

Alkylated Polycyclic Aromatic Compounds in Road Runoff Are an Environmental Risk and Should Be Included in Future Investigations

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Abstract: Polycyclic aromatic compounds (PACs) and metals are important contaminants in road runoff. Vital mitigation measures against aquatic contamination from road runoff include the use of sedimentation ponds. However, it has been demonstrated that ecosystems in sedimentation ponds might be affected by road runoff. Sediments from six natural ponds and 27 sedimentation ponds were investigated to determine the environmental risk of contaminants. The amount of traffic on the respective roads associated with the sedimentation ponds was correlated with the environmental concentrations. Quantification included seven metals, the 16 US Environmental Protection Agency polycyclic aromatic hydrocarbons, alkylated PACs, dibenzothiophene, benzo[e]pyrene, and perylene. The environmental risk was assessed according to the European Union Water Framework Directive guidelines. Sedimentation ponds had a higher level of contaminants than natural ponds, and environmental risks were dominated by the concentration of PACs. Alkylated PACs contributed to the environmental risk posed by PACs and should be included in future monitoring. Only Cu and Zn were measured at concentrations above the environmental quality standards (EQSs), while 13 PACs exceeded the EQS. Sediment concentrations of Cu, Zn, and most PACs correlated significantly with the amount of traffic. The sources of PACs were determined by source apportionment ratios between PACs. Alkylation and pyrogenic indices showed that sources in natural ponds were of mostly pyrogenic origin, whereas in sedimentation ponds they were predominantly petrogenic. Asphalt was the probable main source of PACs. A hierarchical clustering technique was used to investigate both the environmental risks and the pattern of PACs in the ponds and revealed that a few sedimentation ponds were not protecting the environment as intended because the upper sediment layers had not been regularly dredged. *Environ Toxicol Chem* 2022;41:1838–1850. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Roads and traffic have a significant impact on the aquatic environment, and the discharge of polluted storm water from roads is of major concern (Meland, 2016). Runoff from roads contains a mixture of contaminants such as particles, nutrients,

metals, salts, and organic micropollutants. This chemical cocktail is conveyed and discharged to water bodies, where it may cause chemical and ecological damage (Maltby et al., 1995; Meland et al., 2010; Meland, Sun, et al., 2020). To mitigate and prevent environmental harm, the use of various road runoff treatment systems has now become standard practice along major roads in most countries (Meland, 2016). In Norway, as in many other countries, wet sedimentation ponds are often a preferred technology. Important treatment processes in such ponds are settling of particles (with adherent pollutants), sorption processes, and biodegradation. The ponds are also quite rapidly colonized by plants and animals (Meland, Sun, et al., 2020). However, the inevitable pollution content in these systems may potentially be hazardous and in

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the worst case become sink habitats and ecological traps (Clevenot et al., 2018).

With regard to organic pollutants, polycyclic aromatic compounds (PACs) are among the group of chemicals that is most studied in road runoff. They consist of several hundred different substances/compounds and can include metabolites generated by exposed organisms. Despite this vast number of substances, only a selected group of 16 PACs are normally monitored in environmental and toxicological studies. The list of 16 PACs is known as the US Environmental Protection Agency (USEPA) polycyclic aromatic hydrocarbons (PAH16) and have been included in US environmental regulations since the late 1970s (Keith, 2015). Since then, analytical chemistry has evolved considerably; and in a more recent publication, Andersson and Achten (2015) call for an extension of the 16 USEPA PAHs, proposing a list of 40 PACs to analyze in environmental samples. Their argument was mainly that 1) several commonly occurring PAHs possessing high toxicity are not included in the USEPA PAH16 list and 2) alkylated derivatives are not represented despite mounting evidence that their toxic potential may easily surpass that of the parent compounds. They also advocate analyzing heterocyclic PACs.

From vehicles, the main sources of PACs are exhaust, oil and gasoline spill, and tire wear. Wear of asphalt containing bitumen is the major source from road surfaces (Hwang et al., 2018), and the use of studded tires in the winter season increases the rate of road wear. Also, PACs may be released from timber bridges, which are coated by creosote that may sweat and drip (Mahnert & Hundhausen, 2018). As in most other studies, monitoring of PACs in road runoff has normally been limited to the USEPA PAH16. However, recent environmental studies have started to pay attention to the alkylated PAC forms, that is, PACs with one or several carbon chains attached to the ring structure. For example, studies conducted in Alberta, Canada (Athabasca oil sands), where crude oil and heavy bitumen are extracted from oil sands, have documented high levels of alkylated PACs in surrounding water, sediment, and biota (Kurek et al., 2013; Wang et al., 2014). Similarly, previous investigation has documented high levels of alkylated PACs in sediment collected from sedimentation ponds receiving road runoff and tunnel wash water (Girardin et al., 2020; Grung et al., 2021; Meland et al., 2019). A recent Norwegian study on water samples obtained from a noise barrier, built from scrap tires, showed similar findings (i.e., the alkylated PACs summed up to 94% of the total PAC concentrations [Meland, Hultman, et al., 2020]). Based on these studies, we speculate that monitoring programs limited to only 16 PAHs severely underestimate the real total concentrations of PACs in road runoff. Consequently, the environmental risk they constitute is also underestimated. Because of the limited number of studies reporting alkylated PACs, this statement with regard to risk is encumbered with great uncertainties. The present study aims to close some of these uncertainties by assessing PACs and alkylated PACs in sediment from 27 Norwegian sedimentation ponds receiving road runoff and tunnel wash water in comparison with six natural ponds.

MATERIALS AND METHODS

Description of sedimentation ponds and natural ponds

The ponds (whether natural or receiving runoff) and the amount of traffic on the roads (annual average daily traffic [AADT]) are listed in Supporting Information, Table S1. The AADT varied between 0 (natural ponds) and 66,000, with a median of 23,400. The locations of the sampled ponds are shown in Figure 1. All ponds are denoted by a three-letter abbreviation, and natural ponds are denoted with an _R suffix to differentiate them from sedimentation ponds.

Sedimentation ponds ($n=27$) located along highways E6 and E18 in Oslo, Viken, Vestfold and Telemark counties were investigated. All sedimentation ponds received road runoff from road, tunnel, or both. All sedimentation ponds differ according to parameters such as AADT, size, age, amount of vegetation, and water level. However, all sedimentation ponds are constructed similarly including two basins, one slam basin (forebay) and one main basin. All sediment samples were collected from the main basin. The first basin removes larger particles through sedimentation, followed by an accumulation in the bottom sediment of the pond. Smaller particles settle in the main basin before treated water is discharged to the recipient water body. Photos of selected sedimentation ponds are shown in Supporting Information, Figure S1.

Six natural ponds were investigated in the present study. The natural ponds were unconnected to heavy traffic roads or other known sources of emissions for the pollutants of interest. The natural ponds were placed in residential areas or in fields connected to woodland.

Collection of sediment samples

Sediment samples from the ponds were collected in 2017 and 2018 by means of a Van Veen grab, sampling approximately the upper 5 cm of sediment layers. Each sample was made by mixing three to five subsamples from different places in the pond, depending on local factors such as pond size and accessibility. The Van Veen grab was cleaned and disinfected with Virkon S between each sampling. The sediment was sampled in 100-ml glass jars that had been heated to 600 °C prior to sampling, and samples were stored at –20 °C prior to analysis.

