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Research article

Characterization of nutrients and contaminants in fish sludge from Atlantic salmon (*Salmo salar* L.) production sites - A future resource

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ABSTRACT

A total of 47 fish sludge samples from commercial land-based Atlantic salmon (*Salmo salar*) farms in Norway were assessed for their nutrient composition, presence of various legacy contaminants and a wide spectrum of contaminants of emerging concern, veterinary medicines as well as selected salmonid pathogenic bacteria and virus. The aim was to document the levels of desirable and undesirable components in fish sludge in relation to a potential future use of sludge as invertebrate feed. The samples had variable, but relatively high protein and fat contents, indicating a high load of undigested feed in some of the sludge samples. Fatty acid analysis showed the presence of essential omega-3 fatty acids. In terms of undesirable substances, 43% and 84% of the sludge samples contained levels of arsenic and cadmium, respectively, which exceeded the EU Maximum Levels established for complete animal feed. The concentrations of copper, zinc, iron and aluminum were highly variable in the sludge samples. The concentrations of dioxins, sum PCB6, and chlorinated pesticides were all below the Maximum Levels for animal feed. Of the 18 per- and polyfluoroalkyl substances (PFAS) only one compound (L-PFOS) was present at measurable levels. None of the samples had detectable levels of veterinary medicines, salmonid virus or bacteria. Performing a suspect and non-target screening of the sludge samples identified 18 compounds, including four pharmaceuticals, plastic-related products and the UV filter benzophenone, warranting further investigations. Overall, the results from this study show that fish sludge is a nutrient-rich resource; however, undesirable substances, originating from the feed or from treatment of sludge may be present.

1. Introduction

Norway is the world's largest producer of Atlantic salmon (*Salmo salar* L.) with a production of 1.5 million tonnes in 2020 (FAO, 2022), requiring around 2 million tonnes of fish feed (Aas et al., 2022). Large amounts of feed end up as uneaten feed (spillage) or as respiratory- or excretory products, and ultimately end up as waste in farming practices (Wang et al., 2012). With global and national aims for more sustainable, circular food production systems and reduced food losses (Fetting, 2020), there is an increasing number of initiatives investigating fish sludge as an untapped resource, i.e. its application as a fertilizer (Brod et al., 2017, 2023) or as feed for invertebrates (Schmitt et al., 2019; Wang et al., 2019; Anglade et al., 2023; Liland et al., 2023; Rossi et al., 2023).

Fish sludge consists of excrements and feed residues and does not include fish that has died during farming which ends up as fish silage. Only a small fraction of fish sludge is collected today, most of which originates from land-based farms. Land-based systems include both recirculing aquaculture systems (RAS) and flow-through aquaculture systems (FTAS) where the effluent water is filtered before discharge. The collected sludge is often destined for regular waste treatment, e.g. combustion, digested with other effluents for bio-gas or into fertilizer products, which are mostly exported to Asia (Gebauer, 2004, Sawatdeenarunat et al., 2019). The treatment technologies for fish sludge are mainly designed to lower costs and reduce odour and include de-watering and drying processes (Brod et al., 2023). Fish sludge is

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potentially a large source of organic material, with an estimate of more than 500,000 tonnes (dry matter; DM) generated annually by the Norwegian salmon industry alone, with the greatest potential volumes for retrieval lying in collection of sludge from grow-out net-pens at sea (Aas and Åsgard, 2017). Fish sludge is a material high in energy, often containing high amounts of feed spillage (up to 50%) (Ytrestoyl et al., 2016). Feed spillage (or feed waste) is referring to the loss of fish feed pellets or formulated feed, not eaten by the fish (Føre et al., 2023). Since a certain degree of feed spillage is likely in aquaculture, fish sludge is considered a valuable source of protein, fat, including marine omega-3 long-chain (C20-24) polyunsaturated fatty acids (LC-PUFA) and minerals (Ytrestoyl et al., 2016; Liland et al., 2023; Malzahn et al., 2023a). It has been shown that it is possible to use fish sludge in the feed cycle as fertilizer in aquaponics (Zhanga et al., 2021), or as feed for protein-rich low-trophic organisms such as insect larvae (Schmitt et al., 2019; Liland et al., 2023) or polychaetes (Anglade et al., 2023; Malzahn et al., 2023a). Finding ways to upcycle, rather than down-cycle, this resource will be crucial for the development of more feasible circular solutions for fish sludge.

With the establishment of the Green Deal, the European Commission (EC) is aiming for a green transition toward 2030. An important part of the proposed way forward is to reuse by-products from food production. However, when by-products are reused, food safety hazards can accumulate or recirculate in the food chain (Lopes et al., 2011; van Asselt et al., 2023). Previous studies have shown that non-essential elements originating from the feed, i.e. arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and the essential elements zinc (Zn), cupper (Cu) and iron (Fe), can be present in highly variable concentrations in fish sludge (Salazar and Saldana, 2007; Ytrestoyl et al., 2016; Liland et al., 2023, Pettersen., submitted). Although organic contaminants can be considered a non-exhaustive list of compounds with a range of chlorinated, brominated, and fluorinated compounds, there is to our knowledge only a few studies in the scientific literature which present data on organic legacy pollutants such as dioxins, PCBs and brominated flame retardants (PBDEs) in fish sludge (Hellou et al., 2005; Brod et al., 2023). Further, for the identification and detection of unknown and emerging contaminants that may overlooked with the targeted methods, suspect and non-targeted screening methods (NTS) are used (Hollender et al., 2023). Unlike conventional targeted methods that rely on the availability of reference standards of the targeted contaminants, NTS enables the screening of several hundreds to thousands of chemicals based on accurate mass without the need for reference standards. To our knowledge, NTS of organic contaminants in fish sludge has not yet been conducted. Furthermore, the risk of transferring microbial hazards to humans when using sludge from hatcheries or marine aquaculture farms as agricultural fertilizers was assessed to be low in a risk assessment performed by the Norwegian Scientific Committee for Food and Environment (VKM, 2011). However, it was emphasized that spread of diseases from fish to other fish producing facilities cannot be ruled out if sludge is used as fertilizers. Few data on the occurrence of veterinary drugs and microbial hazards in fish sludge from land-based production facilities are available in the literature (Pettersen submitted), and hence, little is known about the possible risks involved in re-introducing fish sludge into food production chains.

In the EU, the EC has set regulations for ensuring safe food, from farm to fork, with actions concentrated around the four main areas of food hygiene, animal health, plant health, and contaminants and residues, aiming to protect consumers, farmed animals and the environment (EC, 2020). Maximum levels (MLs) have been established for certain undesirable substances, including heavy metals (Cd, Pb and Hg) and As, dioxins, PCBs, and chlorinated pesticides in animal feed and feed materials (EC, 2002/32 and amendments) (EC, 2002). For organic fertilizers, the products should not lead to exceedances of legal limits in the final product (EU No, 2019/1009) (EU, 2019), and MLs have been established for metals (i.e. Cd, Cu, nickel (Ni), Pb, Zn, Hg, and chromium (Cr)) in soil, and sewage sludge (86/278/EEC) (EC, 1986). The use of animal by-products in the feed- or food production chain is strictly regulated within the EU (EC 1069/2009) (EC, 2009) and utilizing fish sludge as feed or fertilizers is currently not permitted. Any future changes to the legislation would require a risk assessment of the safety of using fish sludge in a circular production. This will require data on the occurrence of both chemical and biological hazards plausibly found in fish sludge, their fate throughout the value chain, as well as documentation on the quality and safety of the final product.

The aim of this study was to examine fish sludge from a range of commercial facilities for salmonid production in Norway to acquire representative data on nutrient composition, presence of inorganic and organic contaminants (target and non-target contaminants), veterinary medicines, and presence of bacteria and viruses. By generating this data, we aim to diminish the knowledge gap on fish sludge composition, and to generate data that ultimately enables an evaluation of the safety of utilizing this resource as an integrated part of a circular bioeconomy.

