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Tributyltin (TBT) Pollution in the Coastal Waters of West Brittany as Indicated by Imposex in *Nucella lapillus*

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ABSTRACT

Tributyltin (TBT) pollution in west Brittany coastal waters was assessed in 1992, 1993 and 1994 using the bioindicator of imposex (superimposition of male sexual characters on females) in the neogastropod Nucella lapillus. *Imposex was detected at all 75 stations sampled, from Kerfissien on the north coast to Ile de Sein in the south-west, indicating widespread pollution even at sites having full exposure to the Atlantic. Imposex levels in the Bay of Brest were found to be high; female sterilization was prevalent and certain populations appeared to have been exterminated, notably in the northern part of the Bay.*

The evidence suggests that the 1982 legislation, banning the use of TBT-based antifouling-paints on vessels less than 25 m, is being ignored in certain areas; some small ports, like those of the islands, are contaminated despite the fact there are few vessels eligible to use this paint.

INTRODUCTION

Tributyltin (TBT) is a very toxic molecule which has been used in marine antifouling-paints since the mid-1960s. Its effect on marine biota was first described by Alzieu *et al.* (1980) who correlated shell thickening and production decline in the cultured oyster *Crassostrea gigas* with TBT pollution in Arcachon Bay (France). In the 1980s, many research groups worked on TBT effects on different species, in particular Gibbs *et al.* (1987) who introduced two indices of TBT pollution in the dog-whelk *Nucella lapillus* (L.), based on the imposex phenomenon as defined by Smith (1971), i.e. the superimposition of male sexual characters upon the normally gonochoristic female of neogastropods. Bryan *et al.* (1986, 1987, 1988) and Gibbs & Bryan (1986, 1987) demonstrated that imposex is

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initiated on exposure to TBT at a concentration of less than 1 ng/litre; when the TBT level exceeds 5 ng/litre sterilization of the female results through oviduct blockage (Gibbs *et al.*, 1988). Thus, in more polluted areas *N. lapillus* populations have been exterminated.

The phenomenon of imposex as an indicator of TBT pollution has been little studied in French waters despite the fact that some of the earliest work was carried out in the region by Feral (1980). More recent observations were provided by Gibbs *et al.* (1991*a*) and Oehlmann *et al.* (1991, 1993). Such data indicated TBT pollution to be a widespread problem; acute concern for the health of the semi-enclosed inlet of the Bay of Brest prompted the present study. This bay contains diverse maritime facilities including shipyards, a naval base, fishing vessel docks and leisure-boat marinas; consequently, TBT input from various activities has been considerable.

This study uses the methods devised by Gibbs *et al.* (1987) to assess TBT contamination in the west Brittany coastal waters. *N. lapillus* is a neogastropod belonging to the family Muricidae. It lives on the middle–lower reaches of the rocky intertidal shore where it feeds primarily on mussels and barnacles (Feare, 1970; Berry & Crothers, 1974; Hughes & Dunkin, 1984). The main advantages in using this organism are that it has a direct development without a planktonic stage, it is relatively immobile (Hughes, 1972; Menge, 1978*a*,*b*; Pechenik *et al.*, 1984), and it is present throughout the region. Thus, it is possible to assess the relative degrees of TBT contamination at different geographical sites.

MATERIALS AND METHODS

The coastline surveyed extends from Kerfissien (St 1) in northern Brittany to Ile de Sein (St 65) to the south-west and includes the offshore islands of the Archipel de Molène (Sts 66–75) (see Fig. 1). A total of 75 stations were examined in 1992 (February–July), in 1993 (March–June) and in 1994 (March–May). Where possible, 40 adult *N. lapillus* were collected at each site, adults being recognized by their 'toothed' appearance (Cowell & Crothers, 1970). Samples were analysed at the latest within a few days of collection. The animals were extracted by crushing their shells in a vice. Penis lengths were measured by means of a binocular microscope equipped with an ocular micrometer, and the Relative Penis Size Index (RPSI) calculated as defined by Bryan *et al.* (1986) and Gibbs *et al.* (1987):

$$\text{RPSI} = \frac{(\text{mean penis length of females})^3}{(\text{mean penis length of males})^3 \times 100}$$

