Determination of emamectin benzoate in marine sediments in the vicinity of fish farms in Norway with a history of SLICE® treatments.

(emamectin benzoate)

SLICE® (emamectin benzoate, EMB) is a medicated feed premix indicated for the treatment and prevention of sea lice infestations in salmon. When fed at a dose rate of 50 µg EMB/kg body weight/ day for 7 consecutive days, SLICE kills all parasitic stages of sea lice (*Lepeophtheirus* sp. and *Caligus* sp.)*.

Since its' introduction nearly 20 years ago, SLICE has established a proven record of reliable field performance and has become one of the leading medicines for sea lice control.

Following the use of SLICE, the marine environment is exposed to EMB, primarily due to excretion of faeces from treated fish. Because of it's properties, e.g., low water solubility and high adsorption potential to particles, EMB will be present in the sediment rather than the water column. Here, EMB has the potential to cause adverse effects to benthic faunal communities, particularly crustaceans, if sediment EMB residues exceed defined safe concentrations, known as environmental quality standards (EQS)**.
A field monitoring study was recently conducted to investigate the distribution of EMB in marine sediments in the vicinity of five fish farms located in the South and mid regions of Norway, with a focus on selecting farms with different historic patterns of use.

Study design

Selection of fish farms:

The five fish farms were selected based on their previous use pattern of SLICE and their hydrodynamic characteristics.
SLICE treatments took place between 2013 and 2016. The SLICE treatment history per farm is presented in Table 1.

TABLE 1

SLICE treatments	2013	2014	2015	2016
Farm A	No treatment			
Total amount EMB (g)		2030	650	1690
Dose (µg/kg/day)		77	46	83
Number of treatments days		9	9	8
Number of treatments		1	1	1
Farm B	No treatment		No treatment	
Total amount EMB (g)		450		1790
Dose (µg/kg/day)		48		92
Number of treatments days		8		20
Number of treatments		1		2
Farm C	No treatment		No treatment	
Total amount EMB (g)		1190		5550
Dose (µg/kg/day)		53		99
Number of treatments days		8		17
Number of treatments		1		2
Farm D	No treatment	No treatment		
Total amount EMB (g)			197	250
Dose (µg/kg/day)			53	79
Number of treatments days			7	9
Number of treatments			1	1
Farm E		No treatment		
Total amount EMB (g)	1680		2540	1040
Dose (µg/kg/day)	87		47	75
Number of treatments days	7		15	10
Number of treatments	1		1	1

Table 1. Slice use at each farm, 2013 - 2016

With regards to hydrodynamic characteristics, low and high energy sites were selected. The hydrodynamic characteristics, represented by current speed at various depths, are presented in Table 2.

*Check your local package insert for details.

**The EQS is based on toxicological studies conducted in the laboratory, for which representative test organisms (e.g. benthic crustacea) are used. Large safety factors are applied to the toxicological endpoints from these studies to derive the EQS. The safety factor ensures that any uncertainty is covered, such as intra and inter-laboratory variation and variation in the sensitivity of different species, the need to extrapolate from laboratory study results to the field, and from short term (acute) to long term (chronic) toxicity.

TABLE 2

Depth	Average current speed (cm/sec)	Classification [1]
Farm A		
5 m	7.4	Strong
15 m	6.1	Strong
Spreading depth [2]	6.0	Very strong
Bottom [3]	2.1	Medium strong
Farm B		
5 m	2.9	Weak
15 m	-	-
Spreading depth [2]	2.1	Medium strong
Bottom [3]	1.0	Very weak
Farm C		
5 m	15.7	Very strong
15 m	11.5	Very strong
Spreading depth [2]	6.0	Very strong
Bottom [3]	8.0	Very strong
Farm D		
5 m	6.9	Strong
15 m	4.4	Very strong
Spreading depth [2]	3.7	Strong
Bottom [3]	2.5	Very strong
Farm E		
5 m	17.8	Very strong
15 m	7.1	Very strong
Spreading depth [2]	7.0	Very strong
Bottom [3]	9.3	Very strong

Table 2. Hydrodymanic characteristics per farm

[1] - As developed by Rådgivende Biologer AS for measurements from mechanical current meters
[2] - Measured midway between the bottom of the net pen and the depth at which bottom current is measured (see below)
[3] - Measured as close to sea bottom as possible, but no deeper than 100 m below the bottom of the net pen
Color-coding: Green – very weak, blue – weak, yellow – medium strong, orange – strong, red – very strong

***EMB refers to the salt form of the mix of emamectin B1a and B1b. In the mixture, emamectin B1a represents the larger portion (>90%).