Analyses of PACs and metals

The analyses of PACs were performed at the Norwegian Institute for Water Research's research laboratory and metals at Eurofins Norway (www.eurofins.no); both laboratories are accredited by Norwegian accreditation (NS-EN ISO/IEC 17025). All methods were validated according to internal procedures.

The method utilized for analyses of PACs are described in detail in Meland et al. (2019), and the analytical details are therefore not described in the present study; but a brief summary is given. Internal standards were added to sediment

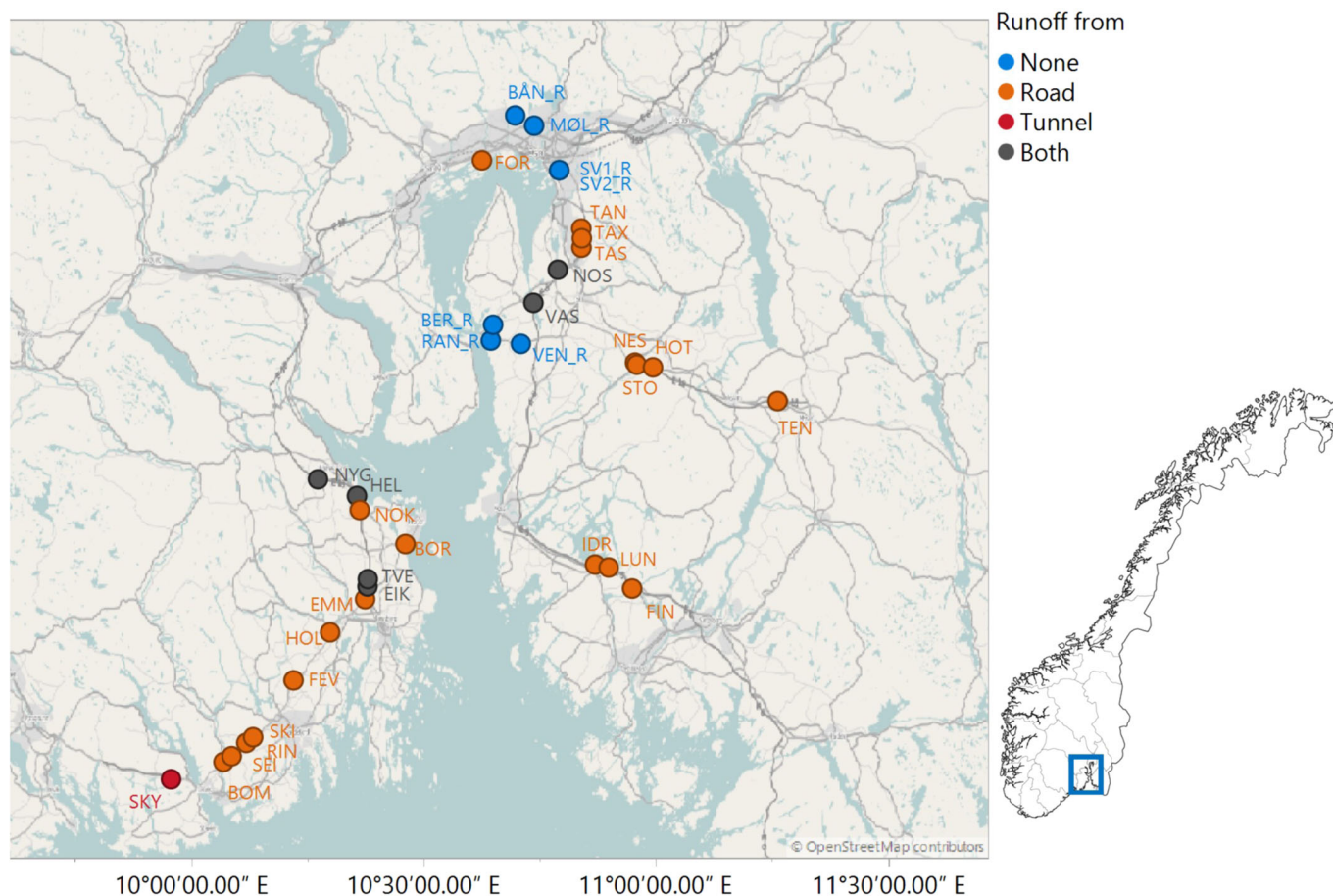


FIGURE 1: Map of sedimentation ponds and natural ponds that were included in the investigations. The ponds are colored according to which type of runoff they received. Each pond is named by three-letter abbreviations listed in Table S1 (Supporting Information). Natural ponds have a suffix of R. One natural pond (SV_R) was sampled twice, and the two samples are denoted SV1_R and SV2_R.

samples and extracted with dichloromethane. The extract was further cleaned by means of gel permeation chromatography before analysis by gas chromatography coupled to a mass spectrometer in single ion monitoring mode. A total of seven internal standards were used for quantification, and 37 PACs were analyzed, including the PAH16 compounds (Table 1). Alkylated PACs of naphthalenes (C1–4), fluorenes (C1–3), dibenzothiophenes (C1–3), phenanthrenes/anthracenes (C1–4), pyrenes/fluoranthenes (C1–2), and chrysenes (C1–2) were also analyzed. In addition to the PAH16 and alkylated PACs, dibenzothiophene, perylene, and benzo[e]pyrene were analyzed. Retention times for the standards employed in the analytical method have been included in Supporting Information, Table S2. The method also builds on identifications of non-target methods employed in Grung et al. (2017). Two samples of certified reference material (SRM 1944; National Institute of Standards and Technology [NIST]) and two blank samples were analyzed with the samples. The median of deviations between measured concentrations and certified values for all PAHs of interest were within 2 standard deviations of the reference material. The medians of the deviations for the two NIST samples were –13% and –4%, while the ranges were –41% to +28% and –40% to +24%, respectively.

The following metals were analyzed at Eurofins: Zn, Cu, Pb, As, Cd, Cr, and Ni. However, three reference ponds (four samples) were not analyzed for As and Cr (BÅN_R, MØL_R, SV1_R, and SV2_R) because they were part of a sampling campaign in 2017 and were not included in the suite of metal analyses at that time.

Comparison with Water Framework Directive guidelines

The Water Framework Directive (WFD) specifies environmental quality standard (EQS) values for priority substances (European Commission, 2013). In Norway, both the priority substances and the river basin–specific pollutants are classified into five environmental classes based on their ecotoxicity. The five classes are *background*, *good*, *moderate*, *poor*, and *very poor*. The PACs and metals that are assigned with EQS values (European Commission, 2013) and classification schemes according to Norwegian regulation (Direktoratgruppen vann-direktivet, 2018) are shown in Table 1. The *moderate*, *poor*, and *very poor* classes are based on ecotoxicity effects, whereas for compounds that have data about concentrations in pristine

TABLE 1: Polycyclic aromatic compounds analyzed with abbreviation used in text and figures

