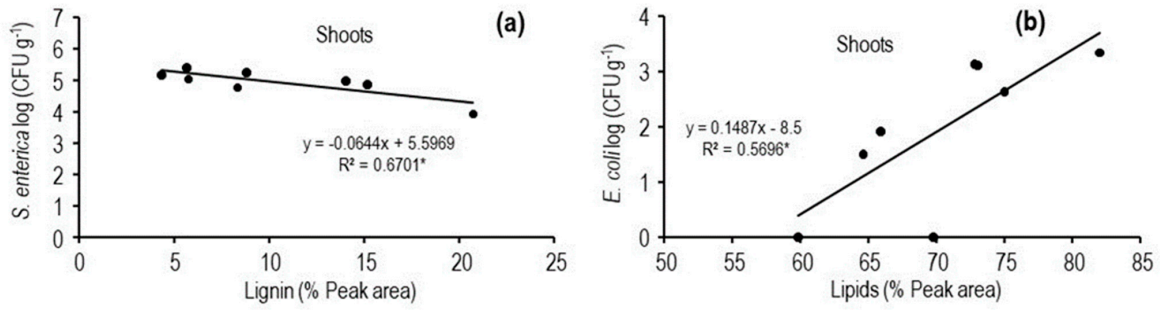


Figure 2. Correlation between (**a**) the log (CFU g^{-1}) of *S. enterica* and the lignin (% Peak area) content in shoots of *S. europaea*; and (**b**) between the log (CFU g^{-1}) of *E. coli* and the lipid (% Peak area) content in shoots of *S. europaea*. * means significance level of correlation coefficient is 0.05

In the data set from bulk soil samples (Table S3), lignin content (% peak area) was negatively correlated (p = 0.0494; r = −0.81) with the abundance of *E. coli* log (CFU g^{-1}) (Figure 3a). Furthermore, pH values and the abundance of *E. coli* (p = 0.0306; r = −0.85) (Figure 3b) and of *L. monocytogenes* (p = 0.0013; r = −0.97) (Figure 3c) and *S. enterica* (p = 0.0283; r = −0.86) (Figure 3e) were negatively correlated. In the rhizosphere soil (Table S2) we found a positive correlation (p = 0.0356; r = 0.84) between the colonisation density of *L. monocytogenes* and the pH (Figure 3d). The correlation network graph (Figure 3f) visualizes that there were strong and weak relationships between the data determined for the bulk and rhizosphere soil samples.