2. Material and method

2.1. Samples

Commercial aquaculture companies in Norway were contacted and asked to participate in this study aiming to get a better picture of the composition of Norwegian salmonids aquaculture sludge. It was asked for sludge samples routinely collected on-site, including samples of both partially de-watered (2–40% DM) and dried sludge, when applicable. In total, 30 positive responses, and 47 samples of fish sludge were received. The samples were collected in 2022 and 2023 from different regions in Norway, as presented in Tables S–1. The participation from the industry actors was voluntary, and no monetary compensation was given to the suppliers of the samples. The suppliers of sludge were Atlantic salmon smolt or post-smolt producers using either RAS or FTAS (see Supplementary, Tables S–1 for an overview). The sludge samples were collected by employees from each aquaculture facility, frozen or cooled, stored in airtight containers, and sent to the Institute of Marine Research (IMR), Bergen. Meta-data collected for each sample included information on the life stage of the fish, the salinity and temperature of water in the production system at the time of sampling, feed type used and if any outbreak of diseases had occurred during the past three months (Tables S–1). Around 1–2 kg of wet and/or dried sludge was received from each facility. Aliquots of the sludge and freeze dried for the different analyses, except for the fat, bacteria and virus analyses where the content was determined on samples as they were received (wet or dried). All analytical methods were performed at accredited laboratories, in accordance with ISO-17025 by the Norwegian accreditation authorities.

2.2. Methods

Methods applied in this work are described in supplementary file, S-Methods.

2.3. Statistical analysis and data treatment

The amount of feed spillage contained in the different sludge samples was determined by following equation, described by Ytrestoyl et al. (2016)

$$\textit{Feed spillage}\ (\%) = 9.2x - 136$$

where x = energy content in kJ/g.

Descriptive statistics and a multiple *t*-test was conducted using GraphPad prism (version 8.1.1).

The correlations between minerals and variables such as ash and crude protein, were performed in Excel Microsoft 365 using the Pearson correlation coefficient. Pearson correlation coefficient (r) calculates the

Table 1

The mean concentrations with standard deviation, range (minimum to maximum) and 25–75% percentile, for dry matter (g/100g dw), crude protein (g/100g dw), crude lipid (g/100g dw), ash (g/100g dw) and energy content (kJ/g dw) in fish sludge samples. The estimated contribution of feed spillage in the samples is expressed as percentage (%). Concentrations in fish sludge are compared with levels in commercial fish feed[b].

	Dry matter ($n = 47$)	Dry matter of dried samples ($n = 14$)	Crude protein (N x 6.25) ($n = 47$)	Crude lipid ($n = 47$)	Ash ($n = 47$)	Energy ($n = 45$)	Estimated feed spillage (%) [a] ($n = 43$)
Mean ± SD	51.5 ± 40.2	94.7 ± 2.6	28.4 ± 10.1	10.2 ± 5.8	20.2 ± 7.2	18.0 ± 2.0	31 ± 18
Range (min – Max)	1.6–98.0	89.5–98.4	4.7–57.0	0.5–28.8	3.9–42.2	14.0–22.4	1–70
25–75% percentile	5.9–88.8	93.2–96.5	22.0–32.9	5.9–14.0	15.7–22.1	16.4–19.2	17–41
Commercial fish feed [b]	91–96	–	33–51	16–37			

[a] The feed spillage is estimated using the procedure defined by Ytrestoyl et al. (2016).

[b] Data from the Norwegian monitoring program for fish feed, samples collected and analysed in 2022 ($n = 80$) (Sele et al., 2023).

strength and direction of the relationship between two variables, regardless of the units in which they have been measured. A value close to ±1 indicates a strong positive or negative correlation and a value close to zero indicates no correlation. Statgraphics Centurion XV Version 15.2.11 (StatPoint Technologies, Inc., Warrenton, VA, USA) was used for the analysis of principal components (PCA). The PCA method converts a set of possibly correlated variables into a set of linearly uncorrelated ones through an orthogonal transformation. The resulting principal components (PCs) are linear combinations of the original set of variables that have been decomposed into two matrices called loadings and scores which represent the weight of the original variables and samples, respectively. The PCA method uses the varimax rotation algorithm which maximizes high- and low-value factor loadings, and minimizes mid-value factor loadings.

For PCA, the sum of elements and contaminants (sum dioxins + dl-PCBs, sum PCB6 and sum PBDE7 were used (Supplementary data, Tables S–3B), with a sample:variable ratio (33:11) higher than 2, and acceptable for exploratory analysis. Contaminants which were included in the PCA were those which had results for more than 30 samples. For PCA, the elements were presented as non-essential elements (sum of As, Cd, Hg, Pb and Ni) and as essential elements 1 (the elements were most <10 mg/kg dw; sum of Cr, Co, Mo, Se and V) and essential elements 2 (the elements >10 mg/kg dw; sum of Cu, Fe, Mn and Zn).

3. Results and discussion

In this study fish sludge samples from salmonid production were collected and found to contain valuable nutrients such as essential amino acids and fatty acids which can be efficiently upcycled by extractive species; however, they also contained non-essential elements and other contaminants.

3.1. Nutrient composition of fish sludge

Both wet and dried sludge samples were retrieved, and the DM content varied accordingly from 1.6 to 98.4 g/100g (Table 1). The sludge samples which had not been dried, only de-watered, had a dry matter content between 1.98 and 37.4 g/100g ($n = 28$). For the dried sludge, the DM content varied from 89.5 to 98.4 g/100g ($n = 14$). There are several different technologies available on the Norwegian market for drying fish sludge, using either high (>100 °C) or low temperatures (40–50 °C). Some of the samples were dried using existing systems for low temperature drying, such as customized drum filters and belt dryers, but limited information was provided on the exact temperatures used for drying (Supplementary data, Tables S–1). There is currently limited knowledge on how drying techniques affect the nutrients in the sludge, and the need for systematic studies on how sludge processing affects the nutrient composition have been emphasized by others (Liland et al., 2023). Processing methods are also expected to influence the profile of both organic and inorganic contaminants in the sludge.

The concentrations of crude protein (CP) and crude lipid (CL) ranged from 4.7 to 57.0 g/100g dw and from 0.5 to 28.8 g/100g dw, respectively, and there was a weak correlation between the CP and CL content of the sludge samples ($r = 0.4224$, $P < 0.05$; $n = 45$; Supplementary, Figs. S–1A). Earlier studies have shown that salmon fish sludge contained CP levels from 15 to 38 g/100g dw, and CL levels from 14 to 38.5 g/100g dw (Schmitt et al., 2019; Anglade et al., 2023; Liland et al., 2023; Rossi et al., 2023). Thus, the mean CP and CL in the current study lie within concentrations reported earlier for fish sludge, although a larger variation was found in this study, likely reflecting a higher number of samples taken from different production facilities. In comparison, commercial feeds for salmonids contain CP from 32 to 59 g/100g dw and CL from 13 to 39 g/100g dw ($n = 82$) (Sele et al., 2023), hence some of the sludge samples have similar content in CP and CL to salmon feed. Similarly, also for the energy content, which is usually around 20 kJ/g in salmonid diets and with a minimal requirement of 18.8 kJ/g (NRC, 2011), some of the sludge samples in this study are within the same range in energy (Table 1). However, it is apparent that there are large variations in sludge characteristics, likely because of samples being retrieved from different aquaculture systems, different processing techniques or different feeds being used at the different fish farms.

The ash content ranged from 3.9 to 42.2 g/100g dw, with a mean ash content at 20 g/100g dw. These levels are close to what is seen in other studies of sludge from salmonids, ranging from 4.8 to 27 g/100g (Schmitt et al., 2019; Anglade et al., 2023; Liland et al., 2023). No correlation was apparent for the ash content with CP ($r = 0.1648$, $p = 0.27$, $n = 47$) or CL ($r = 0.01971$, $p = 0.89$, $n = 45$) (Supplementary data, Figs. S–1). This may be explained by ash representing the inorganic fraction, which may originate both from non-digestible minerals and metals in the feces and in the feed spillage, whereas the CP and CL will mainly originate from the feed spillage alone.