Compound	Abbreviation	<i>m/z</i>	WFD
Naphthalene- <i>d8</i>		136	
Acenaphthylene- <i>d8</i>		160	
Biphenyl- <i>d10</i>		164	
Dibenzothiophene- <i>d10</i>		192	
Pyrene- <i>d10</i>		212	
Benzo[<i>a</i>]anthracene- <i>d12</i>		240	
Perylene- <i>d12</i>		264	
Naphthalene	N	128	PS
C1-naphthalenes	N_C1	142	
C2-naphthalenes	N_C2	156	
C3-naphthalene	N_C3	170	
C4-naphthalenes	N_C4	184	
Acenaphthylene	ACY	152	RBSP
Acenaphthene	ACN	154	RBSP
Fluorene	F	166	RBSP
C1-fluorenes	F_C1	180	
C2-fluorenes	F_C2	194	
C3-fluorenes	F_C3	208	
Anthracene	A	178	PS
Phenanthrene	P	178	RBSP
C1-phenanthrenes/anthracenes	P_C1	192	
C2-phenanthrenes/anthracenes	P_C2	206	
C3-phenanthrenes/anthracenes	P_C3	220	
C4-phenanthrenes/anthracenes	P_C4	234	
Dibenzothiophene ^a	D	184	
C1-dibenzothiophenes	D_C1	198	
C2-dibenzothiophenes	D_C2	212	
C3-dibenzothiophenes	D_C3	226	
Fluoranthene	FLA	202	PS
Pyrene	PY	202	RBSP
C1-pyrenes/fluoranthenes	PY_C1	216	
C2-pyrenes/fluoranthenes	PY_C2	230	
Benzo[<i>a</i>]anthracene	BaA	228	RBSP
Chrysene	C	228	RBSP
C1-chrysenes	C_C1	242	
C2-chrysenes	C_C2	256	
Benzo[<i>b,j</i>]fluoranthene	BbjF	252	PS (b not j)
Benzo[<i>k</i>]fluoranthene	BkF	252	PS
Benzo[<i>e</i>]pyrene ^a	BeP	252	
Benzo[<i>a</i>]pyrene	BaP	252	PS
Perylene ^a	PER	252	
Indeno[1,2,3- <i>cd</i>]pyrene	IP	276	PS
Benzo[<i>ghi</i>]perylene	BgP	276	PS
Dibenz[<i>ac/ah</i>]anthracene	DahA	278	RBSP
PAH16			RBSP
Arsenic			RBSP
Cadmium			PS
Chromium			RBSP
Copper			RBSP
Lead			PS
Nickel			PS
Zinc			RBSP

^aNonalkylated PACs that are not included in PAH16.

The coloring scheme of the cells is used in figures of fingerprinting of polycyclic aromatic compounds (PACs). The mass-to-charge ratios used for gas chromatographic–mass spectrometric quantification are also shown. The PACs and metals that are included in the Water Framework Directive are marked as either European Union priority substances or as river basin–specific pollutants.

m/z = mass-to-charge ratio; WFD = Water Framework Directive; PAH = polycyclic aromatic hydrocarbon; PS = priority substance; RBSP = river basin–specific pollutant.

areas, a *background* classification was made. The EQS (which is based on chronic toxicity) is the limit between *good* and *moderate* classes (equivalent to chemical classification of the WFD). Chronic toxicity (which correlates to the maximum allowable concentration [MAC] of the WFD) is the limit between the *medium* and the *poor* classes. A concentration above five times the MAC is the limit between the *poor* and the *very poor*

classes. The five classes are shown in relevant figures with the following colors: blue, green, yellow, orange, and red. For more details of these classes, see Bakke et al. (2010).

For comparisons of environmental risk between different groups of chemicals, the environmental class was normalized to achieve a continuous number. Supporting Information, Figure S3, explains the process of normalizing class with

three PACs. Within each environmental class, the environmental concentration was expressed as a number by interpolation between the upper and lower limits for that class (Classes 1–5). For concentrations above Class 5, the resulting environmental class was normalized so that each increment in concentration of Class 5 gave an increase of +1 class.

$$\text{Environmental class for conc.} > \text{conc. Class 5} \\ = \frac{[\text{conc.}] - [\text{conc. Class 5}]}{[\text{conc. Class 5}]} + 5 \quad (1)$$

This means that an environmental Class of 6 is 10 times the EQS MAC, while Class 7 is 15 times the EQS MAC. This normalization is only used in the presentation of environmental class in boxplots; otherwise, Class 5 (very bad) is used for classification. The purpose of the normalization was to prioritize which contaminants represent the most hazard and environmental risk.

PAC source apportionment

Polycyclic aromatic compounds can come from different sources, and the pattern and ratios between PACs can be used as information about the sources (Stogiannidis & Laane, 2015). Among the most useful source indices is the pyrogenic index (PI), which indicates whether the source of the PACs is petrogenic or pyrogenic. The PI is the ratio of the sum of the concentrations of USEPA priority unsubstituted three-to-six ring PAHs to the sum of the concentration of the five target alkylated PAH homologs (N, F, D, P, C). The PI ranges from 0.8 to 2.0 for pyrogenic sources and is much lower for petrogenic sources (Stogiannidis & Laane, 2015). The equation for calculating the PI is as follows:

$$\text{PI} = \frac{\text{ACY} + \text{ACE} + \text{A} + \text{PY} + \text{FLA} + \text{BaA} + \text{BbF} + \text{BkF} \\ + \text{BeP} + \text{BaP} + \text{BghiP} + \text{IP} + \text{DahA}}{\text{N}(1-4) + \text{P}(1-4) + \text{D}(1-3) + \text{F}(0-4) + \text{C}(0-4)} \quad (2)$$

In these investigations, we did not measure F₄, C₃, and C₄, so these compounds were not included in our calculations of the PI. The calculations of the PI were quality-assured by assuming an asphalt isomeric fingerprint, and a new PI was calculated with the nondetermined isomers (Stogiannidis & Laane, 2015, Supporting Information, Figure S25). The missing data did not significantly change any PI and, most importantly, did not alter the conclusions regarding PAHs being either petrogenic or pyrogenic. In addition, the analytical method employed did not separate benzo[b]fluoranthene from benzo[j]fluoranthene, which means that we used benzo[b,j]fluoranthene in calculations.

A recently developed index by Hindersmann et al. (2020) identified the source of petrogenic and pyrogenic PACs by utilizing the ratio of nonalkylated PACs ($\Sigma\text{C0-PACs}$) to C₂-alkylated PACs ($\Sigma\text{C2-PACs}$). This is calculated as the relative ratio $\Sigma\text{C0}/(\Sigma\text{C0} + \Sigma\text{C2})$ and referred to as the *PAH alkylation index*. In our case, the alkylation index was calculated as follows:

Alkylation index

$$= \frac{\text{N} + \text{F} + \text{A} + \text{P} + \text{D} + \text{FLA} + \text{PY} + \text{BaA} + \text{C}}{(\text{N}_{\text{C2}} + \text{F}_{\text{C2}} + \text{D}_{\text{C2}} + \text{P}_{\text{C2}} + \text{PY}_{\text{C2}} + \text{C}_{\text{C2}})} \quad (3) \\ + (\text{N} + \text{F} + \text{A} + \text{P} + \text{D} + \text{FLA} + \text{PY} + \text{BaA} + \text{C})$$

In addition, we used two other indices which also attempt to determine pyrogenic–petrogenic differences by investigating the ratios between high- and low-molecular weight PAHs. We employed two different ratios from the equations of Stogiannidis and Laane (2015).

$$\text{L/H} = \frac{\text{P} + \text{A} + \text{FLA} + \text{PY}}{\text{BaA} + \text{C} + \text{BkF} + \text{BaP} + \text{IP} + \text{DahA} + \text{BgP}} \quad (4)$$

$$\text{LPAH/HPAH} \\ = \frac{\text{N} + \text{ACE} + \text{ACY} + \text{F} + \text{P} + \text{A}}{\text{FLA} + \text{PY} + \text{BbF} + \text{BkF} + \text{BaP} + \text{IP} + \text{DahA} + \text{BgP}} \quad (5)$$

Statistical treatment

Data treatment, statistical analyses, and graphical outputs were performed with the computer program JMP, Ver 15.2.0 (SAS Institute). Whenever assumptions of normality and equal variance on data or log-transformed data were met, a *t* test or analysis of variance (ANOVA), followed by Tukey's post hoc test, was performed for group comparisons, while the least squares method was used for regression analysis. When the assumption of equal variance was not met, Welch's *t* test was used. The level of statistical significance was set at $p < 0.5$. For linear correlation of PAC concentrations with AADT, the sum of the alkylated analogues was added to the parent nonalkylated analogue before linear regression. For data below the limit of quantification (LOQ), half of the LOQ was used.