Sediment sampling

Sediment samples were collected in spring/ summer 2017 in the vicinity of each farm, with a total of ten sampling stations per farm. Hydrodynamic and bathymetric conditions were considered in the localization of the sampling stations. The stations were located in different directions and distances from the fish farm, considering the prevailing current direction:

1. Downstream (DS) at increasing distances; i.e. cage edge (CE) (0 m), 30 m , 75 m, 125 m, 250 m, 500 m, 1000 m, and 1500 m
2. Upstream (US) at a single distance of 500 m (proposed negative control)
3. Perpendicular (PP) at a single distance of 500 m (proposed negative control)

Per station, two sediment samples were collected using a 0.1 m² van Veen grab. Appropriate measures were employed to avoid cross contamination; i.e., the grab, and other equipment that had been in contact with the sediment, was thoroughly cleaned after each sampling. Additionally, sampling started at proposed negative control stations (US-500 and PP-500), followed by the DS stations in decreasing distance to the farm. From each grab, three subsamples were collected which represented the top 2 cm surface layer of sediment, and were analyzed for EMB.

Analysis of EMB

EMB was measured using a newly developed ultrasensitive analytical method, validated under Good Laboratory Practice (GLP), for the determination of emamectin B1a over the range 0.0015 – 2.5 µg/kg wet weight (ww) sediment, whilst incurred residue stability (i.e., the stability of field samples when stored) was demonstrated in a non-GLP trial. Deuterated emamectin B1a*** was used as an internal standard and sediment samples were extracted with acidified ethyl acetate and the extract interference removed using polymeric strong cation exchange solid phase extraction (SPE). Final extracts were analysed using liquid chromatography combined with triple-quadrupole mass spectrometric detection (LC-MS/MS).

Results

EMB concentrations in sediment:

The distribution pattern of EMB was site specific, as were the concentrations detected (Figure 1). Generally, higher concentrations of EMB were found downstream of the farm, with respective maximum concentrations detected between CE (Farm B) and 500 m DS (Farm E).

Beyond station 500 m DS, and at stations located upstream and perpendicular to the farms, EMB concentrations were low (<0.37 µg/kg ww). EMB concentrations were compared against the respective EQS determined by the Scottish Environment Protection Agency (SEPA) for far-field stations (i.e. >100 m from cage edge, defined as the area outside the zone of effects) which is 0.763 µg/kg ww (SEPA 2005, 2018).

EMB concentrations at all far-field stations, at all farms, were well below this EQS.

The site-specific EMB distribution pattern can be explained by the hydrodynamic conditions and bathymetric features as described below for each farm.

Farm A

At Farm A, highest EMB concentrations were detected at stations CE, 75 m DS and 250 m DS, whilst concentrations were clearly lower at all other stations. Farm A is located at the rim of a basin which extends in a downstream direction from the farm. The slope of the basin sharply decreases from station 75 m DS to station 250 m DS. Beyond this point, the basin floor is relatively flat.

Strong currents likely allow for an initial transport from underneath the cages to station 75 m DS, followed by an enhanced transport downslope to station 250 m DS, with little EMB remaining on the slope itself, i.e., at station 125 m DS.

EMB is then transported gradually over the flat seabed, with concentrations decreasing evenly over distance.

Farm B

At Farm B, highest EMB concentrations were detected at station CE followed by station 30 m DS. Concentrations were clearly lower at all other stations, with these continuously decreasing over distance. This even EMB distribution pattern is likely the result of an even and weak current, in combination with the farm's location at the rim of a basin with a continuously and moderately descending slope.