The range in CP, CL and energy content are likely linked to amount of organic matter and feed spillage in the sludge. In the current study, the mean estimated feed spillage in the sludge was 30% when using the approach for calculation described by Ytrestøyl and colleagues (2016). This was lower than the estimated average 50% feed spillage, found in the study by Ytrestoyl et al. (2016). At the same time, some samples in this study contained very low and very high feed spillage (from 1 to 80%), reflecting large variations. The variations in feed spillage may reflect different production systems, and different operational procedures, which have been seen to also vary over time within individual production facilities (Ytrestoyl et al., 2016).

3.1.1. Fatty acid and amino acid profiles

The mean level of the essential FA eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) of fish sludge ($n = 19$) were 1.6 and 1.9 mg/g DM, respectively (Table 2). The concentrations

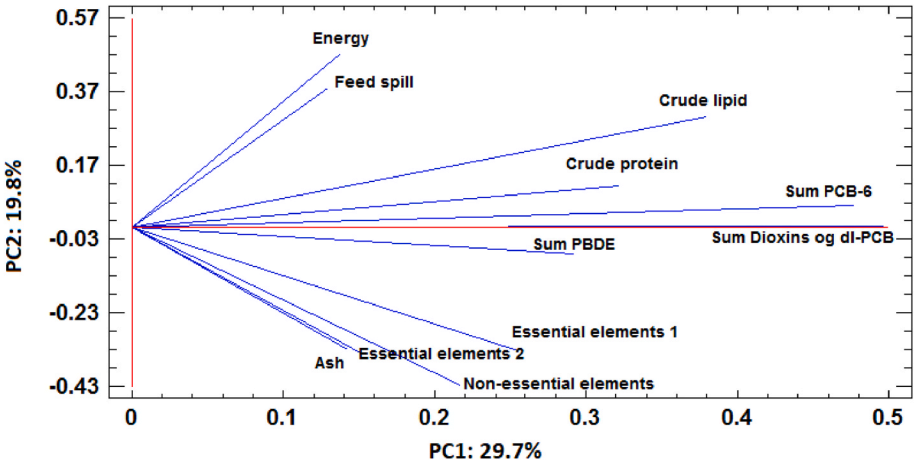

Fig. 1. Principal component analysis to establish correlations between the variable's crude protein (CP), crude lipid (CL), ash, energy, feed spill, non-essential elements (sum of As, Cd, Hg, Pb and Ni), essential elements-1 (sum of Cr, Co, Mo, Se and V), essential elements-2 (sum of Cu, Fe, Mn and Zn), sum dioxins + dl-PCB, sum PCB-6 and sum PBDE7.

Table 2
Fatty acid and amino acid concentrations in fish sludge (mg/g dw, n = 18), mean with standard deviation, and range (minimum to maximum). Concentrations in fish sludge are compared with levels in commercial fish feed[a].

	Fish sludge		Commercial fish feed[a]
	Mean ± SD	Min – Max	Min – Max
Fatty acids			
16:0	10 ± 5.5	0.9–18	–
18:1n-9	18 ± 18	0.6–62	19–114
22:1n-9 (erucic acid)	0.3 ± 0.2	0.0–0.7	0.5–2.9
18:2n-6	18.2 ± 7.5	0.4–24	5.2–37
20:4n-6 (arachidonic acid)	0.3 ± 0.2	0.0–0.8	0.6–1.6
20:5n-3 (EPA)	1.6 ± 1.8	0.0–6.1	8.4–20.0
22:6n-3 (DHA)	1.9 ± 1.9	0.1–7.3	9.7–23.8
Sum SFA	64.5 ± 46	4.3–154	16.4–25.3
Sum MUFA	18.7 ± 10	2.6–36	40–53
Sum PUFA	28 ± 24	1.0–83	28–33
Sum n-6	9.0 ± 7.7	0.2–20.4	7.1–38.4
Sum n-3	6.7 ± 6.5	1.0–83	33.5–62.7
Ratio n-3/n-6	0.8 ± 0.4	0.2–2.0	1.1–8.9
Essential amino acids			
Arginine	10.8 ± 7.1	1.6–25.4	25–29
Histidine	4.0 ± 2.7	0.7–9.8	8–13
Isoleucine	9.0 ± 4.9	1.4–18.7	15–17
Leucine	16.5 ± 10.0	2.7–35	28–31
Methionine	4.0 ± 2.5	0.8–9.6	11–12
Phenylalanine	10.2 ± 5.8	1.4–20.4	17–20
Threonine	8.8 ± 4.5	1.5–17.3	16–17
Valine	11.0 ± 5.8	1.6–21.2	18–19
Non-essential amino acids			
Alanine	12.6 ± 6.4	2.0–22.2	19–20
Aspartic acid	19.0 ± 10.6	2.8–40	39–40
Glutamic acid	28.9 ± 20.8	3.6–70	72–80
Glycine	11.4 ± 5.1	2.4–20.5	20–22
Proline	10.1 ± 6.2	1.7–21.6	20–24
Serine	9.6 ± 5.4	1.6–19	19–21
Tyrosine	6.7 ± 4.0	1.2–13.8	13–14
Hydroxyproline	61.6 ± 0.4	1.1–2.3	1–2
Calculated True Protein	15.7 ± 8.8	2.4–32.8	–

[a] Data from the national monitoring program for fish feed, data for smolt feeds analysed for fatty acids (n = 9) and for amino acids (n = 5), samples collected and analysed in 2022 (Sele et al., 2023).

were in a similar range to values previously reported for salmon sludge in previous studies (Liland et al., 2023). Ytrestoyl et al. (2016) reported concentrations of EPA and DHA of 6.8–12.2 and 8.3–10.3 mg/g, respectively, whereas treatment of fish sludge decreased the content of these omega-3 FAs by 30–70% compared to raw fish sludge. In the current study, the sum of saturated fatty acids (SFAs) was higher (64.5 mg/g) in fish sludge compared to the levels generally found in commercial salmonid feed (16.4–25.3 mg/g; Sele et al., 2023), while the levels of sum of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were lower than those found in commercial fish feed (Table 2). These results are as expected since it has been shown that unsaturated fatty acids are more easily digested and absorbed than SFAs in salmon (Sargent et al., 1999) and as a result, a relatively higher content of SFAs can be expected in the feces. The mean amino acid content of fish sludge (n = 18) was found to be lower than the minimum value reported in commercial fish feed (e.g., arginine in fish sludge was 2.5 times lower than the level typically present in fish feed, Table 2). These results may reflect the higher apparent digestibility of AA, observed in healthy Atlantic salmon (75–95 %, Guillaume et al. (2001)). Overall, the nutrient composition varied considerably among fish sludge samples, probably due to the different feed composition, feed spillage and production practices employed by the farmers.

3.1.2. Essential elements (macro- and microminerals)

Macro- and microminerals are essential for animals, including farmed Atlantic salmon (NRC, 2011), and can be added to animal feed to meet their nutritional requirements. In this study, the concentrations of the macrominerals phosphorous (P), calcium (Ca), magnesium (Mg) and potassium (K) (Table 3) were comparable with those reported in previous studies of sludge from Atlantic salmon (Brod et al., 2017; Schmitt et al., 2019; Anglade et al., 2023). Although few samples were analysed (n = 10), a clear correlation was seen for K and Mg with the ash content of the sludge samples (Supplementary data, Figs. S–2), whereas no correlation was apparent for Ca (r = −0.1729, p = 0.63, n = 10) and P (r = −0.1975, p = 0.58, n = 10). In a study by Brod et al. (2023), fish sludge was shown to have an unbalanced nutrient composition with low nitrogen (N) to P ratio and a low level of K when compared to crop requirements. In the same study, fish sludge that was treated by anaerobic digestion had an equally good N fertilisation effect as mineral N fertiliser (Brod et al., 2023). There is currently focus on the potential use of fish sludge as organic fertilizers, and on techniques for recovering essential elements, such as P, for further use (Brod et al., 2017, 2023). The level of sodium (Na) in fish sludge is likely to reflect whether the sludge is from a freshwater or saltwater-systems, and the sludge samples we analysed which had the highest levels of Na (at 48 and 110 g/kg) was confirmed by the metadata to be from farms that used brackish water (Supplementary, Tables S–1).