Vas deferens sequence stages of females were determined following the method described by Gibbs *et al.* (1987). This scale extends from stage 0 for females with no visible male character to stage 5 where vas deferens tissue has developed to the extent that the oviduct is blocked, preventing the expulsion of egg-capsules, and stage 6 where aggregated capsules are present in the capsule gland. Females whose ovaries were partially or totally transformed into a testis (indicative of exposure to TBT levels exceeding 25 ng/litre; Gibbs *et al.*, 1988) were ranked as VDS stage 7 (see also Fioroni *et al.*, 1991; Gibbs *et al.*, 1991*b*; Oehlmann *et al.*, 1991). The VDS index (VDSI) was calculated as the mean of those stages present in the sample.

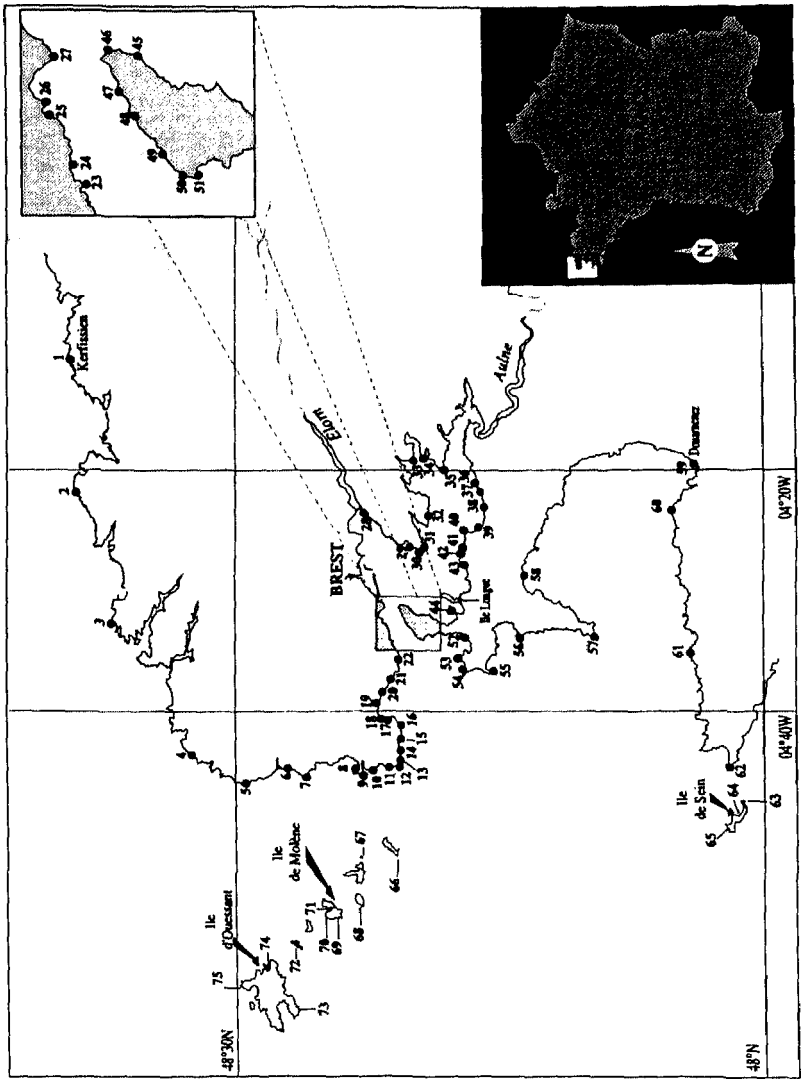

Fig. 1. Map of west Brittany showing positions of sampling stations.