Clustering is a multivariate technique that groups observations together that share similar values across a number of variables. Hierarchical clustering was used as a method for identifying ponds with the same level of toxicity and similar PAC pattern. We used the hierarchical clustering function in JMP and Ward's method for defining the distances between clusters. In the case of PACs, the numbers were log-transformed prior to clustering. In the data sets there were eight data points missing (As and Cr in four samples), which were imputed by multivariate normal imputation to calculate pairwise covariances to construct a covariance matrix for the response columns. Then, each missing value was imputed by a method that is equivalent to regression prediction using all the predictors with no missing values for the given observation. If the constructed covariance matrix was not definite, missing values were imputed using their column means.

RESULTS AND DISCUSSION

Environmental risk

A compilation of concentrations of PACs and metals in both natural and sedimentation ponds is given in Supporting Information, Table S2 (detection frequencies, minimum concentrations, quartiles [10%, 25%, 75%, and

90%], median, and maximum concentrations are given). Environmental classes were assigned based on the Norwegian classification scheme (Direktoratsgruppen vanndirektivet, 2018). The environmental classes are shown in a mosaic plot where metals, individual PAH16, and alkylated PACs were assessed as exerting the same level of toxicity as the nonalkylated parent (Figure 2). The fact that alkylated PACs show the same toxicity as parent PACs has previously been discussed by Andersson and Achten (2015) and was elaborated by Grung et al. (2021).

For metals, 13 of the ponds (38%) did not exceed the EQS (i.e., annual average [AA]) for any metals. Only five ponds (15% of all ponds) had metals in exceedance of the MAC EQS (Class 4, orange color), and all of these were sedimentation ponds for road runoff. The level of Cu was higher than the EQS in all five ponds, while Zn also exceeded the EQS MAC in sedimentation pond VAS. Sedimentation ponds with metal exceedances were among those with the highest AADT (>50,000), except for sedimentation pond FOR (AADT 23,800). Four of the seven natural ponds exceeded the EQS (Class 3, yellow), and a natural pond (MØL) was the pond with the highest class mean. This pond was from inside Oslo city and <400 m from a heavily trafficked ring road (Ring 3 with AADT 62,600). In addition, there are many other smaller roads surrounding the pond, which probably explains the findings. However, the EQS MAC was not exceeded for metals in any natural ponds.

For PAH16, more than half of the ponds ($n = 19$, 56%) did not exceed the EQS. However, the number of ponds where the MAC EQS was exceeded was higher (11 ponds, 32%) than for metals (five ponds, 15%). The same four natural ponds that had exceedances of the EQS for metals also exceeded the EQS for some of the PAH16. The MAC EQS was also exceeded in three of the natural ponds (MØL, RAN, and BÅN). The sedimentation ponds with the highest class means were the ones with the highest AADT, except for sedimentation pond FOR. By adding the alkylated PACs in the assessment of nonalkylated PAH16, the assessments for all ponds showed increased risk compared with only including PAH16 (Figure 2C vs. B). All ponds had at least one PAC above the EQS. Also, four sedimentation ponds had concentrations of PACs greater than five times the acute toxicity levels (EQS MAC, red color), which indicates that acute toxicity for sediment-dwelling organisms may be the case in these ponds.

Comparison of risk among analyzed compounds

For a prioritization of the different metals and PACs investigated, the environmental concentrations were normalized to the environmental class as described in *Materials and Methods*. The boxplots in Figure 3 show that among the PACs the Ns (including alkylated C1–C4 naphthalenes), Cs (including alkylated C1–C2 chrysenes), and PYs (including alkylated C1–C2 pyrenes/fluoranthenes) were the PACs with the highest geometric mean of normalized class (see Equation 1, Supporting Information, Figure S3, and explanation in *Comparison with Water Framework Directive guidelines*). One

sedimentation pond (FEV) contained oil and had very high concentrations of Ns, Fs, and Ps (58,000, 52,000, and 45,000 ng/g dry wt, respectively, compared to medians of 1000, 35, and 168 ng/g dry wt for the same compounds in the data set). For this pond, quantification was performed on a subsample which had five times the amounts of internal standards to account for the higher concentrations. This sedimentation pond (FEV) was registered by the road authorities as being polluted with oil. The high concentrations of small-ring PACs like N, F, and P confirm that pollution by an oil spill had taken place.

Correlations of concentrations of PACs and metals with AADT

All compounds investigated were tested for correlation with AADT in the sedimentation ponds ($n = 26$). Because FEV had 110 times the median PAC concentration in the data set, the pond was excluded from linear regression; but data for linear regressions with FEV included are shown in Supporting Information, Table S3. A correlation of individual PAH16 with roadside soil has previously been found in South Korea (Kim et al., 2019). Unfortunately, in that study only PAH16 were investigated. In a Danish study of sedimentation ponds, no correlation between metals and PACs with AADT was found (Grauert et al., 2012). For PACs, the alkylated PACs were included in the sum of the nonalkylated isomer. The natural ponds were not included in the linear correlations. All of the four- to six-ring PACs investigated and the metals Zn and Cu correlated significantly with AADT (for details, see Table 2). Major sources of Zn are tires and galvanized steel from, for example, traffic signs, guardrails, and so forth, while brakes are an important source for Cu (Meland et al., 2010). Therefore, the positive correlation between traffic density and Cu and Zn levels is not unexpected. Also, PACs are associated with traffic, so it is expected that PACs correlate with AADT. However, the smaller-ring PACs did not correlate equally well with AADT. One sedimentation pond (FEV) had much higher concentrations of PACs than the other ponds (the sum PACs were 110 times the median of the data set [1500 ng/g dry wt] and >9 times the second highest sum of PACs). This sedimentation pond had previously been polluted by an oil spill. The FEV sedimentation pond was therefore excluded, and the linear regressions were done on the 26 remaining sedimentation ponds. The resulting data are shown in Table 2. For metals other than Cu and Zn, nonsignificant linear correlation of concentration versus AADT were observed, but all PACs (excluding N) correlated significantly with AADT. When checking the correlation with AADT with all individual alkylated PACs, the data did not change significantly other than for naphthalenes, where N, N_C1, and N_C4 correlated significantly with AADT and N_C2 and N_C3 did not. This observation shows that PACs, Zn, and Cu in sedimentation ponds were strongly correlated with the amount of traffic. The three PACs with the highest R^2 values were PY (four rings), P (three rings), and BgP (five aromatic rings, six rings in total), indicating that this applies to PACs with both small and large ring sizes. However, the smaller-ring PACs like N,