For the essential microminerals Co, Se, V, Mo, Cu, Mn, Zn and Fe, large ranges in concentrations were found in fish sludge (Table 3). These elements are essential for animals, including Atlantic salmon, and are

Table 3
Concentrations of the macroelements (g/kg dw, n = 10) and microelements (mg/kg dw, n = 47) in fish sludge, mean concentration with standard deviation, range (minimum to maximum), and the 25–75% percentiles. The EU limits for maximum content in animal feed, under the regulation for feed additives (EC, 1831/2003) and the concentrations reported in commercial fish feed for salmonids are given.

Elements	Mean ± SD	Range (min – max)	25–75% percentile	*EU maximum content -feed additives (mg/kg)* [a]	*Commercial fish feed* [c]
Macro-elements					
Phosphorous (P)	24.7 ± 6.9	12.0–34.0	19.5–30.5	–	10–27
Calcium (Ca)	41.5 ± 12.2	24.0–59.0	31.3–55.5	–	5–25
Magnesium (Mg)	7.0 ± 4.8	3.0–19.0	4.0–8.1	–	1.9–2.9
Potassium (K)	1.8 ± 1.2	0.7–4.8	1.1–2.1	–	7.2–12
Sodium (Na)	20.3 ± 34.4	2.1–110	3.1–21.0	–	2.5–25
Micro-elements					
Cobalt (Co)	0.81 ± 0.52	0.20–2.5	0.45–1.1	1.0	0.07–0.38
Selenium (Se)	1.6 ± 0.6	0.27–3.2	1.2–1.9	0.5	0.32–2.4
Vanadium (V)	3.3 ± 2.2	0.98–10.0	1.7–4.0	–	–
Molybdenum (Mo)	3.0 ± 1.8	1.0–13.0	2.1–3.5	2.5	0.35–3.2
Chromium (Cr)	6.5 ± 11.5	1.0–82	3.0–6.3	–	0.19–11
Copper (Cu)	28 ± 59	10.0–420	15–25	25	5.7–23
Manganese (Mn)	212 ± 129	44–750	130–260	100	19–116
Zinc (Zn)	478 ± 155	200–1100	410–570	180 [b]	110–340
Iron (Fe)	1006 ± 638	280–2500	470 - 1400	750	52–830

[a] Maximum limit set for additives for feed in EU, found in the European Union Register for Feed Additives.

[b] Maximum limit for zinc in feed for all fish species other than salmonids is 150 mg/kg.

[c] Data from the Norwegian monitoring program for fish feed, samples collected and analysed in 2022 (Sele et al., 2023). Results for the macro elements represent smolt feeds for salmonids (n = 26), whereas the data for micro elements represents all feeds for salmonids (starter, smolt and grower feeds, n = 80).

important for e.g. antioxidant activity, wound healing and the immune system (Lall and Kaushik, 2021). The concentrations of Co, Se, V and Mo were within the same levels typically found in commercial salmonid feeds (Sele et al., 2023) (Table 3). The minerals originate naturally from feed materials, e.g. fish meal is known to be a rich source for Se and Zn (Prabhu et al., 2018; Sele et al., 2023) and for some minerals, supplementation is common practice to meet fish's requirements. In the EU, mineral supplementation is regulated under the EU feed additive regulation (Regulation (EC) No 1831/2003), which sets limits for the maximum content of additives permitted in animal feeds. In fish feed, the levels of Se, Mo and Zn have been reported to exceed the maximum limits set for feed additives in animal feed (Prabhu et al., 2020; Sele et al., 2023). Studies have also shown that the dietary Se required to maintain improved health status of farmed Atlantic salmon fed plant-based diets exceed the regulatory limits (Prabhu et al., 2020). Several of the fish sludge samples exceeded these limits (Table 3).

The mineral levels were not correlated with the ash content, using Pearson correlation (Supplementary data, Tables S–3). However, when normalizing the mineral concentration with ash content, considering that ash will mainly contain inorganic components (i.e. elements), negative correlations were found for Zn (−66%), Se (−66%), Mo (−55%), Co (−55%) (Supplemented data, Tables S–4), suggesting lowered concentrations of Zn, Se, Mo and Co when the ash content is higher. Correlations were also observed between elements, i.e. between Cu and Cr (93%), and Zn and Mn (82%). These correlations may reflect the source of the sludge, or chemical forms of the elements. The chemical forms of the minerals will affect the bioavailability hence uptake, for example the organic form of Se, selenomethionine or Se-yest, is more bioavailable than inorganic chemical forms in rainbow trout (*Oncorhynchus mykiss*) (Fontagne-Dicharry et al., 2015). Several of the micro minerals, i.e. Fe, Cu, Mn, Se and Zn are supplemented in fish feeds, and the uptake and bioavailability of the elements varies, depending on source, chemical forms, and other dietary components (Prabhu et al., 2018; Silva et al., 2019). Particularly high concentrations of Cu, Mn, Zn and Fe were found in some fish sludge samples, reaching 420 mg Cu/kg dw, 750 mg Mn/kg dw, 1100 mg Zn/kg dw and 2500 mg Fe/kg dw. These levels are higher than concentrations generally seen in commercial fish feed for salmonids (Table 3) and may be explained by low bioavailability of these elements, but it may also indicate that there are other sources for the elements, e.g. through sludge processing procedures. It has been documented that Cu and Zn tend to accumulate in the sediments at aquaculture production sites, and are of environmental concerns (Dean et al., 2007). Although wide concentration ranges observed in fish sludge in the present study, the mean Cu concentrations in fish sludge is comparable with the range of Cu levels in cattle and chicken manure (Zhang et al., 2011). Fe can originate from feed spillage and from the use of flocculants in the processing of sludge. The sludge samples with the highest concentrations of Fe in this study did use flocculants of polymers.

3.2. Non-essential elements (metals and metalloids)

All sludge samples (n = 47) were analysed for the content of the metals Cd, Hg, Pb and the metalloid As (Table 5), which are non-essential elements, and can cause environmental problems and health implications due to their toxic properties. The concentrations of Cd, Hg, Pb and As in the sludge samples are similar to those reported for fish in previous studies (Schmitt et al., 2019; Brod et al., 2023; Liland et al., 2023). No clear correlations of these metals with the ash content of the fish sludge were observed (Supplementary data, Tables S–3).

For some elements, the chemical forms, often referred to as element species will affect bioavailability and toxicity. For Hg and As, methylmercury (MeHg) and inorganic forms of As (iAs), arsenate and arsenite, are known to be of greater toxicological concern than other element species. Speciation analyses for MeHg and iAs were conducted on several fish sludge samples (n = 10; Table 5). MeHg accounted for 32–100% of total Hg in the sludge (data not shown), whereas in fish feed, MeHg generally comprises >70% of total Hg (Sele et al., 2023). The range in the proportion of MeHg in sludge may consequently reflect the proportion of feed spillage in the different sludge samples.