The bottom of the basin, between 600 m and 1300 m downstream, has a constant depth of approximately 225 m. At approximately 1200 m DS, the seabed rises to approximately 200 m depth, which is due to the fact that the gradient cuts the northern slope of the basin. Due to the low current velocities there is likely no significant initial transport away from station CE.

However, if transport takes place, an even distribution into the basin along the continuously descending slopes can be assumed.

EMB concentrations were clearly different for the two grab samples collected at station CE. In one sample concentrations were 4.60 – 4.98 µg/kg ww across the three subsamples, whilst in the other grab these concentrations were clearly exceeded.

This difference can likely be attributed to either patchy sediment conditions (with differing properties within a short distance) resulting in pockets of EMB, or to a patchy distribution of feed pellets and/or faeces and thus EMB underneath the farm. Also, the grab samples may have been taken from slightly different areas of the seabed with varying EMB residues because, although these were collected at the same GPS position, movement of the boat and the submerged grab can result in collection of sediment from slightly different locations.

Farm C

For Farm C, the highest EMB concentrations were found at 250 m DS. Concentrations continuously increased from station CE to this point. From 250 m DS concentrations decreased with further distance. The bathymetric gradient shows an underwater valley, with steadily decreasing slope from CE to 500 m DS. The bottom of the valley is relatively flat beyond this point.

The higher EMB concentrations found between 30 m DS and 500 m DS are presumably due to enhanced transport along this gradient, with the generally strong but even current likely causing a majority of the particles transported away from the farm to settle in the area of 250 m DS.

For stations 75 m DS and 500 m PP, no sediment samples could be collected due to hard bottom conditions.

Farm D

For Farm D, the highest EMB concentrations were found at 125 m DS, with concentrations continuously increasing from station CE to this point. Beyond 125 m DS concentrations continuously decreased with further distance.

This distribution pattern is likely the result of strong currents, in combination with the farm's location over the deeper area of an underwater valley. In the downstream direction from the farm, the floor of the valley rises continuously and moderately. The highest EMB concentrations found at 125 m DS are likely due to initial transport away from the farm, followed by settling of the particles in an area where there is equilibrium between current speed and resistance from rising seafloor.

For stations 1000 m DS and 1500 m DS, no sediment samples could be collected, either because of hard bottom conditions or rocks blocking the closure of the grab.

Farm E

For Farm E, the EMB concentrations were low at all stations, with the highest concentration detected at station 500 m DS. The bathymetric gradient shows shallow water with a relatively flat seabed from CE to a point beyond station 250 m DS.

From that point the seafloor descends relatively steeply towards station 500 m DS, forming a narrow basin. From station 500 m DS to station 1000 m DS, the seafloor descends further, but less steeply.

Beyond station 1000 m DS the seafloor is relatively flat, with station 1500 m DS being located on the floor of the basin. Strong currents likely allow for an initial transport from underneath the cages, over the flat seabed and downhill to station 500 m DS, which is located towards the end of a steep slope. EMB is then transported gradually over the further descending flat seafloor, with concentrations decreasing evenly over distance.