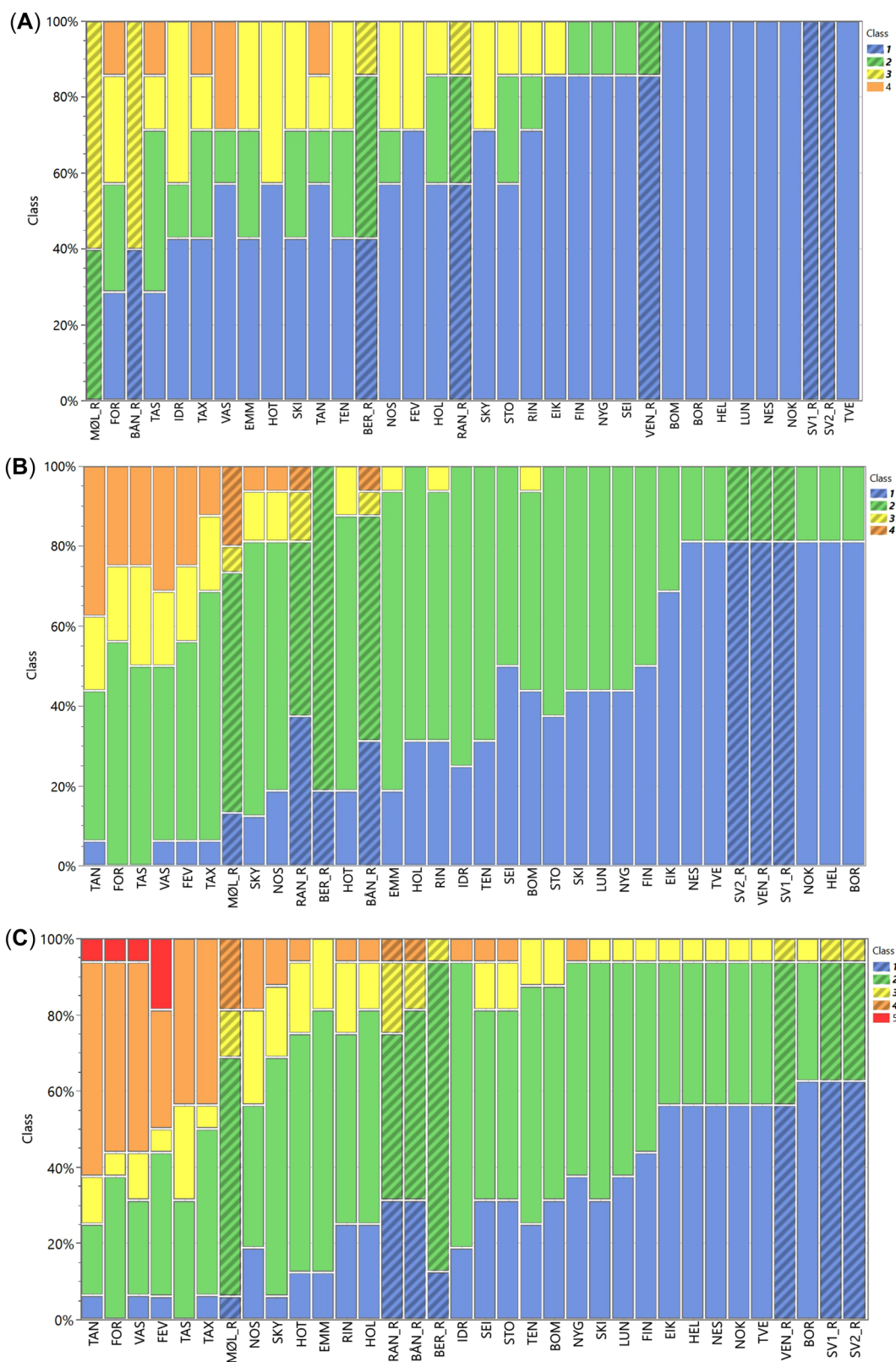


FIGURE 2: Mosaic plot of environmental class (see section *Comparison with WFD guidelines* for explanation) for (A) metals, (B) polycyclic aromatic hydrocarbon (PAH) 16 (all individual polycyclic aromatic compounds [PACs]) and (C) PACs (alkylated isomers included for all PAH16, e.g., all alkylated N included in assessment of N). The areas of each pond and class correspond with the number of compounds assessed for each pond. For three natural ponds, As and Cr were not analyzed; hence, these bars are narrower. The natural ponds are shaded for ease of identification. The ponds are ordered by the geometric mean of class, so the ponds with the highest environmental risk are located to the left. For definitions of pond acronyms, please see Figure 1 and Table S1 (Supporting Information).

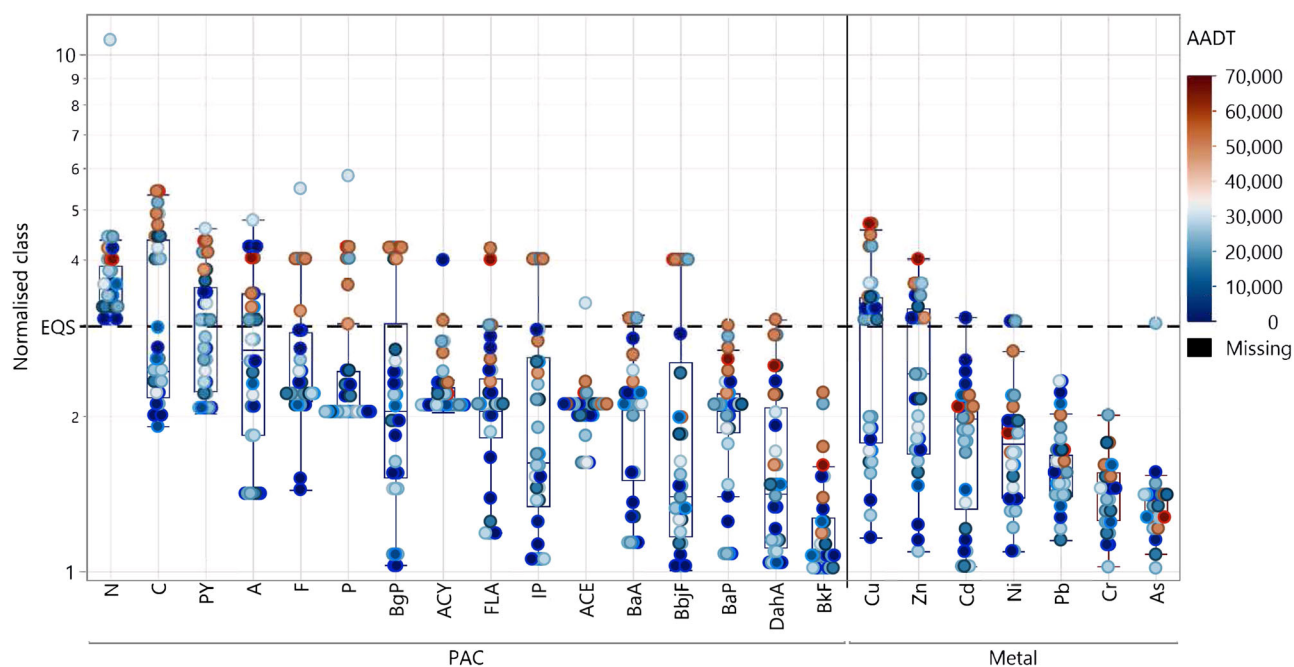


FIGURE 3: Boxplot of environmental class (see Equation 1; Supporting Information, Figure S3; and text for calculation) for individual polycyclic aromatic hydrocarbon 16 (alkylated polycyclic aromatic compounds [PACs] included) and metals. The contaminants are ordered by contaminant group (PAC and metal) and by the geometric mean of class for each compound. Each point represents a pond colored by the annual average daily traffic (scientific colour map vik (Cramer 2018)). The y-axis (normalized class) is presented on a log₁₀ scale. EQS = environmental quality standard; AADT = annual average daily traffic.