Inorganic As (iAs) concentrations in fish sludge were between 0.057 and 0.42 mg/kg, whereof all samples were well below the MLs for iAs in feed of 2 mg/kg. The ratio of iAs to total As ranged from 2 to 24%, which is higher than the ratio of iAs normally seen for fish feed (<5%) (Silva et al., 2023). The high proportion of iAs in some of the samples may reflect that iAs is more prone to excretion via feces than the organic As species, such as arsenobetaine (AB), which is known to have a higher bioavailability and is accumulated in fish muscle tissue (EFSA, 2009). Speciation analysis of organic As shows that AB was the major As species present in the sludge samples, but also the simple methylated As form dimethylarsinate (DMA) and the higher methylated forms (TMAO, TMAP and AC) were present in lower concentrations (Supplementary

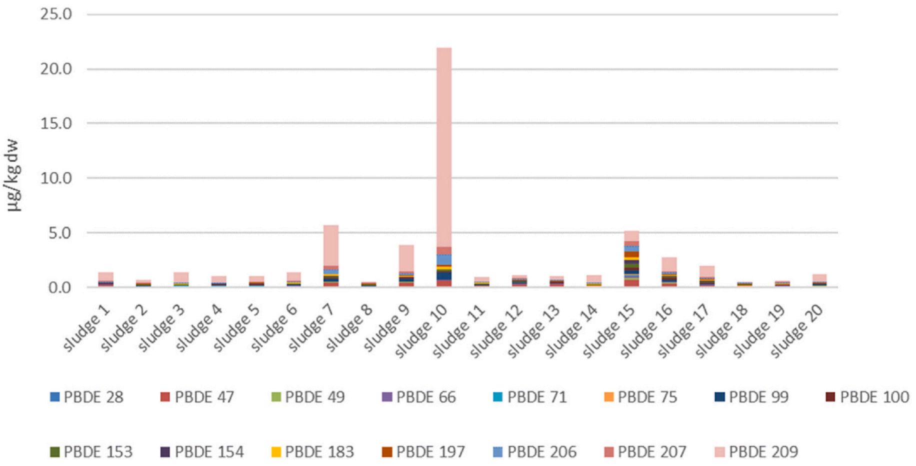

Fig. 2. The congener profile of PBDEs in aquaculture sludge samples (n = 20), showing the concentrations (µg/kg dw) of the measured congeners, PBDE28 to PBDE209.

Table 4

Concentration of cadmium (Cd), mercury (Hg), methyl mercury (MeHg), lead (Pb), arsenic (As), inorganic As (iAs), nickel (Ni) and aluminum in sludge samples (mg/kg dw, n = 47), with mean and standard deviation, range (minimum to maximum) and the 25–75% percentiles. For comparison, the EU maximum levels (MLs) given in the EC2002/32, and concentrations reported for commercial fish feed are also given.

	Cd	Hg	MeHg [b]	Pb	As	iAs [b]	Ni	Al [c]
Mean ± SD (n = 47) [a]	0.71 ± 0.32	0.048 ± 0.021	0.024 ± 0.019	1.0 ± 1.3	1.68 ± 0.76	0.18 ± 0.10	5.5 ± 7.6	1843 ± 5031
Range (min- max)	0.22–1.6	<0.017–0.089	0.009–0.074	0.10–5.6	0.44–3.3	0.057–0.42	1.6–51	220–20,000
25–75% percentile	0.42–0.92	0.03–0.07	0.014–0.028	0.4–0.77	1.0–2.2	0.11–0.23	2.8–4.5	382–980
EU ML feed for animal (mg/kg) [d]	0.5	0.1	–	5	2	2	–	–
EU ML feed for fish (mg/kg) [d]	1	0.2	–	5	10	2	–	–
EU ML feed material of fish (mg/kg) [d]	2 [e]	0.5	–	10	25	2	–	–
Commercial fish feed [f]	0.04–0.83	<0.006–0.11	<0.003–0.08	0.02–0.2	1.0–7.5	0.009–0.10	0.30–3.9	–

[a] Mean is calculated for the UB LOQ when concentrations are below LOQ.
[b] n = 10.
[c] n = 15.
[d] EU limits are set for samples with a 12% moisture content.
[e] ML set for feed materials of animal origin, not specified for fish.
[f] Data from the Norwegian monitoring program for fish feed, samples collected and analysed in 2022 (n = 80) (Sele et al., 2023).

data, Figure S-3). It should be noted that 62–85% of total As was not identified by the methods applied. This may be due to some of the As being present is in the form of lipid-bound As (arsenolipids), which is not detected by this method. Arsenolipids are the major As species in marine oils and have also been identified in fish feed (Sele et al., 2012).

In EU, the elements Cd, Hg, Pb and As are regulated under the feed directive for undesirable compounds (EC, 2002/32), with established MLs in animal feed and feed materials. If fish sludge is to be considered as a future feed resource, and when comparing the concentrations in sludge with the EU MLs for complete feed for animals, several of the sludge samples exceeded the ML for Cd of 0.5 mg/kg (84% of the samples) and As of 2 mg/kg ((for most animal species); 43% of the samples). One sample exceeded the ML for Pb of 5 mg/kg. All sludge samples were below the ML set for Hg in animal feed (Table 4). For some production animal species, such as fish, specific MLs deviating from the majority have been set, being higher for Cd and As. When comparing the concentrations of Cd, Hg, Pb and As we found in the sludge samples with MLs feed material made of fish, aquatic animals or thereof, none of the sludge samples exceeded the MLs.

For the non-regulated elements Ni and Al, wide variations in concentrations were apparent (Table 4). High levels of Al have also been observed in previous studies of fish sludge, with Al concentrations up to 30 g/kg dw (Teuber et al., 2005; Salazar and Saldana, 2007). In the processing of sludge, flocculants are being used for enhancing e.g. the drying process, and the application of Al-based flocculant in wastewater treatment results in a large amount of Al-rich sewage sludge (Chang et al., 2022). The sludge sample with the highest Al concentration, contained 20 g/kg and it was reported that aluminum chloride (AluPac) had been used as a flocculant at the site where this sample originated. No ML have been established for Al in feed or feed materials. The toxicity of Al depends on several factors, including animal species, form of aluminum, and duration and level of exposure (EFSA, 2008).

3.3. *Legacy organic pollutants*

Organic pollutants refer to a class of environmental contaminants that can have natural origins, but anthropogenic activities significantly contribute to their release into the environment, and their presence can have adverse effects on ecosystems, and human- and animal health. Some organic pollutants resist degradation, and these persistent organic pollutants (POPs) are considered priority pollutants for both feed and food safety, and includes the dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs), non-dioxin-like PCBs, brominated flame retardants (BFRs), and organochlorine pesticides (OCPs), such as DDT and hexacyclobenzene (HCB) (Glencross et al., 2020). POPs are all halogenated structures, known to be persistent, can bioaccumulate, and may have many different chemical congeners. Several of them are regulated with MLs in both food (EU 203/915 and amendments (EU, 2023),) and feed (EC, 2002/32 and amendments (EC, 2002),). None of the sludge samples exceeded the EU MLs set for the regulated organic pollutants in the EU feed directive (EC, 2002/32).

3.3.1. *Dioxins and PCBs*

Samples (n = 37) were analysed for dioxins, dl-PCBs and PCBs, with

Table 5

Concentrations of the dioxins and PCBs (sum dioxins (sum PCDD/PCDF), sum dioxin-like PCBs (dl-PCB) and sum dioxin and dl PCBs (TEQ2005 ng/kg dw, n = 37), sum PCB6 and sum PCB7 (UB, μg/kg dw, n = 37)), the brominated flame retardants PBDE (sum PBDE7 (μg/kg dw, n = 37), sum PBDE8 (μg/kg dw, n = 20)), organochlorine pesticides (n = 10, μg/kg dw), sum polycyclic aromatic hydrocarbons (PAHs, n = 20, μg/kg dw) and per- and polyfluoroalkyl substances (PFAS, n = 10, μg/kg dw) in fish sludge. The sum and mean are calculated as upper-bound LOQ. For comparison, the EU maximum levels (MLs) given in the EC2002/32, and concentrations reported for commercial fish feed are also given.

