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Trace metals accumulation in biofilms of the upper and middle reaches of Otamiri river in Owerri, Nigeria

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Abstract

This study utilized biofilms as model in ecotoxicology to estimate pollutant loading of a natural water body. Water samples were collected from six sampling locations sited between the upper and middle courses of the Otamiri River in the southeastern city of Owerri, Nigeria and fixed with conc. HNO_3. Biofilms were grown in microcosms housing serially arranged sterile glass slides at the sampling locations, harvested after 1, 2 and 3 weeks, minced in sterile sample bottles with distilled water and fixed with conc. HNO_3. Natural biofilms were also collected from submerged surfaces and fixed. Pb, Cu and Cd contents were determined in samples with atomic absorption spectrophotometer. The studentized t-test was used to compare trace metals levels in water column and biofilms, while single factor ANOVA was used to determine spatial homogeneity in mean variance. Mean Pb concentrations ranged from 1.5950-3.2900 (2.4303 ± 0.0835) mg/kg, Cu from 4.2934-7.5020 (5.6212 ± 0.1938) mg/kg and Cd from 0.0308-1.0559 (0.2082 ± 0.0005) mg/kg in the slide biofilms. However, they ranged from 0.0017-0.0267 (0.0150 ± 0.0003), 0.0333-0.6067 (0.2047 ± 0.0929) mg/L and totally undetected, respectively in water columns. Trace metals levels in slide and natural biofilms differed very markedly from those in water column (sig. t-values = 0.000, each), even as levels in slide and natural biofilms did not (sig. t-value = 0.747) at $P<0.05$. Pb and Cu concentrations increased from location 1 to 6 in both water columns and biofilms, even as there was homogeneity in spatial mean variances in slide [$F_{(1.1458)}<F_{crit(4.1300)}$] and natural biofilms concentrations [$F_{(1.2812)}<F_{crit(4.1300)}$] at $P<0.05$. Although mean Pb and Cu levels were below regulatory limits and Cd undetected in water columns, their average concentration exceedances were between 32 and 70 times higher in the biofilms. Results question the assignment of water potable based on regulatory standards alone.

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Introduction

Biofilms are consortium of microorganisms which form on solid surfaces in aqueous or wet environments (Costerton *et al.* 1994). They could be found in surface and ground waters, in drinking water piping and wastewater treatment plants, and on other technical equipment such as in the medical field (Wanner and Bauchrowitz, 2006). Biofilms execute a niche and so, interact strongly with their environment; are greatly affected by, as well as in return, affect the physical and chemical conditions in their enmeshing habitats.

Biofilms prefer to live in sessile communities (Flemming and Wingender, 2001) and include bacteria, algae, amoebas, ciliates and fungi in a great variety of compositions. Sunlight favours the growth of photoautotrophic components of biofilms such as algae and cyanobacteria and they conduct photosynthesis and thus, build up their biomass from inorganic substances. By this function therefore, these autotrophs are primal species in the trophic chain. However, in the absence of sunlight, biofilms are formed mostly by heterotrophic bacteria, which degrade organic substances, with the less frequent chemoautotrophic bacteria which utilize inorganic substances (Wanner and Bauchrowitz, 2006). In streams, algae-dominant autotrophic biofilms are mostly found on the riverbed and bacterial-dominant heterotrophic biofilms are found in the pore systems under the river bed (Lock, 1993). As biomass producers and decomposers therefore, biofilms are important components in the trophic web.

In biofilms, microorganisms are embedded in a slimy matrix which consists of extracellular polymeric substances that are excreted by the organisms themselves. These polymeric substances contain mainly high-molecular polysaccharides, proteins, other carbohydrates (such as uronic acid), and small amounts of lipids and nucleic acids (Wanner and Bauchrowitz, 2006).

As microorganisms, biofilms have been particularly utilized as interesting models in ecotoxicology to estimate the pollutant loading of natural water bodies and the hazard potential of toxic substances. Their suitability for this purpose lies in the central role they play in ecosystem metabolism and interaction with toxic substances (Doering and Uehlinger, 2006), and on the other hand because, as immobile biological elements, they accumulate pollutants over a long period of time and may thus reveal chronic impacts (Wanner and Bauchrowitz, 2006). Examples of such pollutants are the trace metals (Pb, Cd, Cu, Zn, Al, etc), which are recalcitrant in the environment.