FIGURE 1

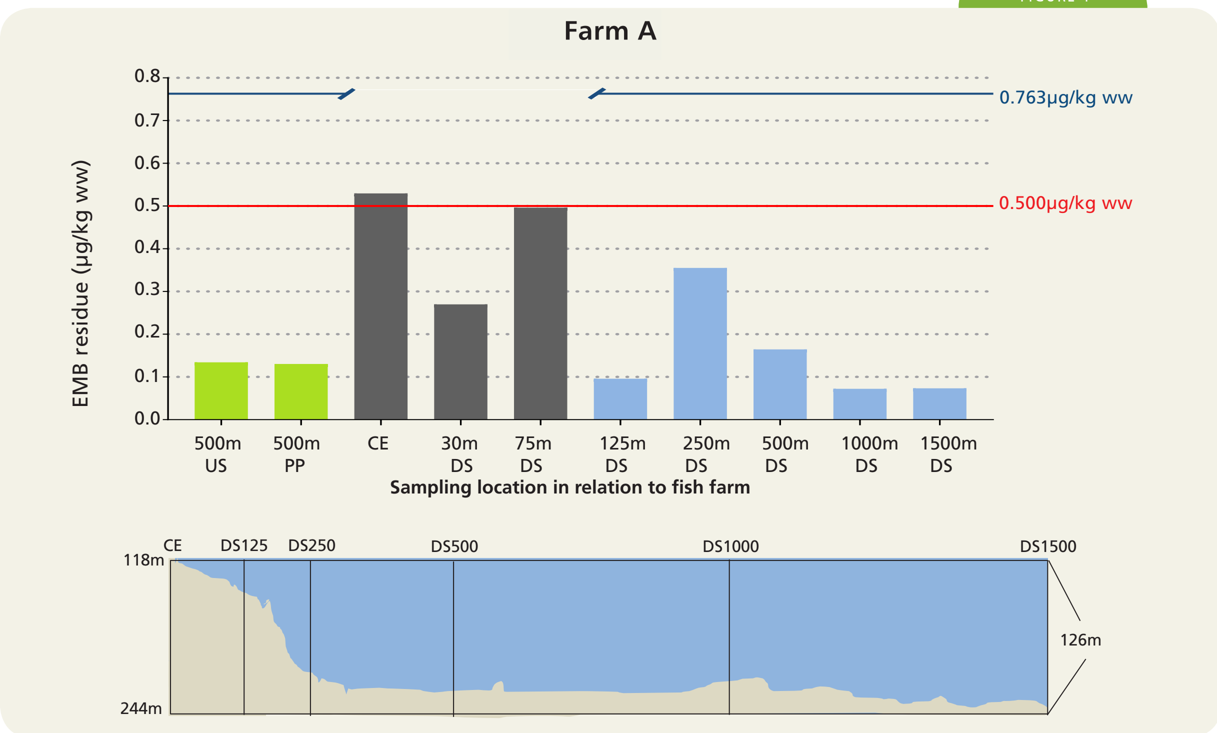