	Dioxins and PCBs (n = 37)					Brominated flame retardants (n = 37)		Organochlorine pesticides (n = 10)					PAHs (n = 20)	PFAS (n = 10)
	Sum dioxins (PCDD/PCDF)	Sum dl-PCB	Sum dioxins and dl-PCB (UB)	Sum PCB6[a] (UB)	Sum PCB7[b] (UB)	Sum PBDE7[c] (UB)	Sum PBDE8[d] (UB)	Sum dieldrin and aldrin	Sum DDT	Sum Toxaphene	Sum Chlordane	HCB	Sum PAH4	L-PFOS
Mean ± SD (n = 37)	0.38 ± 0.15	0.31 ± 0.21	0.69 ± 0.33	2.2 ± 1.4	2.5 ± 1.5	0.48 ± 0.46	2.28 ± 4.28	0.90 ± 0.27	3.00 ± 0.82	2.11 ± 0.14	0.88 ± 0.07	0.56 ± 0.13	1.89 ± 0.71	0.27 ± 0.20
Range (min – max)	0.14–0.74	0.08–0.92	0.25–1.51	0.83–6.3	0.93–7.1	0.18–2.3	0.31–19.8	0.54–1.33	1.98–4.25	2.0–2.4	0.75–0.95	0.50–0.89	1.1–3.7	<0.06–0.62
25–75% percentile	0.26–0.47	0.17–0.33	0.26–0.78	1.2–2.7	1.3–3.0	0.24–0.49	0.74–2.05	0.6–1.1	2.4–3.7	2.0–2.2	0.80–0.94	0.50–0.55	1.4–2.2	<0.06–0.44
EU ML feed for farmed animal[e]	0.75	–	1.5	10	–	–	–	10	50	–	20	10	–	–
Commercial fish feed [g]	0.1–0.4	0.1–0.4	0.3–0.7	1.4–5.5	–	0.20–0.42		0.9–2.4	2.8–9.1	2.1–18.2	0.8–6.2	0.6–4.6	1.1–2.7	<LOQ [f]

[a] Sum PCB6 is the sum of six indicator PCBs: PCB-28, -52, −101, −138, - 153 and 180.

[b] Sum PCB7 is the sum of seven indicator PCBs: PCB-28, -52, −101, −118, −138, −153 and 180.

[c] Sum PBDE7 is the sum of seven PBDEs: PBDE-28, -47, −99, −100, −153, −154, −183.

[d] Number of samples, n = 20 for the sum of PBDE8. PBDE8 is the sum of eight PBDEs; PBDE-28, -47, −99, −100, −153, −154, −183 and −209.

[e] EU MLs are set for 12% moisture content.

[f] All PFAS compounds analysed were below the limit of quantification (LOQ) for the method. The LOQs were higher for the method used in Sele et al., 2023 than for the method used in the present study.

[g] Data from the national monitoring program for fish feed, samples collected and analysed in 2022 (n = 20) (Sele et al., 2023).

concentrations between 0.14 and 0.74 ng TEQ2005/kg dw for sum dioxins, 0.08–0.92 ng TEQ2005/kg dw for sum dl-PCBs, 0.83–6.3 μg/kg dw for sum PCB6 and 0.93–7.1 μg/kg dw for sum PCB7 (Table 5, and Supplementary data, Tables S–5).

Few studies have investigated the levels of dioxins and PCBs in fish sludge (Hellou et al., 2005; Brod et al., 2023). Hellou et al. (2005) studied sludge samples collected beneath and up to 100 m away from an aquaculture site in the Bay of Fundy, Canada, and found trends with higher organic carbon and PCBs below the cages compared to 50m away (Hellou et al., 2005). In fish sludge from five Norwegian salmonid production facilities, both raw and processed sludge samples were analysed for this group of undesirable substances in the study by Brod et al. (2023). A somewhat larger variation in concentrations was seen for dioxins and PCBs results the present study compared to Brod et al. (2023), but it should be noted that we have report UB (upper bound LOQ) results as recommended for dioxins and PCBs (FAO/WHO, 2018), whereas Brod et al. (2023) reported lower bound (LB) results. In salmonid feeds, fish oil and fish meal are the feed ingredients considered as the major sources of dioxins and PCBs (Kelly et al., 2008; Berntssen et al., 2011) and therefore the likely source for dioxins and PCBs in the fish sludge samples analysed in the present study. A higher inclusion of plant feed ingredients, replacing the marine feed ingredients has been shown reduce the levels of dioxins and PCBs in fish feed (Berntssen et al., 2011). The levels of dioxins, dl-PCBs and PCB6 were within the same range in the sludge as those reported in commercial salmonid feeds (Sele et al., 2023) (Table 5). If considering fish sludge as a feed resource and comparing the concentrations in sludge to the EU MLs for complete feed for animals, all samples were under the EU MLs for animal feed (EC2002/32 and amendments). However, some of the sludge samples had concentrations of sum dioxins (PCDD/PCDF) and sum dioxins and dl-PCBs (0.74 and 1.5 TEQ2005 μg/kg dw, respectively) close to the EU MLs for feed. It should be noted that the EU MLs for feed are given for a 12% moisture content, whereas the concentration in sludge is presented in dw.

3.3.2. *Polybrominated diphenyls (PBDEs)*

Polybrominated diphenyls (PBDEs) are a group of brominated flame retardants (BFRs), which are anthropogenic chemicals widely used in commercial products to improve their resistance to fire. The occurrence and persistence of several BFRs in the environment have raised concerns regarding their presence in the environment, food and humans (EFSA, 2011; EFSA, 2024), and led to bans on the production and use of certain formulations. In this study, the following PBDE congeners PBDE28, 35, 47, 49, 66, 71, 75, 77, 85, 99, 100, 118, 119, 138, 153, 154 and 183 were analysed, in addition to some samples (n = 20) that were analysed for the additional heavy PBDE congeners (PBDE 196, 197, 206, 207 and 209). EFSA has considered eight congeners of primary interest in terms of food and feed safety (EFSA, 2011) PBDE-28, -47, −99, −100, −153, −154, −183 and −209, which herein is the sum PBDE8 presented in Table 5. The occurrence of some of these congeners, particularly PBDE 206 and PBDE-209, were variable in the sludge (Fig. 2), with PBDE209 accounting for 13–83 % of the sum (total) PBDE congeners measured (Supplementary data, Tables S–5). One sample was found to contain a notably high concentration of PBDE-209 (18 μg/kg dw). In the study by Brod et al. (2023), variable proportions of the PBDE 209 congener were observed, ranging from 29 to 70% of total PBDE. Similarly, in sewage sludge the PBDE209 congener was found in variable concentrations and was the dominant congener (Cincinelli et al., 2012). PBDEs may be mixed into polymers, and leach from products into the environment (EFSA, 2011). Fish feed and feed materials are known to contain PBDE (Pietron et al., 2023), as well as other POPs (Glencross et al., 2020). In fish feeds, the congener PBDE47 and PBDE209 are the more common congeners (Pietron et al., 2023). Contamination of fish sludge from processing technologies (i.e. chemical treatment and drying) or transportation may occur, however, to our knowledge, there are no conducted systematic studies on this available.

3.3.3. *Organochlorine pesticides*

Organochlorine pesticides (OCPs), such as DDT and chlordane are persistent in the environment due to their historic widespread use as plant protection products, and is a group of POPs that can accumulate along the food chain (van Asselt et al., 2023). In this study, samples of fish sludge ($n = 10$) were analysed for the OCPs dieldrin, aldrin, sum DDT, toxaphene, chlordane and HCB (Table 5). All concentrations of alpha-, beta- and delta- HCH, endrin, mirex, octachlorstyrene and trans-nonachlor, were below the LOQs of the method (Supplementary data, Table-S-5).