TABLE 1
Relative Penis Size Index (RPSI) and Vas Deferens Sequence Index (VDSI) Values at Stations 1-75 in 1992, 1993 and 1994

ST	*RPSI92*	*RPSI93*	*RPSI94*	*VDSI92*	*VDSI93*	*VDSI94*	*N*	*st%*	*ST*	*RPSI92*	*RPSI93*	*RPSI94*	*VDSI92*	*VDSI93*	*VDSI94*	*N*	*st%*
1	2	3	3	2.63	2.31	2.37	99	0.0	39	41	NS	NS	5.25	NS	NS	5	100.0
2	5	14	15	3.50	4.00	4.00	21	0.0	40	37	38	23	4.75	4.32	4.06	67	31.3
3	6	5	9	3.75	3.86	4.00	37	0.0	41	45	54	22	4.77	4.27	4.15	76	34.2
4	5	8	7	3.95	4.00	4.00	56	3.6	42	32	47	31	4.12	4.33	4.17	69	18.8
5	22	18	25	4.07	4.15	4.00	38	7.9	43	32	43	28	4.47	4.33	4.15	59	23.7
6	22	18	23	4.00	4.00	4.00	26	0.0	44	34	NS	NS	4.40	NS	NS	5	40.0
7	30	25	29	4.24	4.24	4.00	75	14.7	45	39	32	41	4.64	4.27	4.23	65	29.2
8	36	42	38	4.15	4.24	4.07	60	16.7	46	23	24	20	4.18	4.09	4.08	49	10.2
9	26	28	22	4.20	4.27	4.00	48	14.6	47	33	19	36	4.28	4.00	4.25	60	18.3
10	16	29	23	4.15	4.16	4.00	62	9.7	48	19	31	22	4.22	4.40	4.30	53	17.0
11	32	31	30	4.17	4.26	4.17	67	14.9	49	30	31	28	4.55	4.29	4.13	77	23.4
12	39	41	43	4.09	4.19	4.26	80	15.0	50	31	35	39	4.37	4.13	4.00	75	12.0
13	43	21	28	4.15	4.13	4.13	65	15.4	51	22	35	32	4.33	4.27	4.13	66	13.6
14	64	54	32	4.50	4.29	4.07	67	17.9	52	52	28	31	4.18	4.00	4.00	65	3.1
15	30	40	30	4.22	4.13	4.00	53	11.3	53	19	12	16	4.19	4.08	4.07	53	17.0
16	43	57	35	4.40	4.31	4.21	59	22.0	54	27	23	25	4.22	4.06	4.13	56	14.3
17	50	29	30	4.78	4.36	4.36	58	34.5	55	13	13	13	4.06	4.00	4.17	70	4.3
18	36	23	NS	4.41	4.12	NS	57	24.6	56	9	11	13	3.93	4.00	4.00	57	1.8
19	29	33	35	4.13	4.13	4.25	59	11.9	57	9	12	19	3.95	4.00	4.00	52	0.0
20	16	15	33	4.29	4.00	4.00	58	5.2	58	18	27	23	4.11	4.00	4.00	71	2.8
21	38	60	47	4.27	4.33	4.07	70	17.1	59	33	37	27	4.08	4.14	4.12	69	11.6
22	37	42	30	4.20	4.16	4.36	60	13.3	60	31	27	27	4.21	4.09	4.25	84	11.9
23	17	33	26	4.29	4.35	4.29	64	28.1	61	8	8	NS	3.95	3.90	NS	62	0.0
24	31	41	27	4.00	4.35	4.31	70	17.1	62	8	8	6	3.83	3.95	3.87	67	0.0
25	62	57	34	4.54	4.77	4.38	61	41.0	63	11	11	NS	4.05	4.00	NS	48	2.1
26	41	45	28	4.61	4.31	4.25	92	31.5	64	17	38	NS	5.33	5.70	NS	13	92.3
27	41	50	32	4.00	4.20	4.31	62	12.9	65	5	4	NS	3.50	4.00	NS	51	0.0
28	75	**	**	6.00	**	**	10	100.0	66	12	14	NS	4.08	4.00	NS	40	5.0
29	66	NS	**	5.00	NS	**	4	75.0	67	12	19	NS	4.14	4.00	NS	38	5.3
30	49	49	33	4.36	4.60	4.62	68	38.2	68	9	12	NS	4.00	4.00	NS	32	0.0