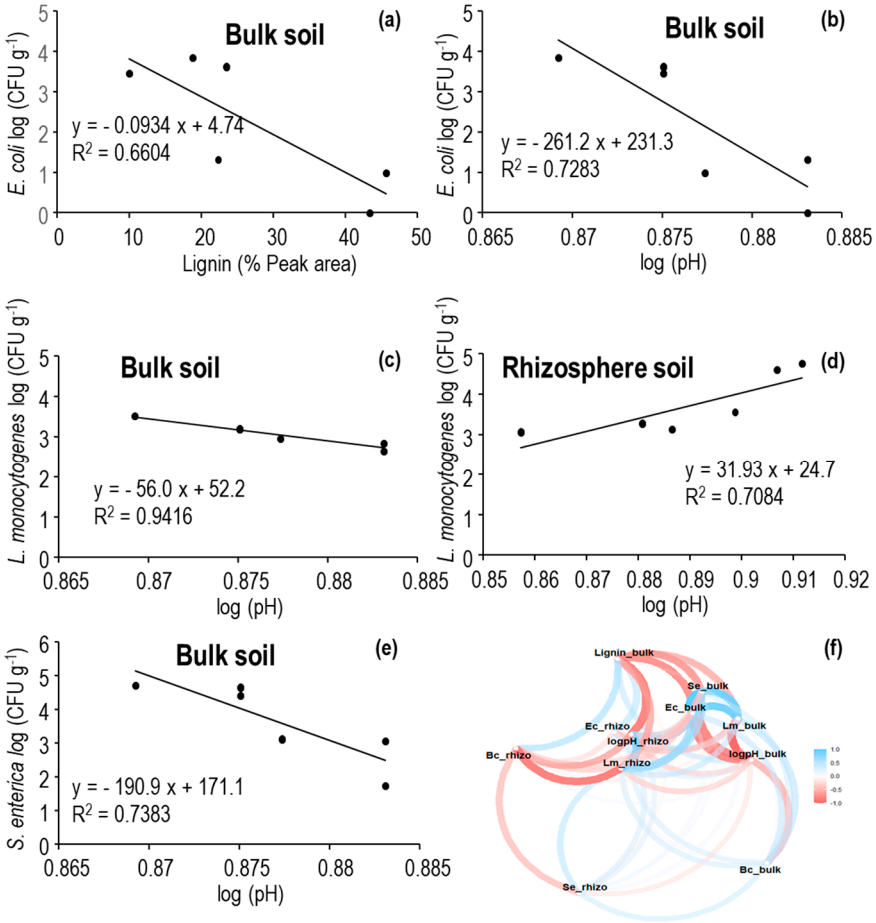


Figure 3. Correlation between the lignin content (% peak area) and the abundance of *E. coli* log (CFU g^{-1}) (**a**), between the pH and *E. coli* log (CFU g^{-1}) (**b**), between the pH value and *L. monocytogenes* log (CFU g^{-1}) (**c**), and between the pH value and *S. enterica* log (CFU g^{-1}) (**e**), and in the rhizosphere soil, between the colonisation density of *L. monocytogenes* log (CFU g^{-1}) and the pH (**d**), and visualization of interdependencies between these data in a correlation network graph (**f**).

4. Discussion

The abundance of HPMOs is affected by environmental conditions and the specific controls for defined bacterial species were investigated in the present study. The results of the present study focus on soil characteristics and microbial dynamics in old marsh and young marsh sites in France, as well as on their potential implications for HPMOs. According to our results (Figure 1), the lignin content found in shoots was lower than in the rhizosphere soil, suggesting both that *S. europaea* has a low lignin content and that the rhizosphere soil is a complex system where the activity of the plant roots plays a crucial role. This is confirmed by the same significant difference at both sites analysed in France. The low lignin content in *S. europaea* (Figure 1) confirms results reported by other studies where *S. europaea* was compared to other halophilic plants [29,30]. Until now, no studies