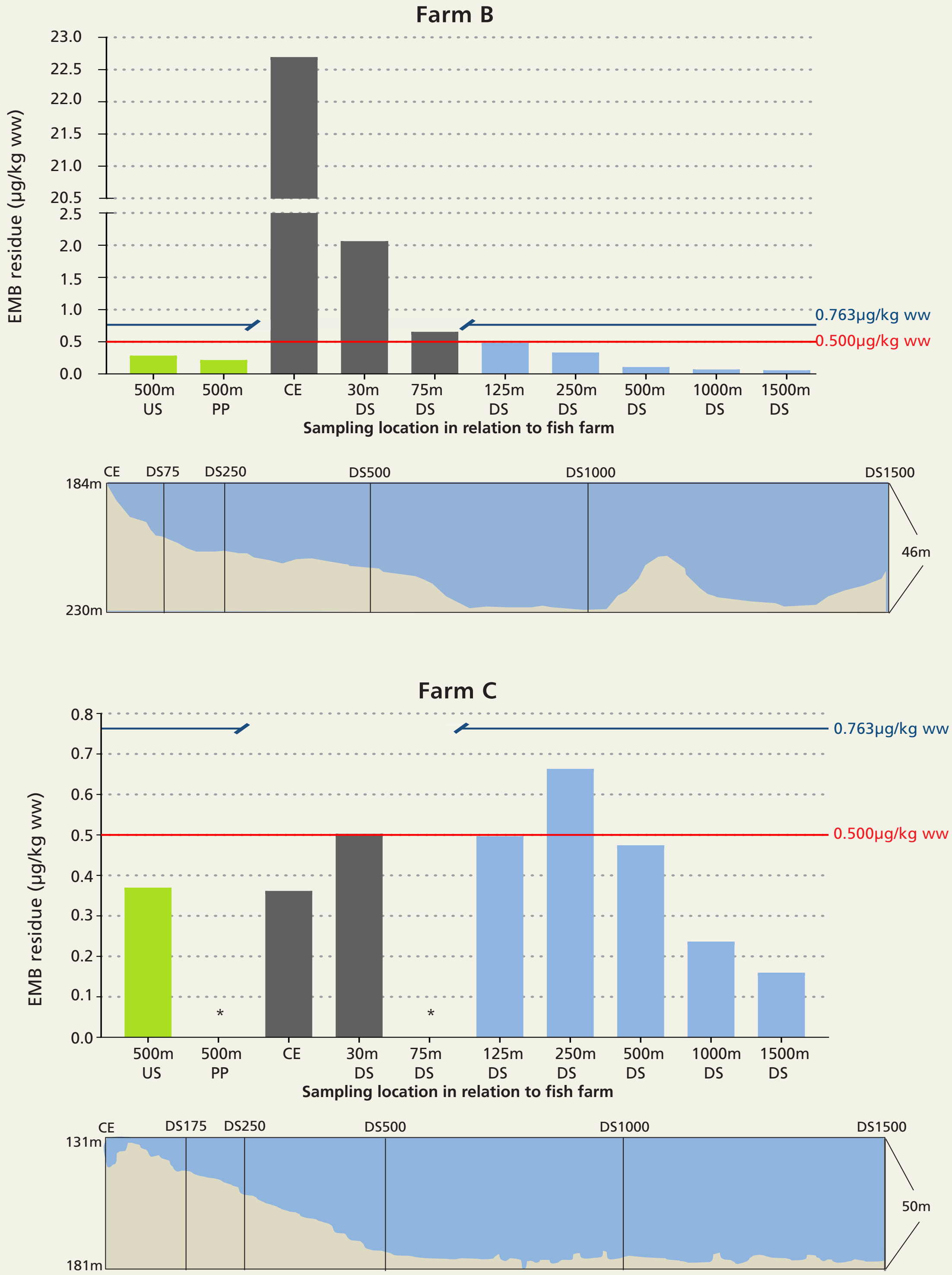