Though they are important trace nutrients for water organisms, they can also be toxic at elevated enough concentrations. An exploratory determination of levels of some trace elements of the Otamiri River, one of the major river systems in Owerri, the capital of Imo State, southeastern Nigeria revealed concentrations that were below permissible limits by regulatory bodies, or even undetected by analytical instruments used. However, even low metal concentrations can have negative impacts on water organisms as well as local consumers, especially when considered on the merit of their bioaccumulative potentials over a length of time. Unfortunately, current researches in this area have been concentrated on the comparison of concentrations with these regulatory standards as criteria for assigning the river water potable. This current research therefore investigated the accumulation potentials of some heavy metals of environmental and public health importance (Cu, Pb and Cd) in consortium of resident biofilms of Otamiri River against background levels in water columns. The study approaches are as follows:

- - Determination of the concentrations of the trace elements in slide and natural biofilms of the river

- Comparison of the concentrations of the trace elements in biofilms with water column levels as well as regulatory standards, and
- Determination of spatial variation in trace metals concentrations in biofilms.

Materials and methods

Study area

Owerri, the capital city of the southeastern Imo State, Nigeria lies within latitude 05° 29´ 06s and longitude 07° 02´ 06s (Fig. 1). The area experiences a longer wet (which lasts from April to November) than dry season (which lasts the rest of the year) (Victor *et al.* 2011). Mean daily maximum temperature range between 28 and 35 °C, while daily minimum values range between 19 and 24 °C, with average humidity of up to 80%. The vegetation is dominated by semi-deciduous forest that had been altered by agricultural and other anthropogenic activities (Onweremadu *et al.* 2008) and the dominant top-soil is moderately humus in composition. The Otamiri River, one of the two major rivers that traverse the city rises from Egbu and courses through the city into the neighbouring Rivers State. The river provides domestic source of water and serves for fisheries as well as artisanal sand mining activities by local inhabitants.

Sampling locations

Six sampling locations (SL) designated along the course of the river were utilized in this research. SL 1 and SL 2, the reference locations, were situated at Egbu, the upper course of the river. SL 3 and SL 4 were situated at Umuchima, about 23km from SL 2, while SL 5 and SL 6 were situated about 2km from SL 4 in the Federal University of Technology, Owerri (FUTO). With the exception of the reference sampling locations, the other locations were in areas of ongoing sand mining and greater inputs of anthropogenic pollutants.

Water sample collection

Water samples were collected in 250mL plastic containers and fixed with conc. HNO_3 in the ratio of 2:500.

Establishment of biofilm microcosms

Biofilms were grown and investigated under relatively natural conditions in the so called microcosms, consisting of plastic containers housing serially arranged sterile glass slides (Figure 2). Three such microcosms were installed at each sampling location at pelagial zones, fastened with inextensible ropes to riparian holdfasts (such as vegetation) and allowed in place. Serial harvests were made after 1, 2 and 3 weeks from the date of installation.

The microorganisms contained in the water body colonized the surfaces of glass slides provided in the microcosms, thus forming mixed biofilms. After harvests, biofilms were scraped off the surfaces of glass slides and transferred into sterile sample bottles, minced with distilled water and fixed with 2 drops of conc. HNO_3. However, biofilms were also collected from natural, submerged surfaces at the same locations and also fixed.

Laboratory analysis

The trace metals concentrations were determined with the use of a Varian 600 AA Atomic Absorption flame atomization Spectrometer, as adopted from APHA (1998).

Statistical analysis

Descriptive statistics was utilized in the transformation of data. The studentized t-test of significant variation was used in the comparison of trace metals levels in water column and biofilms, while the test of spatial equality in mean variance of trace metals concentrations was conducted with the single factor ANOVA. Comparisons were also made between exceedance times in trace metals accumulations on biofilms and water column levels.