27	41	30	32	4.00	4.20	4.31	62	12.9	65	5	4	NS	3.50	4.00	NS	31	0.0
28	75	**	**	6.00	**	**	10	100.0	66	12	14	NS	4.08	4.00	NS	40	5.0
29	66	NS	**	5.00	NS	**	4	75.0	67	12	19	NS	4.14	4.00	NS	38	5.3
30	49	49	33	4.36	4.60	4.62	68	38.2	68	9	12	NS	4.00	4.00	NS	32	0.0
31	39	43	34	4.56	4.24	4.25	94	26.6	69	7	11	NS	4.00	4.00	NS	37	0.0
32	38	45	NS	4.62	4.60	NS	40	47.5	70	7	8	NS	4.00	4.00	NS	33	0.0
33	20	19	30	4.27	4.00	4.00	53	15.1	71	33	46	NS	5.00	5.00	NS	14	78.6
34	21	35	NS	4.45	4.12	NS	28	25.0	72	7	NS	NS	3.69	NS	NS	13	0.0
35	46	42	NS	4.43	4.17	NS	38	18.4	73	7	6	NS	4.00	4.00	NS	53	0.0
36	32	25	37	4.72	4.30	4.22	70	30.0	74	39	69	NS	4.14	4.83	NS	13	30.8
37	43	22	NS	4.68	4.31	NS	32	16.0	75	7	11	NS	3.53	4.00	NS	46	0.0
38	40	54	20	4.50	4.31	4.00	68	25.0									

N, Total number of females analysed. **, No nucella found. st%, Percentage of sterilized females. NS, Not sampled.

At some polluted sites a few males and females proved to be aphallic (i.e. without penis) and comparable to those described as having 'Dumpton Syndrome', a condition found in one area in south-east England (see Gibbs, 1993). The syndrome is a male genital defect, characterized by an underdevelopment of the male tract; this genetically-based abnormality is also reflected in females, which then exhibit lesser imposex developments. Affected males and females were here regarded as abnormal and omitted from the calculations of the RPS and VDS indices. Detailed observations on the occurrence of aphally will be presented in a further paper (in preparation).

RESULTS

The results of the survey are summarised in Table 1. Since the data for the three years sampled are similar, no trends being apparent, mean values are shown in Fig. 2. For descriptive purposes, it is convenient to divide the survey area into four regions: northwest Brittany, Bay of Brest, Bay of Douarnenez, and the Archipel de Molène.

Northwest Brittany

From Kerfissien (St 1) to Pte Saint-Mathieu (St 12), RPSI values increase from 3 to 41 indicating an increasing gradient of TBT pollution. Sterilised females were not found at Sts 1–3 but were present from Sts 7 to 12. From St 12 to the mouth of the Bay of Brest (St 27), RPSI values range from 22-52 with a mean of about 40. Sterilized females were found at every station.

Bay of Brest

In this bay, 20 stations were sampled (St 27–46). Overall, RPSI values are high indicating widespread TBT pollution. The lowest RPSI values are obtained at the mouth of the bay, at Pte des Espagnols (St 46) and in the southeastern part (St 33–34) close to the freshwater inputs from the Rivière de Daoulas. Sterile females (VDS stages 5 and 6) occurred throughout the bay.

In two areas of the bay, pollution appears severe. In the north-east (St 28 and 29), few animals were taken despite intense searches of the suitable habitats present; at St 28, 40 adults (possibly imported—see Discussion) were discovered in 1992 and all females were sterile (VDS stages 5 and 6 but also one stage 7), and at St 29 just 16 were collected in 1992 (3 of the 4 females were sterile) and one male in 1994. In the south of the bay between St 43 and St 45, a restricted naval area, the species is scarce and in 1992 only 9 adults were found at Ile aux Morts (St 44); 3 of the 5 females appeared capable of breeding and, significantly, juveniles were also present.

To the south of the mouth of the Bay of Brest (Sts 46–50), RPSI values are similar to those in the north (Sts 23–27), and further south (Sts 51–57) values are low except at Camaret (St 52) which is a fishing port.