TABLE 2: Linear regression analysis of investigated compounds and annual average daily traffic in sedimentation ponds (FEV and natural ponds excluded)^a

Significant	Compound	ANOVA _p	R ²	R ² Adjusted	Slopes
Yes	PY	0.000033	0.52	0.50	0.045
	P	0.000077	0.49	0.46	0.059
	BgP	0.000077	0.49	0.46	0.0045
	C	0.000093	0.48	0.46	0.054
	D	0.000093	0.48	0.46	0.037
	Zn	0.000095	0.48	0.45	0.0096
	Cu	0.00014	0.46	0.44	0.0016
	FLA	0.00017	0.45	0.43	0.0074
	BeP	0.00046	0.41	0.38	0.0054
	PER	0.00087	0.38	0.35	0.00095
	A	0.001	0.37	0.34	0.00063
	BaP	0.0012	0.36	0.33	0.0019
	BaA	0.0026	0.32	0.29	0.0013
	BbjF	0.0042	0.29	0.26	0.0036
	IP	0.0051	0.28	0.25	0.0014
	F	0.0057	0.28	0.25	0.02
	BkF	0.0066	0.27	0.24	0.00095
	ACE	0.0082	0.26	0.23	0.00023
	DahA	0.0083	0.26	0.23	0.00035
	ACY	0.048	0.15	0.12	0.0002
No	N	0.21	0.065	0.026	0.021
	Cr	0.23	0.059	0.020	0.00023
	Pb	0.30	0.044	0.0041	0.00016
	Ni	0.62	0.01	-0.031	0.000094
	As	0.88	0.00098	-0.041	0.0000068
	Cd	0.90	0.00062	-0.041	0.00000035

^aThe alkylated polycyclic aromatic compounds are included with the nonalkylated equivalent (N, F, P, D, PY, C). For each correlation, the *p* value (ANOVA), R², R² adjusted, and slopes are listed (with two significant digits). The compounds are ordered by the R². The number of sedimentation ponds included was 26. Abbreviations for compounds are defined in Table 1. ANOVA = analysis of variance.

ACY, and ACE correlated less well with AADT. In 1982, Pb in the vicinity of roads was correlated with AADT (Rodríguez-Flores & Rodríguez-Castellón, 1982); but since the introduction of lead-free petrol (1983) and later a ban on lead petrol in 1996 in Norway (Throne-Holst, 2000), there has been no correlation (Figure 4).

Hierarchical two-way cluster of environmental class

A two-way hierarchical cluster method was employed to look for differences between the ponds (Figure 5). Four clusters were considered appropriate because the distance graph to the bottom right of Figure 5A shows an elbow at three to four clusters, indicating that the differences between clusters were larger at Clusters 1–4 (18–5.9) than the others (5.6–1.3). The differences in the data set are indicated by the length of the “legs” of the clusters, where more similar ponds have shorter legs. Cluster 1 (green pond names in Figure 5A) consisted of 15 ponds that were characterized with the median normalized environmental class of high-ring PACs (green line in Figure 5B), the low median normalized environmental class of small-ring PACs, and the high/median environmental class of the metals to the right in Figure 5B (As, Cd, Cr, Ni, and

Pb). The cluster included four natural ponds (see second to last paragraph in this section for discussion of these ponds). Cluster 2 (blue pond names in Figure 5A) consisted of 13 ponds that were characterized by the lowest toxicity for all contaminants measured. Two of the natural ponds (three samples, VEN_R, SV1_R, and SV2_R) were included in this cluster, but the cluster contained more sedimentation ponds than natural ponds. Cluster 3 (dark gray color) was represented by only one pond (FEV). The pond was noted as having received an oil spill, which resulted in a predicted higher than average toxicity for the smaller-ring PACs (A, F, P, ACE, ACY, N) that are expected to be associated with oil spills. The environmental class for larger-ring PACs were medium to high, and metals not correlating with AADT (metals except Cu and Zn) were medium to low. Cluster 4 contained five sedimentation ponds (orange color) that were characterized by the highest levels of environmental class for larger-ring PACs (four or five rings), quite low levels for small-ring PACs (two or three rings), and the highest levels of Cd, Cr, Ni, and Pb, although none of these metals have means above environmental Class 3 (i.e., concentrations exceeding EQS).

Also, the analytes measured have been clustered (bottom of Figure 5A), and the largest difference among contaminants was between the compounds in the green box (metals [As, Cd, Cr, Ni and Pb] and ACY) versus the other PACs and the two metals

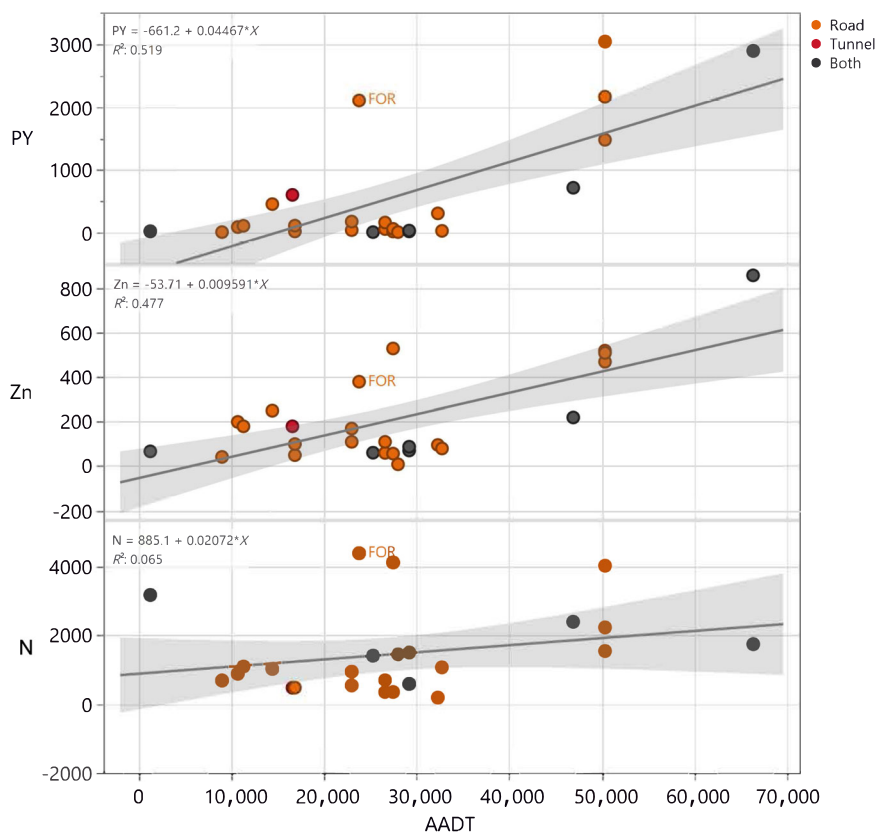


FIGURE 4: Linear regression of contaminants with annual average daily traffic for PY, Zn, and N. The alkylated polycyclic aromatic compounds are included with the nonalkylated equivalent (N, F, P, D, PY, C). The ponds are colored according to which type of runoff they received. The linear correlations are shown in dark gray, and the 95% confidence bands of the linear correlations are indicated. The FEV sedimentation pond and natural ponds were excluded from the data; thus, the number of sedimentation ponds was 26. AADT = annual average daily traffic.