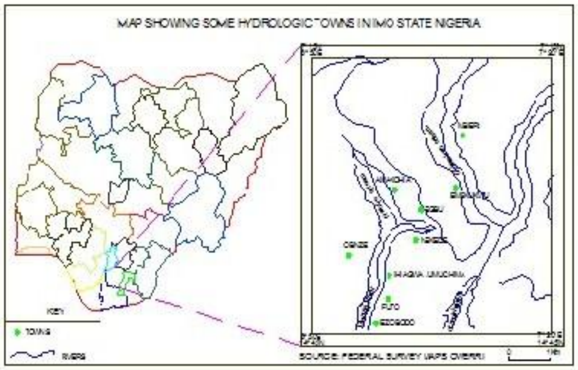

Fig. 1. Location map of Nigeria showing Otamiri River in Imo State.

Fig. 2. Six sets of glass microcosm assemblages.

Results

Trace metals concentrations in biofilms and water columns

Whereas mean lead concentration ranged from 1.5950-3.2900 (2.4303 ± 0.0835) (Table 1) and 1.7033-3.1234 (2.4200 ± 0.0694) mg/kg (Table 2) in slide and natural biofilms, respectively, mean concentrations in water column ranged from 0.0017-0.0267 (0.0150 ± 0.0003) mg/l (Table 3). For copper, concentrations ranged from 4.2934-7.5020 (5.6212 ± 0.1938) mg/kg in slide biofilms, 4.3867-6.8100 (5.6302 ± 0.1144) mg/kg in natural biofilms, and 0.0333-0.6067 (0.2047 ± 0.0929) mg/l in water column. While cadmium concentrations ranged from 0.0308-1.0559 (0.2082 ± 0.0005) mg/kg in slide biofilms and 0.0347-0.0560 (0.0431 ± 0.0006) mg/kg in natural biofilms, it was generally undetected in water columns.

In a pairwise comparison between slide biofilms and water column, as well as between natural biofilms and water columns, the studentized t-test revealed that concentrations of trace elements differed by between 2 and 3 mean units on the average after the 3 weeks sampling period (Table 4). However, between slide and natural biofilms, concentrations differed by an insignificant 0.06 mean units only. At 0.637, 0.580, and 0.956, the correlations between slide biofilms and water column, natural biofilms and water columns, and slide biofilms and natural biofilms, respectively were statistically significant at $P<0.05$.

Albeit, trace metals concentrations in slide biofilms perfectly differed significantly from those in water columns; and so did concentrations in natural biofilms from those in water columns (sig t-values = 0.000 each) at $P<0.05$. Conversely, slide and natural biofilms did not differ significantly in metals concentrations (sig. t-value = 0.747) as $P>0.05$.

Table 1. Trace metals concentrations in slide biofilms of Otamiri River in Owerri, Nigeria.

Parameters (mg/kg) Limits	Weeks	SL 1	SL 2	SL 3	SL 4	SL 5	SL 6	WHO/FME (mg/l)
Pb	1	1.5300	2.0000	2.7500	2.4200	3.1400	3.1200	
	2	1.6200	1.8000	2.3500	2.3000	2.7200	3.3500	
	3	1.6350	2.1000	2.4000	2.3000	2.8100	3.4000	
	Mean	1.5950 (± 0.0328)	1.9667 (± 0.0882)	2.5000 (± 0.1258)	2.3400 (± 0.0400)	2.8900 (± 0.1277)	3.2900 (± 0.0862)	0.0500
Cu	1	3.8800	5.2000	6.2500	4.8000	6.4200	5.1000	
	2	4.2000	5.6000	6.3000	4.9000	7.2000	5.8000	
	3	4.8002	5.8000	6.5200	5.0000	7.5020	5.9100	
	Mean	4.2934 (± 0.2697)	5.5333 (± 0.1764)	6.3567 (± 0.0829)	4.9000 (± 0.0577)	7.0407 (± 0.3223)	5.6033 (± 0.2537)	0.1000
Cd	1	0.0630	0.0310	0.0400	0.0250	0.0420	0.0200	
	2	0.0408	0.0520	0.0380	0.0350	0.0410	0.0360	
	3	0.0640	0.0600	0.0415	0.0400	0.0422	0.0365	
	Mean	1.0559 (± 0.0076)	0.0477 (± 0.0087)	0.0398 (± 0.0010)	0.0333 (± 0.0044)	0.0417 (± 0.0004)	0.0308 (± 0.0054)	0.0100

SL = sampling location, WHO = World Health Organization, FME = Federal Ministry of Environment