have shown the colonization of *S. europaea* by HPMOs. Furthermore, to the best of our knowledge, the relationships between the composition of plant and soil organic matter (SOM) and the abundance of HPMOs have not yet been investigated for *S. europaea*. The negative correlation between lignin content and the abundance of *S. enterica* in shoots of *S. europaea* and between the abundance of *E. coli* and lignin content in bulk soil suggest that lignin or associated compounds may have antimicrobial properties against these pathogens. Indeed, polyphenols in lignin can damage bacterial cell walls, causing lysis and leakage of internal fluids [31]. While lignin's role in plant defence against plant pathogens is documented as explained in detail below, the impact of lignin on HPMOs within plant tissues has still not been tested. Lignin has been reported to confer resistance to both abiotic and biotic stress [32]. Among these, the growth of plant pathogens was inhibited in plants with high lignin presence [33]. Interestingly, the attack of plant pathogens stimulates the plants to produce more lignin to reinforce their cell walls and block the traffic of the plant pathogens [12,34]. In halophilic plants like *S. europaea*, lignin plays a crucial role in strengthening the cell wall in response to salt stress [35,36]. It has been demonstrated that *Bacillus subtilis*, under specific saline conditions, increased the quantity of lignin in common bean roots, strengthening the cell walls, which was connected to a better plant growth and reduction of oxidative damages [37]. The transcriptomic profile of *Eutrema salsugineum*, another halophilic plant, revealed that salt stress led to the over-expression of lignin-biosynthesis genes, suggesting that enhanced lignin accumulation could have an active role in response to a salt environment [38]. Quantitative trait loci (QTLs) analyses revealed that crown rust resistance mapped onto LG3 and LG7 loci; coincidentally, those mapped genes were connected to cell wall development, including lignin biosynthesis [39]. Interestingly, the importance of lignin is not only linked with stronger immune systems for plants, but also with healthy diet for humans, since lignin derivatives can reduce blood cholesterol, obesity, and diabetes [40]. Moreover, we found a positive correlation between the lipids content and abundance of *E. coli* in the plant material (Figure 3). We assume that the majority of lipids originate from the plant; however, a potential contribution from the bacterial biomass to the total lipid content cannot be ruled out. The above correlation might be based on adequate changes in the plant membrane composition and in its fluidity [15]. Lipids, including phospholipids and sphingolipids, are not only involved in plant development and membrane fluidity, but can also confer salt tolerance in *S. europaea* [41]. One particular lipid molecule—phosphatidylserine—protects cells from biotic and abiotic stress, including pathogens and salinity, respectively [42,43]. Cuticular lipids serve as messenger molecules during pathogen attack [44,45]. A significant contribution of plant bacteria to total lipid content has been reported [46]. Human pathogenic bacteria, such as *E. coli*, *Salmonella* spp., and *L. monocytogenes*, have demonstrated remarkable adaptability in colonizing plant surfaces and, in some cases, internalizing within plant tissues [47]. The presence of these pathogens in fresh produce has been a persistent concern for public health, leading to numerous foodborne illness outbreaks worldwide. However, to the best of our knowledge, no studies have shown if and how the above bacteria interact with *S. europaea*. Understanding the factors that generally influence the abundance of these bacteria in plants is crucial for developing effective strategies to enhance food safety. Moreover, saline soils are less likely to be colonized by HPMOs, as salt has been previously identified as an inhibitory factor for these pathogenic bacteria [48–50]. Increased NaCl concentrations typically inhibit the growth of *E. coli* and its virulence-related traits, including biofilm formation, oxidative resistance, and motility [48]. A 5% NaCl concentration significantly decreased the growth of *S. enterica*, whereas the lowest concentration that affected the growth of *L. monocytogenes* was 7% NaCl [49,50]. However, we must also consider situations where HPMOs might promote plant growth, e.g., by improving the nitrogen nutrition of plants [51]. It is possible that the presence of HPMOs will increase as changes in global climate raise the temperature of soils close to body temperature, which is the optimal temperature for HMPO growth [51]. Thus, in summary, the relationships between plant and soil chemistry and their microbial colonization are fundamental controls

for food quality and will become even more important due to global climate change. The ongoing challenge will be to divide causal and successive changes in chemical plant traits in response to colonization by HPMOs in subsequent inoculation experiments.

5. Conclusions

The new insights into the relationships between the molecular-chemical composition of *S. europaea* L. shoots and soils and the abundance of HPMOs confirm that the methodological approach using selective media and mass spectrometric analyses of plant and soil materials was suitable and can be recommended in forthcoming studies. Our findings suggest that plants' chemical composition and environmental factors play crucial roles in controlling HPMO populations, with potential implications for food safety. the key findings from our research are as follows: (i) lignin content indicates a protective role of lignin against some HPMOs; (ii) the negative correlation of *E. coli* abundance with lignin content in bulk soil indicates a possible direct suppressing effect of soil organic matter composition, as bacteria cannot use lignin as a carbon source; (iii) the positive correlation of lipid content in shoots with the abundance of *E. coli* points to the use of lipids as a carbon source due to their lipolytic enzymes; (iv) the higher soil pH values and salinity associated with lower contamination levels by *E. coli*, *S. enterica*, and *L. monocytogenes* in bulk soil underscore the potential of these fundamental soil parameters as simple natural tools for enhancing food safety in *S. europaea* cultivation. Further research is recommended to explore the mechanisms behind these correlations and to investigate potential applications in agricultural practices. This knowledge could lead to improved cultivation methods and risk assessment strategies for *S. europaea* and potentially other halophilic crops, contributing to safer food production in saline environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112740/s1, Table S1: $\log_{10}$ CFU g^{-1} of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Bacillus cereus* with % peak area of carbohydrate, lignin, and lipid content in shoots of *S. europaea*; Table S2: $\log_{10}$ CFU g^{-1} of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Bacillus cereus* with % peak area of carbohydrate, lignin, and lipid content and log pH in rhizosphere soil; Table S3: $\log_{10}$ CFU g^{-1} of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Bacillus cereus* with % peak area of carbohydrate, lignin and lipid content and log pH in bulk soil; Table S4: Carbohydrate, lignin, and lipid content at two sites in France.

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