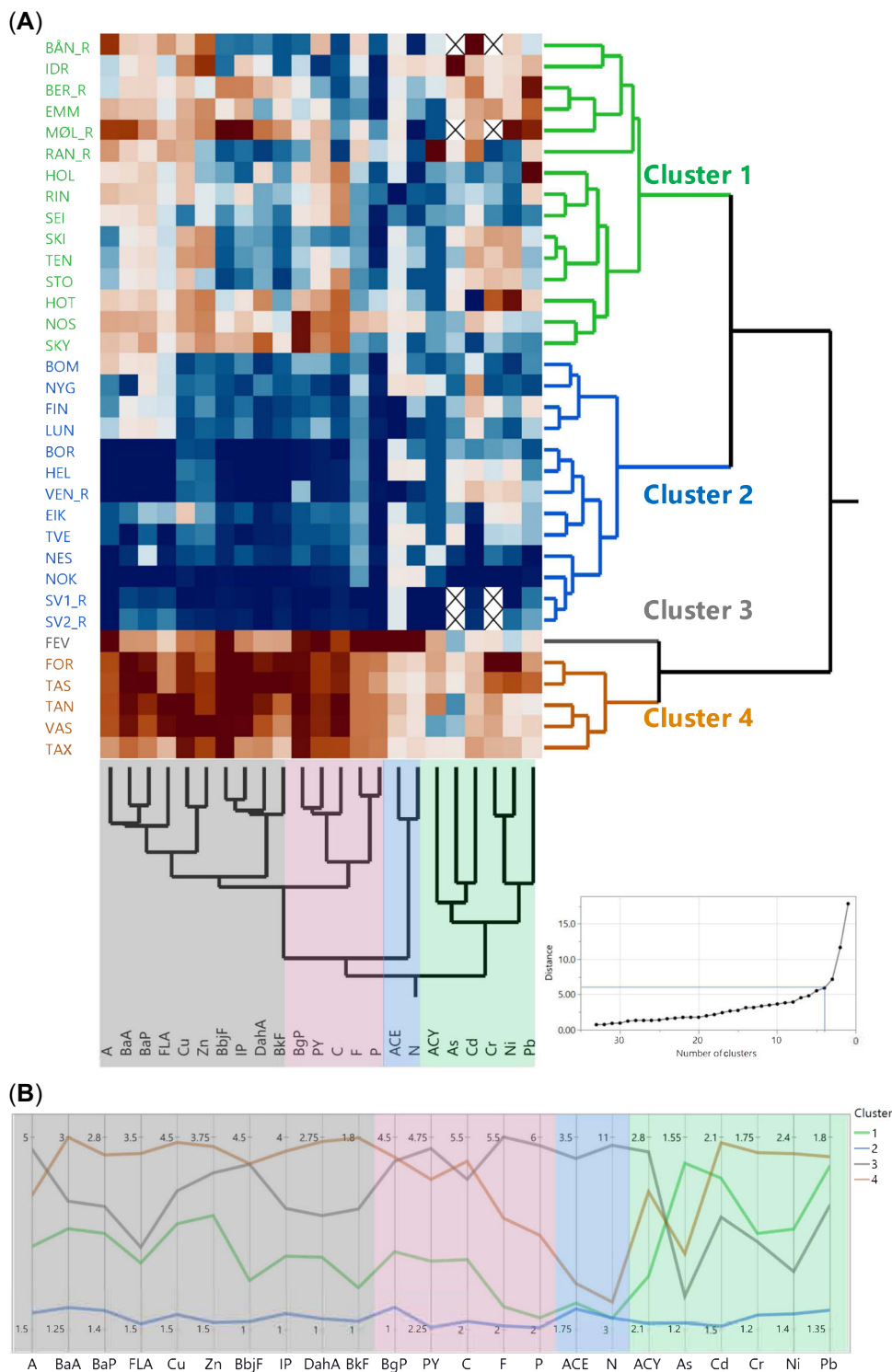


FIGURE 5: (A) Two-way hierarchical cluster of normalized environmental class for polycyclic aromatic compounds (including alkylated isomers) and metals. The hierarchical method employed was Ward, and the eight missing values for As and Cr (shown as crosses) were imputed by singular value decomposition. The normalized probable toxicity is indicated in a color scheme from blue to gray to brown (scientific colour map vik (Crameri 2018)) for each individual compound (B). The means of each cluster for individual compounds are shown in a parallel plot where the highest and lowest normalized environmental classes are given for each compound at the top and bottom, respectively, for each axis.

Cu and Zn. The compounds in the green box were characterized by relatively low environmental class, with ACY as the highest with a scale up to 2.8 (Figure 5A). The next level of cluster was between the blue box (N and ACY) and the other

PACs and Cu/Zn. The main difference was that N/ACY were higher in the oil-polluted sedimentation ponds and lower than the compounds in the gray/pink box for the heavily impacted sedimentation ponds (Cluster 4, orange).

The hierarchical clustering method proved to be useful for detecting differences among the ponds investigated in the present study with regard to prioritization of environmental hazard. The ponds with the highest environmental concern were in Clusters 3 and 4, whereas the ponds with the lowest risk were in Cluster 2. The most polluted sedimentation ponds (Clusters 3 and 4) had the highest AADT, which combined were significantly higher than Clusters 1 and 2 (ANOVA $p=0.0003$, Tukey's post hoc $p=0.0004$ and 0.0007 vs. Clusters 1 and 2, respectively; Supporting Information, Figure S2). There was, however, no difference between AADT for Clusters 1 and 2, so the explanation of the difference in contaminants is unrelated to AADT.

Four of the natural ponds (BÅN_R, BER_R, MØL_R, and RAN_R) occurred in Cluster 1 (green; with likely higher environmental effects than Cluster 2 [blue]) together with sedimentation ponds which received runoff from traffic (AADTs in the range 10,700–47,000). Ponds BÅN_R and MØL_R are located in urban areas near Oslo, and therefore one explanation for the elevated contamination may be that the ponds receive PACs not from runoff directly but maybe a diffuse source of PACs/metals from air or another source.