Spatial variations in metals concentrations in biofilms

Generally, metals concentrations (except for Cd that fairly decreased) increased from sampling location 1 to 6 in the biofilms. While least mean Pb concentrations of 1.5950 and 1.7033 mg/kg were recorded in sampling location 1 on slides and natural biofilms, respectively, maximum levels of 3.2900 and 3.1234 mg/kg were recorded in the biofilms in sampling location 6. The least mean concentrations of 4.2934 and 4.3867 mg/kg were recorded in sampling location 1 on slide and natural biofilms, respectively for Cu, and maximum concentrations of 7.0407 and 6.8100 mg/kg were recorded in sampling locations 5 and 4 on slide and natural biofilms, respectively for the element. Least mean concentrations of 0.0308 and 0.0347 mg/kg were recorded in sampling locations 6 and 4&6, respectively for Cd, and maximum levels of 1.0559 and 0.0560 mg/kg were recorded in location 1 on slide and natural biofilms, respectively for the element. The test of homogeneity in mean variance of concentrations across the locations revealed significant equality (homogeneity) in slide [$F_{(1.1458)}<F_{crit(4.1300)}$] and natural biofilms [$F_{(1.2812)}<F_{crit(4.1300)}$] at $P<0.05$.

Regulatory standards and exceedance times of trace metals concentrations

Although mean Pb and Cd levels were below the World Health Organization and Federal Ministry of Environment's (FME, 2001) maximum permissible limits of 0.0500 and 0.0100 mg/l for drinking water, respectively, their concentrations in both slide and natural biofilms were many folds higher than these in water columns. Exceedances for Pb in slide biofilms were between 32 and 66 times over water column concentration units (Fig. 3), and between 34 and 63 times over this in natural biofilms (Fig. 4). Cu concentrations in slide biofilms exceeded those in water columns by between 43 and 70 unit times (Fig. 5), and in natural biofilms, exceedance was between 44 and 68 unit times (Fig. 6). For Cd, slide biofilms concentrations exceeded levels in water columns by between 3 and 6 unit times (Fig. 7), and in natural biofilms, exceedance was between 4 and 6 unit times (Fig. 8).

Table 2. Trace metals concentrations in natural biofilms of Otamiri River in Owerri, Nigeria.

Parameters (mg/kg) Limits	Weeks	SL 1	SL 2	SL 3	SL 4	SL 5	SL 6	WHO/FME (mg/l)
Pb	1	1.6300	2.0801	2.6100	2.1500	3.1500	3.1002	
	2	1.7000	1.9501	2.2100	2.2500	2.7200	3.1200	
	3	1.7800	2.0600	2.5000	2.4000	3.0000	3.1500	
	Mean	1.7033 (± 0.0433)	2.0301 (± 0.0404)	2.4400 (±0.1193)	2.2667 (± 0.0727)	2.9567 (±0.1260)	3.1234 (± 0.0145)	0.0500
Cu	1	4.0100	6.0920	4.3100	6.7500	6.8000	5.2000	
	2	4.5000	6.2320	4.5000	6.8000	6.3000	5.2000	
	3	4.6500	6.3100	4.8000	6.8800	6.5000	5.5100	
	Mean	4.3867 (± 0.1932)	6.2113 (± 0.0638)	4.5367 (± 0.1426)	6.8100 (± 0.0379)	6.5333 (± 0.1453)	5.3033 (± 0.1033)	0.1000
Cd	1	0.0750	0.0200	0.0420	0.0280	0.0450	0.0260	
	2	0.0430	0.0560	0.0410	0.0320	0.0400	0.0380	
	3	0.0500	0.0610	0.0480	0.0440	0.0460	0.0400	
	Mean	0.0560 (± 0.0097)	0.0457 (± 0.0129)	0.0437 (±0.0022)	0.0347 (± 0.0048)	0.0437 (±0.0019)	0.0347 (± 0.0044)	0.0100

SL = sampling location, WHO = World Health Organization, FME = Federal Ministry of Environment

Table 3. Trace metals concentrations (mg/l) in water columns of Otamiri River in Owerri, Nigeria.