Bay of Douarnenez

In this bay (Sts 57–61), imposex seems less severe. Even on each side of the port of Douarnenez, RPSI values are between 27 and 37 at Plomarc'h (St 59) and between 27 and

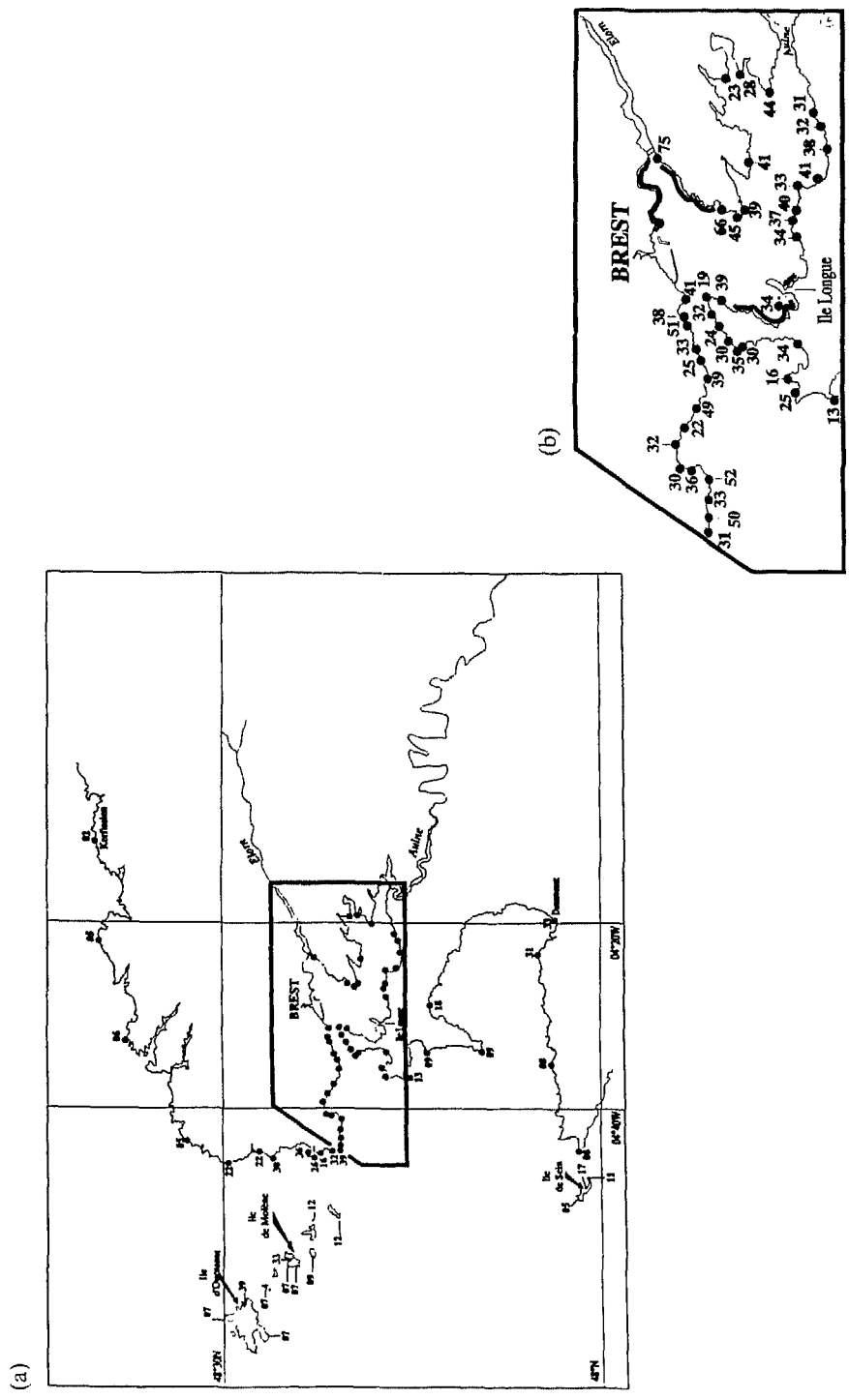

Fig. 2. (a) Distribution of mean RPSI values in west Brittany. (b) Distribution of mean RPSI in the Bay of Brest and adjacent coasts. (▬), Suitable habitats without *Nucella* population.

31 at the Pointe de Leydé (St 60). Importantly, juveniles are present. To the south, at Pte du Raz (St 62) and Ile de Sein (Sts 63–65), RPSI values are low. It should be noted that in the port of Ile de Sein (St 64), only 3 females were observed out of 44 individuals collected in 1992, and two of these females were sterile. This low RPSI value must be viewed with caution, since in 1993 the RPSI was 38 and all 10 females found were sterile.