Cluster 2 ponds were characterized with a low risk of environmental hazard for all measured compounds. Two of the natural ponds (three samples, VEN_R, SV1_R, and SV2_R) were in this cluster and receive no road runoff. The range of AADT of the sedimentation ponds in the cluster was 1300–33,000. Because the likelihood for effects was so low, combined with lower concentrations of PACs, Cu, and Zn which correlate with AADT, we suspect that some of the sedimentation ponds in Cluster 2 are not working properly and/or are not receiving runoff from the roads. To tease out any difference between the clusters in correlation with AADT, a separate linear correlation (Clusters 1 and 4 together and Cluster 2 separately) was compared with no separation of the clusters (Supporting Information, Figure S8) for the seven compounds that correlated best with AADT (Table 2; PY, P, BgP, C, D, Zn, and Cu). Supporting Information, Figure S8, reveals that Cluster 2 ponds do not correlate equally well with AADT, especially the ponds that are outside the confidence band of the correlations (i.e., ponds with high AADT and low concentrations). We suspect that at least some of the sedimentation ponds in Cluster 2 (and possibly in Cluster 1) are not functioning well and/or are not receiving road runoff. Ponds in Cluster 2 were not correlating with AADT (Supporting Information, Figure S8).

Characterization of PAC and their pattern

To characterize the PACs, different ratios for source apportionment of the PACs were applied, and these are shown in Supporting Information, Figure S4 (Hindersmann et al., 2020; Stogiannidis & Laane, 2015). As previously mentioned, one of the most used indices for source identification is the PI (Stogiannidis & Laane, 2015), where a PI >0.8 indicates a petrogenic source. The PI values of the sedimentation ponds were significantly lower than those of the natural ponds (Welch's t test $p=0.0073$), indicating that the sources of PACs in

sedimentation ponds were petrogenic. The PI in the natural ponds had a large range (0.08–1.3 in VEN_R and MØL_R, respectively), indicating ponds with both petrogenic and pyrogenic sources. As stated in *Materials and Methods*, we did not analyze some of the higher alkylated PACs (D_4, F_4, C_3, and C_4), which means that the PI would have been lower (more petrogenic) if those alkylated PACs had been found above the LOQ.

The PAH alkylation index by Hindersmann et al. (2020) confirms this pattern. Their investigation indicated that petrogenic PACs generally have an alkylation index <0.4 , while pyrogenic PAHs have an index >0.6 . All of the sedimentation ponds had an alkylation ratio <0.4 . The t test of log-transformed alkylation ratios showed that the sedimentation ponds had a significantly lower alkylation ratio than the natural ponds ($p < 0.0001$). As can be seen, the alkylation ratio performed better than the PI at differentiating natural ponds from sedimentation ponds. For the natural ponds, all but two had alkylation ratios >0.6 . The natural ponds VEN_R (alkylation ratio 0.3) and BER_R (0.43) both had an alkylation ratio <0.6 , indicating that the ponds were influenced also by alkylated PAC sources, especially VEN_R. Both of these natural ponds are located near farming lands and might have been influenced by accidental spills of gasoline or oil. Neither of the two indices with ratios based on low and high molecular weight showed any differences between the natural ponds and sedimentation ponds.

For the PAC pattern, a hierarchical clustering of the PACs was performed to investigate if this would reveal more information about the pattern of PACs in both the natural ponds and the sedimentation ponds (Supporting Information, Figure S5). Again, four clusters of ponds were selected based on the distance graph. The two easiest clusters to describe were Cluster 3 (light blue), which was characterized with the lowest PAC concentrations (Supporting Information, Figure S5b), and Cluster 4 (purple), which was characterized by the highest PAC concentrations. Cluster 1 (green) ponds had sediments with higher concentrations of PACs than sediments of most ponds in Cluster 2 (orange) except for the alkylated N (PACs in purple background). This means that ponds in Cluster 2 are possibly more affected by oil spills. The same also applies to the sedimentation ponds in Cluster 3 (BOR, HEL, and NOK).

The PAC patterns as bar plots of concentrations of representative patterns from all four PAC clusters are shown in Supporting Information, Figure S5. From Cluster 4 (purple), two sedimentation ponds are shown (FEV and TAN). The note about the oil spill in the FEV sedimentation pond was confirmed by the fact that the PAH concentration in the pond was more than nine times higher than that of the second highest pond in the data set and from the PAC pattern, with dominating high concentrations of alkylated N, F, and P (all small-ring PACs). The PAC pattern of sediments from TAN are more typical for a functional sedimentation pond receiving high amounts of runoff, where most PACs are in quantifiable amounts and the pattern was characterized with a balance of most PACs. The alkylated PACs are visibly present, but larger-ring PACs like C were the PACs at the highest

concentrations. The pattern is very similar to the road asphalt in the supplementary material of Stogiannidis and Laane (2015). The asphalt is therefore possibly a major source to the road runoff in the Cluster 4 ponds, and the studded tires used in the winter season in Norway increase the asphalt emissions (Thorpe & Harrison, 2008). The pattern was in contrast to FEV, where alkylated N₃ was the PAC with the highest concentration. From Cluster 3 (light blue) characterized by low concentrations of PAC, SV2_R is shown in Supporting Information, Figure S5. Of the smaller-ring PACs, only nonalkylated P was detected above the LOQ, while many larger-ring PACs were detected. Alkylated PY and FLA (PY_C2 and PY_C2) were the only alkylated PACs detected. The alkylation ratio and the PI for this natural pond were high (0.83–0.85) and low (0.13–0.20) for the two sediments analyzed from the pond, indicating that the natural ponds have a strong pyrogenic source of PACs similar to the urban runoff shown in the supplementary material of Stogiannidis and Laane (2015).

The two remaining clusters (1 and 2) are exemplified by PAC patterns from HOT (Cluster 1, dark green) and EIK (Cluster 2, brown; Supporting Information, Figure S5). The difference in pattern was that PAC pattern in ponds from Cluster 1 were dominated by alkylated N and C, while alkylated P, D, and PY were present. From the means of the PACs (Supporting Information, Figure S5) it can be seen that the means of alkylated N_{C2-C4} were substantially lower than those of sediments from Cluster 2 (brown). Also, the larger-ring PACs were present in Cluster 1 sediments but not dominant like in Cluster 4 (e.g., SV2_R). For Cluster 2 ponds exemplified by EIK, one can see that alkylated N dominated, while other PACs were present but at much lower concentrations. The dominance of the alkylated N indicates that oil spills or gasoline spills are an important source of PACs in these ponds.

CONCLUSIONS AND IMPLICATIONS

The investigation of PACs and metals in road runoff from sediment ponds compared with natural ponds revealed that sediment-dwelling organisms in these mitigatory ponds are exposed to higher concentrations of PACs and metals than in natural ponds. The risk to sediment-dwelling organisms in sedimentation ponds often exceeded the EQS for PACs, Cu, and Zn. Analyzing an extended number of PACs, including alkylated PACs, and including these in the assessment of environmental risk showed that it is important to investigate their presence in road runoff. The PACs constituted more risk to sediment-dwelling organisms than metals. The pollution pressure of most PACs as well as Zn and Cu in the sedimentation ponds was shown to increase with increasing traffic (AADT) and could therefore be used to prioritize investigations.

The alkylated PACs also indicated that asphalt was the probable source of PACs in sedimentation ponds, and the Norwegian use of studded tires and snow ploughs in the winter season may contribute to concentrations of PACs in

sedimentation ponds. A hierarchical clustering technique revealed that a few sedimentation ponds were probably not functioning well and received spills of oil and/or gasoline.

The value of analyzing more PACs than the usual PAH₁₆ for identifying environmental risk and sources of PACs should not be underestimated. We believe that with today's advanced analytical instrumentation this is easily achievable and will allow a more holistic risk assessment of PACs in the future.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5399>.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (merete.grung@niva.no).

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