Parameters Limits	SL 1	SL 2	SL 3	SL 4	SL 5	SL 6	WHO/FME (mg/l)
Pb	0.0017 (±0.0017)	0.0017 (±0.0009)	0.0233 (±0.0067)	0.0200 (±0.0000)	0.0167 (±0.0033)	0.0267 (±0.0067)	0.0500
Cu	0.4167 (±0.1922)	0.6067 (±0.2987)	0.0733 (±0.0291)	0.0500 (±0.0058)	0.0333 (±0.0033)	0.0480 (±0.0280)	0.1000
Cd	BDL	BDL	BDL	BDL	BDL	BDL	0.0100

SL = sampling location, BDL = below detectable limit, WHO = World Health Organization, FME = Federal Ministry of Environment

Table 4. Pairwise comparison of trace metals concentrations on biofilms and waters columns

	Pairs	Mean	SE	Correlation(r)	Sig. r-value	t	Sig. t-value
Pair 1	SB_{films} W_{column}	2.7532 0.0732	0.5614 0.0387	0.637	0.004	4.986	0.000
Pair 2	NB_{films} W_{column}	2.6978 0.0732	0.5751 0.0387	0.580	0.012	4.741	0.000
Pair 3	SB_{films} NB_{films}	2.7532 2.6978	0.5614 0.5751	0.956	0.000	0.327	0.747

SE = standard error

Discussion

The very high concentrations of the trace metals in biofilms than in water column confirms that biofilms are good candidates for bioaccumulation studies (Doering and Uehlinger, 2006) lending to the investigation of chronic impacts (Meylan and Behra, 2006). Additionally, the study revealed that the assigning of water as clean and potable based on regulatory standards alone could be inadequate and misleading, as just 3 weeks exposure of the biological models revealed several folds of exceedances over them. This applies specifically to persistent pollutants, such as the trace metals in the environment and confirms that even at low or undetectable concentrations, they can still exert negative impacts on water organisms as well as local consumers (Behra *et al.* 2006).

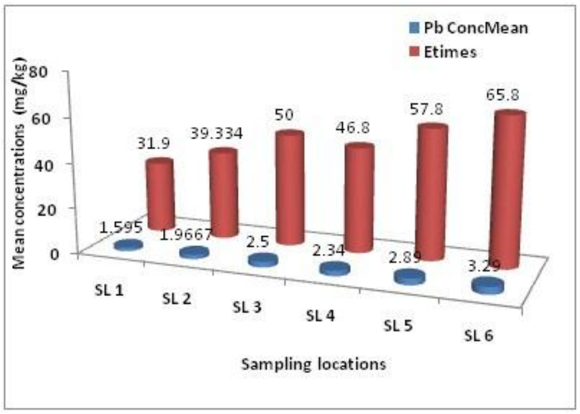

Fig. 3. Spatial exceedance in mean lead accumulations on slide biofilms of Otamiri River.

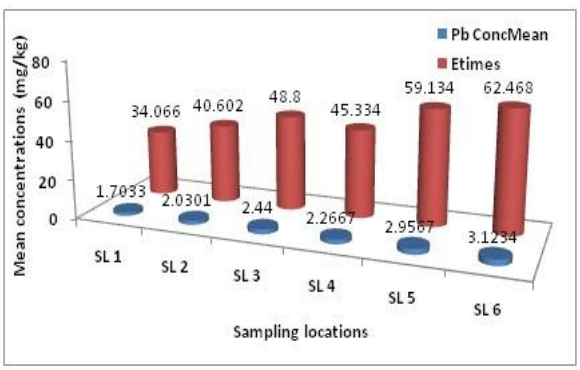

Fig. 4. Spatial exceedance in mean lead accumulations on natural biofilms of Otamiri River.

The statistically significant difference between metal concentrations in the biofilms and water columns reinforces the bioconcentration and biomagnification potentials of the candidate bioaccumulators (Meylan *et al.* 2006) against background water columns. The insignificantly different levels of trace metals on slide and natural biofilms indicate that both surfaces are similar in reception and that the microcosm setups were essentially similar to natural conditions.

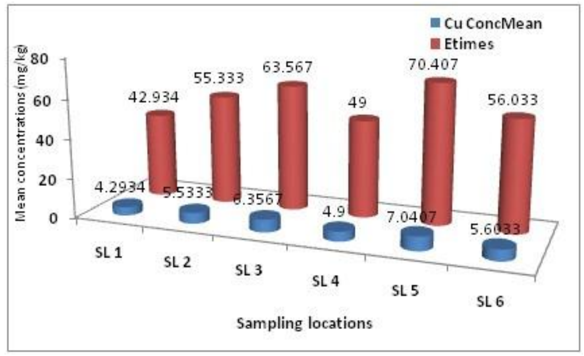

Fig. 5. Spatial exceedance in mean copper accumulations on slide biofilms of Otamiri River.