Fish oil or fishmeal, produced from marine pelagic oceanic fish species, are considered the main source of OCPs in commercial fish feeds (Jacobs et al., 2002; Berntssen et al., 2011). Two isoforms of DDT (p,p' and op) were measured, and the forms DDD, DDE and DDT, and ratios of 1–8 for the sum of DDE to the sum of DDT isoforms were found (Supplementary data, Tables S–5). It is known that DDT is metabolized or degraded to DDE and DDD (Strandberg et al., 1998), and the ratio between the isoforms is therefore indicative of time of the source to DDT in the samples. The ratio of DDE/DDT found in the fish sludges samples analysed in the current study was similar to that previously reported for fish feed (Sanden et al., 2013). These results imply that the source of DDE and DDT in sludge is likely the feed. The concentrations of OCPs in the sludge are well below the EU regulatory limits set in the feed directive (EC, 2002/32) (Table 4). The concentrations found in fish sludge were within the same concentration range as for fish feed (Sele et al., 2023) (Table 5).

With the increasing inclusion rate of plant materials in fish feed, pesticide residues of e.g. pirimiphos-methyl, chlorpyrifos and glyphosate may be present in fish feed (Berntssen et al., 2021). The presence of organophosphate pesticides and herbicides are considered relevant to study in fish sludge, however, was not included in this work.

3.3.4. *Polycyclic aromatic hydrocarbons (PAHs)*

Processing technologies such as drying by heating of raw materials, feed and food, may introduce processing-induced contaminants, such as polycyclic aromatic hydrocarbons (PAHs), generated during incomplete combustion of organic matter. Unrefined plant oils obtained from oilseeds such as soybeans, or rapeseeds may contain elevated levels of PAHs, and the replacement of marine ingredients with plant feed ingredients have shown to increase PAHs in salmon feeds (Berntssen et al., 2010).

In this study, samples ($n = 20$) of both wet and dried sludge retrieved from the same production facilities ($n = 7$), were analysed for PAHs to assess whether the drying process could cause elevated PAHs levels in sludge. The samples were analysed for 16 PAHs, whereof the sum of the four PAHs benzo(a)anthracene, benzo(a)pyrene, benzofluoranthene and chrysene, sum PAH4, are of most relevance in terms of food safety (EU, 2023). Our results showed relative stable concentrations of PAHs in fish sludge, with a mean concentration for sum PAH4 of 1.9 ± 0.7 μg/kg (Table 5). Chrysene and benzo(b)fluoranthene were the major contributors to sum PAH4, accounting for up to 40% and 50% of sum PAH4, respectively. The results did not indicate that the drying methods used for the fish sludge caused elevated levels of PAHs in fish sludge, and the concentrations in wet and dried sludge were not significantly different ($p > 0.17$, multiple *t*-test, Supplementary data, Figure S-4). PAHs formation is often associated with processes involving pyrolysis or combustion of organic matter, and the specific temperatures at which PAHs are formed can vary depending on the precursors present and other conditions, but can be formed at temperatures from a few hundred degrees Celsius (Chanyshev et al., 2017). The results are consistent with the fact that fish sludge is dried using low temperature, and hence, there is lower probability of PAH formation. A likely source of PAHs in the sludge will be from feed spillage, undigested feces, or from contamination by other products or chemicals if used in the processing of sludge. There are no legislative limits for PAH for feed or feed materials, but there are MLs for specific food items, such as smoked fish (12 μg/kg for sum PAH4) are set in the EU legislation (EU, 2023). The results from this study suggest that the risk of finding high levels of PAH in fish sludge is low.

3.4. *Contaminants and nutrient levels in relation to the composition of sludge*

The levels of nutrients and contaminants may be related to the composition parameters, e.g. CP, CL or ash content. To explore this, a PCA was conducted for the nutrient together with the elements and selected organic pollutants (sum dioxins + dl-PCB, sum PCB6 and sum PBDE7). The PCA shows that there was a close relationship between CP and CL, and that the correlation of feed spillage with the other variables followed the order energy > CL > CP (Fig. 1). Furthermore, the PCA shows a clear inverse correlation between energy (and feed spillage) and ash. When including the non-essential elements (Sum of As, Cd, Hg, Pb and Ni), and the essential elements group 1 (sum of Cr, Co, Mo, Se and V) and group 2 (sum of Cu, Fe, Mn and Zn) and the sum of dioxins + dl-PCB, sum PCB6 and sum PBDE7 in the PCA, 49.4% of the total data variability was explained by the first two components (PC1 and PC2) (Fig. 1). The PCA shows that the non-essential and the essential elements (group 1 and 2) and ash were correlated and characterized by negative PC2 values. These data suggest that the elements may be explained by the ash content of the sludge, although, a clear correlation between elements and ash was not observed when using Pearson correlation (Supplementary data, Tables S–3A).

The PCA shows that the sum dioxins and dl-PCB, sum PCB-6, CP and CL are correlated and characterized by positive PC2, whereas the sum PBDE7 seems to be weakly correlated to CP and CL (Fig. 1). These data suggest that the accumulation of dioxins and PCBs is affected by the levels of lipids or proteins in the sludge. Further studies are advisable to increase the number of samples to validate these observations.

3.5. *Emerging contaminants*

Emerging contaminants refer to new substances that are of increased focus due to their potential to pose risks to human and animal health, as well as to ecosystems. Emerging contaminants are substances not regulated or routinely monitored.

3.5.1. *Per -and polyfluoroalkyl substances (PFAS)*

Known as the 'forever chemicals', per- andpolyfluoroalkyl substances (PFAS) have been identified as contaminants of very high environmental concern (REACH) that have similar properties to the POPs (Steindal and Grung, 2021). PFAS are pervasive in today's society, and according to their new definition, over 7 million PFAS exist in PubChem database (Schymanski et al., 2023). Perfluorooctane sulfonic acid (PFOS) and related products have been regulated in the EU since 2006 and were listed in the Stockholm Convention in 2009. PFAS have been measured in many wild and farmed aquatic animals and different fish feeds (Suominen et al., 2011; Zafeiraki et al., 2019). However, to our knowledge, no previous studies on PFAS in fish sludge exist. In this study, sludge samples ($n = 10$) were analysed for a target list of 18 PFAS (see Tables S–2). The results showed that, out of the targeted 19 PFAS, only the liner isomer of perfluorooctane sulfonate (L-PFOS) was detected in 70% of the sludge samples analysed, in concentrations from <LOQ to 0.62 μg/kg dw range (Table 5). PFOS are being produced with electrochemical fluorination (ECF), which yields a complex mixture of both linear and branched isomers with known percentages, and the absence of branched PFOS isomers in the sludge samples can be attributed to their less sportive properties (Houde et al., 2008). PFOS was found to be the predominant PFAS in fish meal, fish oil, and fish feed and in fish from different regions (Berger et al., 2009; Suominen et al., 2011). PFOS has relatively higher partitioning to organic matter, and since fish sludge is rich in various organic matter, the occurrence of PFOS in the sludge is not surprising. In sewage sludge, PFOS was reported to be the dominant

PFAS, and with higher concentration in comparison to the current study (Navarro et al., 2011). PFOS measured in the fish sludge may be attributed to contaminated feed spillage. As of yet, the EU has not set regulatory limits for PFAS in animal feed, but MLs are established for food for commonly detected PFAS (PFOS, PFHxS, PFOA, and PFNA) ranging from 0.001 to 0.5 μg/kg wet weight (EU, 2023).

3.5.2. Non-target screening of contaminants

Targeted analytical methods, which rely on analytical standards, may overlook relevant contaminants. Suspect and non-target analysis, where screening and identification of unknown contaminants using HR-MS and database search, is an effective tool for a more holistic characterization of high number of organic contaminants in various solid waste materials (Altamirano et al., 2023). In this study, fish sludge samples (n = 10) were subjected to non-target analysis using LC-HRMS and several database searches (Supplementary data, S-Methods).