Archipel de Molène

As expected for this Atlantic-exposed region, RPSI values are low but a gradient exists from the Ile de Béniguet (St 66) and the Ile Morgol (St 67) to the south-west of the Ile d'Ouessant (St 73). Sterilized females are found only in these two first islands and in the two fishing ports of the Ile de Molène (St 71) and Ile d'Ouessant (St 74), where RPSI values are higher. Significantly, one female was found in 1993 in Ouessant harbour, and this possessed an ovotestis (VDS stage 7).

DISCUSSION

The Bay of Brest is so polluted that no population of *N. lapillus* has survived in the northern part, which includes the most important harbour facilities. At Keralliou (St 28), the only animals were collected in 1992 and all females were sterile; the collecting site is close to an oyster farm which imports its stock from Cancale (north-east Brittany) and it is possible the *N. lapillus* were an accidental introduction to the site. No *N. lapillus* was found in 1993 nor in 1994. No juvenile was found in all of this part of the bay, between Fort du Portzic (St 27) and the Anse du Caro (St 29) and, with one exception, all females were sterile. The TBT water concentrations in this area exceed 10 ng/litre (Michel, P., pers. comm.). Such concentrations may explain the decline of *N. lapillus* populations on this shore; Crisp & Fischer-Piette (1959) recorded the presence of such a population in the Brest harbour in 1955.

In all of the samples collected in the Bay of Brest (St 28–46) RPSI values were high and sterilised females were found. In the Bay of Douarnenez, RPSI values were lower and sterile females uncommon. The difference in imposex level in the two bays is probably due not only to differences in TBT inputs but also to differences in the flushing rate. Indeed, Le Corre (pers. comm.) estimates the flushing rate in the Bay of Douarnenez to be just a few days, except when the Ushant thermal front is present in summer, whilst Berthois & Auffret (1970) estimated the flushing rate to be about three months in the Bay of Brest.

All *N. lapillus* populations examined showed imposex and it is evident that the background level of TBT pollution in this region is significant, even at sites with full exposure to the Atlantic, for example around Ile de Sein and Archipel de Molène. Similar observations were made by Gibbs *et al.* (1991*a*) along the south coast of Brittany, where even populations at fully-exposed sites such as the Pte de Penmarc'h exhibited well-developed imposex. Gibbs *et al.* (1991*a*) also reported sea water TBT levels in their study area. Some of this TBT pollution in the waters off west Brittany undoubtedly originates from sources to the south-east (e.g. shipyards of St Nazaire, Lorient, Concarneau) and is carried in a north-westerly direction by the residual current flowing along the Armorican Shelf (see Pingree & Le Cann, 1989). This current flows north off west Brittany and explains the different levels of imposex in the north and south of the region. TBT

emanating from the Bay of Brest increases the pollution load so that populations to the north of the bay are more highly affected than those to the south, with mainland values decreasing gradually from the Pte Saint-Mathieu (St 12, RPSI≈41) to Saint-Samson (St 4, RPSI≈7), a distance of about 20km. Even at Atlantic-exposed sites around the islands (Sts 66–68), some 10–15km offshore, the effects of contamination from the Bay of Brest is readily detectable. The degree of pollution of the coastline between the Pte Saint-Mathieu (St 12) and the mouth of the Bay of Brest (St 22) is similar because waters which leave this bay are mixed by a vortex (Salomon & Breton, 1991).

Bailey & Davies (1991) defined uncontaminated sites to have a RPSI of < 1; on this basis virtually the whole of the west Brittany coast must be considered contaminated. Even at Kerfissien (St 1), VDS stage 4 females were found. French legislation banning the use of TBT-based antifouling paints on vessels less than 25 m was introduced in 1982; the effectiveness of this legislation must be questioned, considering that high RPSI and VDSI values and high percentages of sterilised females were found in the isolated fishing ports of Sein (St 64), Molène (St 71) and Ouessant (St 74) (almost all vessels are less than 25 m length in these ports). This disregard for legislation has also been noticed by Alzieu *et al.* (1989) and Sarradin *et al.* (1991). The RPSI values found in the 1992, 1993 and 1994 surveys are similar at each site and no general amelioration in TBT pollution is apparent over the period of the survey.

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