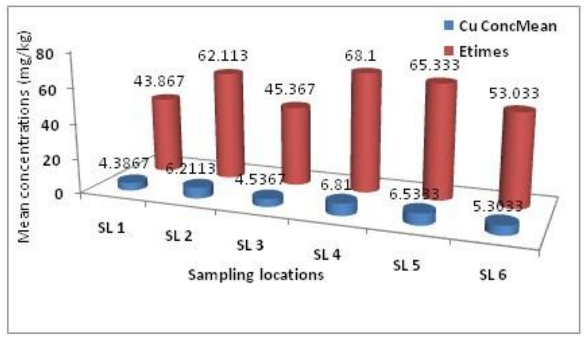

Fig. 6. Spatial exceedance in mean copper accumulations on natural biofilms of Otamiri River.

The general numerical increase in metal concentrations from the reference location to SL 6 (except in Cd) corresponds with increasing anthropogenic inputs along the course of the river in the metropolitan town of Owerri. The Egbu sampling locations receives comparatively lower pollutant inputs due mainly to the suburb disposition of the area, and also as the upper course of the river, the locations are comparatively pristine. Conversely, the trend observed in Cd concentrations indicates the exclusion of the trace element in anthropogenic inputs and possible serial dilutions in water mixture along the river course. However, the measurable levels of Cd in biofilms contrast highly with their undetectable status in water columns. This buttresses the fact that even analytically absent recalcitrant species of pollutants could build up over reasonable periods in the environment, and thus constitute adverse effects.

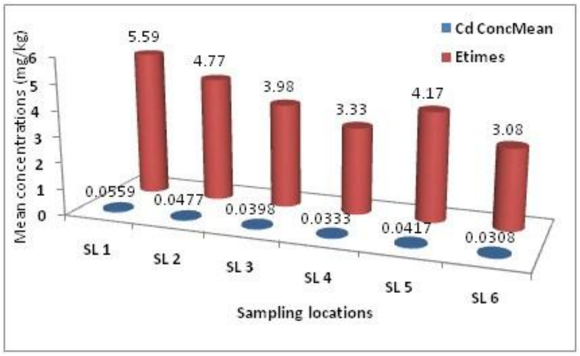

Fig. 7. Spatial exceedance in mean cadmium accumulations on slide biofilms of Otamiri River.

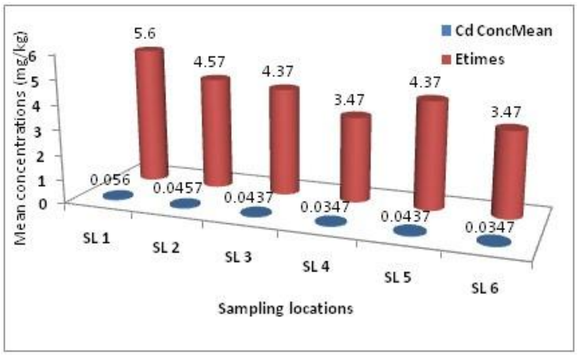

Fig. 8. Spatial exceedance in mean cadmium accumulations on natural biofilms of Otamiri River.

Albeit, the homogeneity observed in mean variance of the trace metals contents across the sampling locations indicate the lotic dynamics of the aquatic ecosystem and its homogenizing abilities, as opposed to lentic partitioning in concentrations.

Summary

The current study revealed the high accumulation potential of a consortium of resident microbial surface colonizers in an aquatic ecosystem. With empirical evidence, it questions the qualification of water as safe and unpolluted based on regulatory standards, especially concerning the recalcitrant and persistent categories. Accordingly, the study revealed that low levels of trace elements in water columns could bioconcentrate and biomagnify over time on biofilms and so, exceed water column and regulatory threshold limits by many folds. The lotic nature of the river did not permit significant spatial variation in concentrations, even though numerical levels majorly increased from the pristine to more perturbed locations, downstream.

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