The results showed thousands of features detected in positive and negative scans. Of these, 18 compounds have been reported as findings and are considered anthropogenic contaminants and annotated with a high confidence (identification level 2) based on mass spectral fit match (>85%) with the mass spectral library mzCloud (Schymanski et al., 2014). The mass spectra of the identified compounds are shown in Supplementary Figures S-5A-G. It should be noted that no quantitative measurements were performed in this study. The presence of four pharmaceuticals (salicylic acid, acetophenone, penbutolol, and tolycaine), the stimulant caffeine, plastic related products (nine homologues polyethylene glycols and caprolactam) and the UV filter benzophenone were detected in the fish sludge samples analysed (Supplementary data, Table S-6). Among these, benzophenone and caffeine were confirmed with the highest confidence (identification level 1a). Many of these contaminants have previously been detected in environmental samples and biota, e.g. the plastic related product caprolactam was found in highway-runoff impacted fish (Du et al., 2017), and the stimulant caffeine was detected in fish silage used for biogas production in Norway (Ali et al., 2019). Caprolactam is an EU regulated additive and monomer used as a raw material in the manufacturing of polyamides polymers (EU, 2011). Caprolactam is typically found in food contact materials, which has the potential to migrate to food (Tsochatzis et al., 2023). Polyethylene glycols (PEGs) are water-soluble non-ionic synthetic polymers with various applications (Thurman et al., 2017). PEGs are considered high volume production chemicals, and many have been detected in waste-water treatment plant effluents (Freeling et al., 2019). A Kendrick mass defect plot with MS1 normalized to C_2H_4O (the repeating unit of PEG), showing polyethylene glycols (PEG5, 6, 8, PEG10-16) homologue series were observed (Supplementary data, Figure S-6). The occurrence of these plastic related chemicals can be attributed to their leaching from different polymers applied in the flocculation process, which was reported at some of the aquaculture facilities which supplied samples. More research is needed to evaluate the risk associated with the detected substances and to measure their concentration.

3.6. Veterinary residues

Veterinary antibiotics are used in livestock farming to control diseases, and outside Europe they can also be used to promote animal growth (van Asselt et al., 2023). Norwegian fish farmers use veterinary prescribed medicines to treat diseases, in addition, sedatives are used prior to handling the fish. Residues of such veterinary pharmaceuticals can be dissolved in water or bind to particles, precipitating in the sludge. All samples analysed (n = 20) showed levels below the LOQ for residues of the veterinary antibiotic's ciprofloxacin (<10 ng/g dw), enrofloxacin (<10 ng/g), flumequine (<40 ng/g), oxolinic acid (<40 ng/g) and trimethoprim (<2 ng/g).

Whereas fish sludge is currently not allowed as organic fertilizer within EU, the use of manure from other animals as agri- and horticulture fertilisers may spread veterinary residues, and the importance of monitoring and controlling veterinary antibiotics to prevent food safety risks has been emphasized (van Asselt et al., 2023). The use of medicines in farmed fish in Norway is registered in the Norwegian veterinary prescription register (VetReg) and annually reported (Sommerset et al., 2023). In 2022, the following antibiotics were used for farmed fish in Norway; florfenicol (397 kg), oxolinic acid (28 kg) and enrofloxacin (0.1 kg). In this study, florfenicol was not analysed due to analytical challenges with the sample matrix. Furthermore, the sites for sludge collection were not targeted specifically for those sites with registered treatment. Hence, future studies on veterinary residues in sludge should be more targeted to analysis of sludge from sites where veterinary medicines have been used to evaluate the presence and stability of the residues in sludge.

3.7. Fish pathogens

Fish sludge samples were analysed for the infectious salmon anaemia virus (ISAV), the infectious pancreatic necrosis virus (IPNV), the piscine orthoreovirus-1 (PRV-1), the piscine myocarditis virus (PMCV), and the Salmon gill poxvirus (SGPV) and for the presence of the bacteria *Mycobacterium salmoniphilum* and *Yersinia ruckeri* (n = 23). The viruses were selected based on previous studies with virus detection in fish and/or environmental samples from hatcheries and sea farms (Mutoloki et al., 2016; Jensen et al., 2019; Dean et al., 2022; Tartor et al., 2022). None of the investigated viruses were detected in the sludge samples (Supplementary data, Table S-7). However, the dilution of the samples to prevent inhibition in the qPCR may have missed detection of viruses, if present in low amounts. One of the sites, providing two sludge samples, in this study reported a previous IPN-infection, and another site reported a PRV-infection, but neither IPNV nor PRV-1 were detected in the sludge samples. This may be because the peak of infection preceded the collection of the sludge sample, and because the amount of virus present is likely to depend on the stage of virus infection. Another explanation could be inactivation/degradation of virus over time in the sludge, as the stability and infectivity of viruses are known to be influenced by both temperature and other environmental parameters. PRV-1 and IPNV are frequently found in hatcheries without apparent disease in Norway (Sommerset et al., 2023). In this study, selection for sludge collection was not designed to specifically target sites with on-going virus infections.

The bacterial pathogens analysed for represents different morphological characteristics as well as connection to disease outbreaks in Norwegian aquaculture. *Y. ruckeri*, a gram-negative bacterium known as the causative agent for "enteric redmouth disease", which can affect salmon before and after sea transfer, and outbreaks are accompanied by shedding of bacteria into the environment. The bacterium may be isolated from subclinical infected healthy fish and in biofilms in fish farms (Sommerset et al., 2023). *M. salmoniphilum*, a gram-positive, acid--resistant bacterium, can be present in natural water sources and has been linked to disease outbreaks in RAS facilities. This group of bacteria is generally robust to environmental changes (Santos et al., 2015). The results showed that none of the investigated bacterial pathogens were detected in any of sludge samples, with the exception of some samples, with inconclusive results (Ct > 35) (Supplementary data, Tables S–7). The results indicates that the two fish pathogens may be present in few samples, however, attempts to isolate live bacteria by culturing from inconclusive samples were unsuccessful. The detection with qPCR is subject to some uncertainty when it comes to sludge as a matrix, as it cannot be ruled out that gene fragments may be similar to unknown or not well characterized bacteria present in the aquatic environment. To our knowledge, there is no literature on the presence of viruses or pathogens in fish sludge from land-based aquaculture farms.

4. Conclusion

This is the first comprehensive analysis of fish sludge, providing occurrence data on nutrients and contaminants, aiming to document the levels of desirable and undesirable substances in relation to a potential future use of fish sludge in feed for invertebrates. The study showed that sludge can contain substantial amounts of protein and fat, but that the content is highly variable and related to the amount of feed spillage. Many of the sludge samples contained levels of As and Cd which exceeded the EU MLs set for animal feed (43% for As and 84% for Cd), whereas all samples had concentrations of the organic pollutants dioxins, PCBs and chlorinated pesticides below the MLs set for animal feed. The elements Cu, Zn, Fe, as well as Ni and Al were found at high, but variable concentrations. None of the samples analysed were found to contain detectable levels of veterinary medicines, viruses or bacteria. A non-target screening analysis identified the presence of pharmaceuticals, plastic related products, and benzophenone, which warrants further investigation. Overall, the results from this study show that fish sludge is a nutrient-rich resource; however, undesirable substances, originating from the feed or from treatment of sludge may be present.

CRediT authorship contribution statement

Veronika Sele: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Aasim Ali:** Writing – original draft, Visualization, Methodology, Formal analysis. **Nina Liland:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Anne-Katrine Lundebye:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Jojo Tibon:** Writing – original draft, Methodology, Formal analysis. **Pedro Araujo:** Writing – review & editing, Formal analysis, Data curation. **Hilde Sindre:** Writing – original draft, Methodology, Investigation, Formal analysis. **Hanne Nilsen:** Writing – original draft, Methodology, Formal analysis. **Andreas Hagemann:** Writing – review & editing, Visualization, Project administration, Investigation, Funding acquisition. **Ikram Belghit:** Writing – review & editing, Visualization, Validation, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Veronika Sele reports financial support was provided by Norwegian Seafood Research Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121103.

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