FIGURE 1



*No sediment sample was collected

FIGURE 1

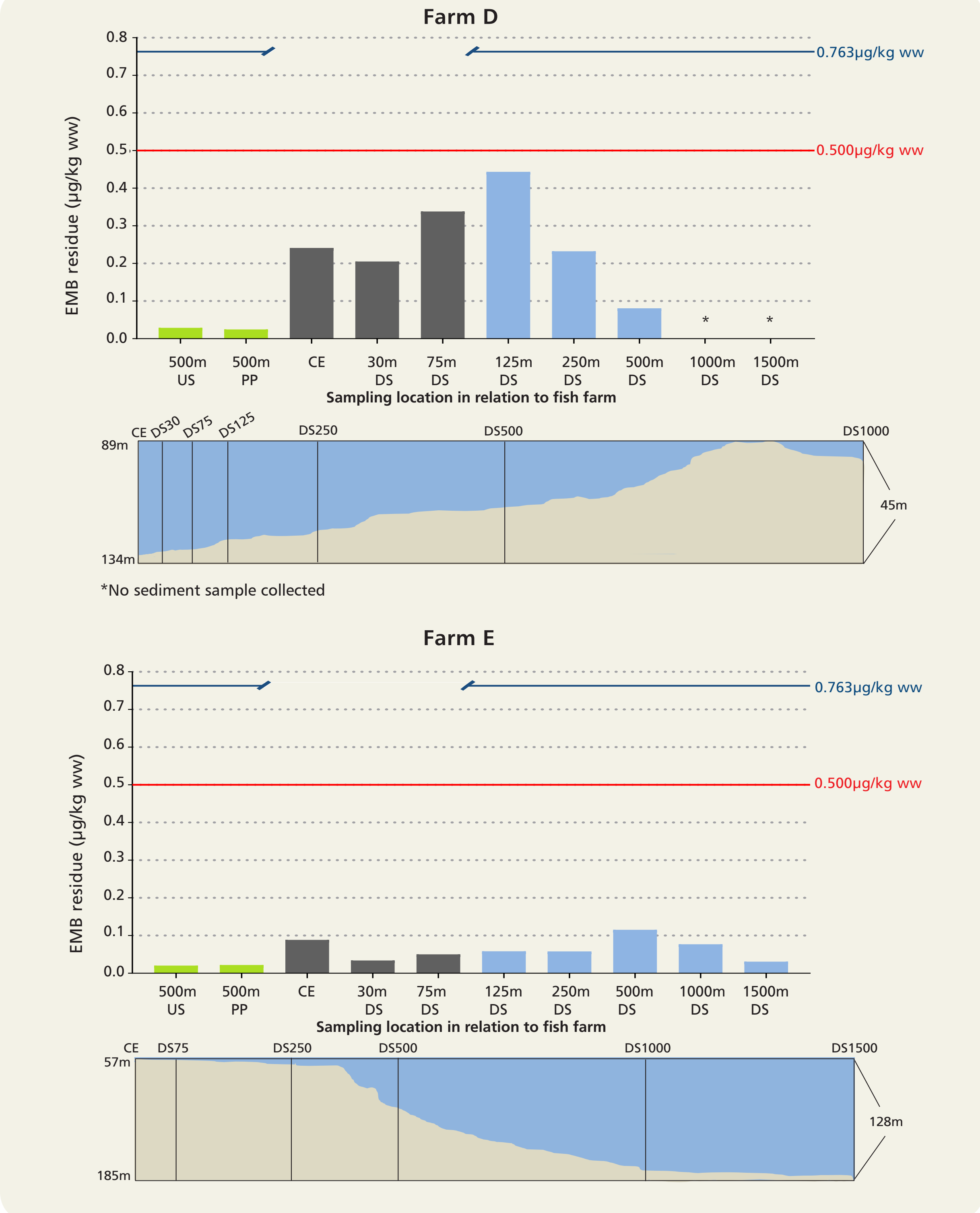


Figure 1: Average EMB concentrations in marine sediment in the vicinity of farms A - E and bathymetric profile from stations CE to 1500 m DS. Green columns – proposed negative control stations (US and PP), grey columns – stations inside the zone of effects, blue columns – far-field stations (outside the zone of effects), blue line - EQS of 0.763 µg/kg ww applicable to far-field stations (>100 m from cage edge), red line – detection limit of routine analytical method (0.5 µg/kg ww).

Conclusions

EMB was detected at all stations at which sediment was successfully collected. This is unsurprising when considering that a new ultrasensitive analytical method was used. This new method is 333-times more sensitive than the routine method. Considering the detection limit of the routine method (0.5 µg/kg ww), then for 40 out of 46 stations analysed EMB would be 'absent' (except for Farm A station CE; Farm B stations CE, 30 m DS, and 75 m DS; Farm C stations 30 m DS and 250 m DS). The distribution pattern of EMB was site specific, due to a combination of the local hydrodynamic conditions and bathymetric features. At stations located outside the footprint of the farm, i.e. at a distance >100 m, the respective far-field EQS, meant to protect all species, was not exceeded in any case. Accordingly, the concentrations detected are defined to be safe for benthic organisms.

The level of EMB residues detected in sediment around the farms does not directly correlate to the amount of EMB used. Instead, the levels are impacted by the local hydrodynamic conditions with high dynamic sites, e.g. Farms C and E, having low EMB concentrations despite the relatively high amount used. In contrast, at the low dynamic site Farm B, EMB residues were high, despite only moderate amounts of EMB being used. Based on the fate pattern of EMB in marine sediment with slow disappearance, the residues found at the farms likely represent a steady state resulting from repeated SLICE use over several years.

In conclusion, based on the findings of this field monitoring study, which encompassed five farms of varying SLICE use pattern and hydrodynamic conditions, risk to benthic fauna around the farm, including crustaceans, is unlikely to occur.

References

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