

Sampling over time: developing a cost effective and precise exposure assessment program

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Studies requiring ambient exposure assessments invariably ask: How often should measurements be taken? Answer to such questions is dictated by budgetary considerations as well as spatial and temporal variability in the data. For example, do we obtain measurements during all seasons, all months within seasons, weeks within months and days within weeks? On one hand, we can obtain a one-time snapshot sample and regard it as representing the “true” mean exposure. On the other hand, we may obtain a large number of measurements over time and then average these in order to represent this “true” mean exposure. The former estimate is the least expensive but may also be the least precise while the latter, may be very precise but prohibitively costly. In this paper, we demonstrate how a pilot study can be undertaken with a potentially promising and feasible sampling plan for the full-scale study. By applying the statistical methodology of variance component analysis (VCA) to the pilot study data and exploiting mathematical relationship between the variance of the overall mean exposure and posited variance components, we can develop a sampling design with decreased sampling costs and/or increased precision of the mean exposure. Our approach was applied to determine sampling design choices for an on-going study that aimed at assessing ambient particulate matter exposure. We conclude that a pilot study followed by the VCA analysis may often lead to sampling design choices that offer considerable cost savings and, at the same time, promise to provide relatively precise estimates of the mean exposure for the subsequent full-scale study.

1. Introduction

Many researchers worldwide are addressing issues related to the ambient environmental exposure and its impact on health of those exposed. Before we can credibly ascertain the health effects of such exposures, it is imperative that we assess the exposures as precisely and accurately as possible. There are WHO guidelines for exposure assessment of fine and ultra fine particulate matter (PM) in epidemiological studies.¹ Frequently asked questions on environmental sampling and analysis involve the type and number of samples needed.² A question not given adequate attention is, “How to allocate these temporally?” Bernard³ alluded to partitioning of the total sample variance in terms of its relevant components and concluded that a well-conceived and cost-effective sampling program must be based on an appropriate statistical design that considers all of the components of variance. Allen⁴ employed multistage cluster sampling design to estimate variance components in a survey of the fish discards with hauls within trips within vessels, and investigated the optimal number of hauls, trips and vessels that will be required to be sampled to achieve certain levels of precision. In another study⁵ comparing two environmental monitoring programs with respect to the cost-effectiveness, temporal variance components were estimated using time series approach. Efficient sampling designs were also investigated in fisheries⁶ in order to reduce sampling frequency without any loss of precision. All these studies investigated achieving efficient sampling design but not quite with respect to sampling in time. In an attempt to address the issue of allocating sample assessments in time, we

previously developed an approach for examining the health effects of workers exposed to solvents.⁷ A feasible solution was to select a reasonable number of total exposure measures and plan how the entire sampling scheme was configured. It was recommended to perform a pilot study first and then use variance component analysis on the results to establish a link between the average exposure assessment and various posited sources of variations. This link allows manipulation and redistribution of exposure assessments permitting an evaluation of alternate sampling designs relative to the pilot design with respect to the *cost* of sampling (in terms of the number of samples) and *precision* in determining the average exposure (in terms of the inverse of the standard error of the mean). Designs of a pilot study can be identified with decreased sampling costs and/or increased precision of the mean level of exposure, depending on the requirements of the full-scale study. In this paper, we have refined the previous application;⁷ the ambient particulate matter (PM) monitoring problem is approached from the viewpoint of sampling theory developed for environmental survey applications.^{8,9} In particular, we invoke the theory of sub-sampling with each “day” of PM sampling considered as the basic unit or element. The entire sampling duration is viewed as a composite of several “nested” stages of clusters of days (such as weeks, months, seasons, etc.). With the proposed multi-stage sub-sampling view of the entire duration of sampling, we provide expressions for estimating precision (inverse of the standard error) of the mean exposure in terms of the variance components that account for the finite population correction (FPC) as well. In the previous application, we effectively assumed the population of clusters (weeks, months, periods etc.) to be “very large”, and thus ignored the FPC. In this paper, with a more realistic scenario and exact expressions for variance, considerable enhancement is afforded, thereby

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leading to credible design choices. We have applied this modified approach to the on-going Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS).

2. Methods

2.1 Ambient PM monitoring study

CCAAPS examines the relationship of exposure to diesel exhaust particles and the development of allergy in infants and young children. Newborns are identified from birth certificate records. Their residential address is used in combination with a geographic information system (GIS) to identify infants residing within either 400 m or beyond 1500 m from a major roadway. Major roadways are defined as those having a daily traffic intensity of 1000 or more heavy-duty diesel vehicles. The children are tested with skin prick tests annually for the development of atopy. The ultimate goal of the CCAAPS, however, is to have more precise measures of exposure than the distance from the highways and traffic intensity. The study protocol called for setting up several air monitoring stations throughout the Greater Cincinnati area. There was no unanimity as to the most precise and cost-effective approach to capture "true" exposure while not exceeding budgetary resources. The sampling scheme had large implications as it was to last for five years.

The pilot study included several sites selected for exposure assessment. Both the ambient PM_{2.5} aerosol (particulate matter with an aerodynamic diameter of up to 2.5 μm) and the PM_{2.5} chemical compositions were monitored at each site with repeated aerosol sampling over time. Sampling and analysis methods are described in detail elsewhere.¹⁰ Among 39 elements identified in the CCAAPS by the X-Ray fluorescence analysis of PM_{2.5} samples, few elements are selected here for presentation purposes, including S (as an indicator of the secondary sulfate source, coal combustion and diesel sources), as well as Fe, Pb, and Zn (as elements associated with industrial processes and motor vehicle traffic). Data collected from a representative site located in the center of the Greater Cincinnati area (Water Tower) are used here to illustrate our approach. Fig. 1 shows the pilot sampling design for that site. The sampling period consisted of phases (cycles or periods) of sampling. Each phase consisted of a certain number of weeks of sampling. (For example, period 1 had one week of sampling, period 2 had two weeks of sampling, and period 3 had three weeks). Likewise, each monitoring week consisted of three to five days of sampling.

Any sampling procedure typically presupposes a clear division of the population of samples (be it spatial or temporal) into a finite number of distinct and identifiable units called the sampling units. The smallest units into which the sampled population can be divided are called the elements of the population, and groups of elements as the strata. When all the elements within a stratum are sampled, the procedure of sampling is called cluster sampling. Thus, in the present set up, one can view a day of exposure assessment as the element of the population and week, month, season, period or phase as different strata. Consequently, an industrial hygiene sampling protocol over time can be described in a nested data structure,

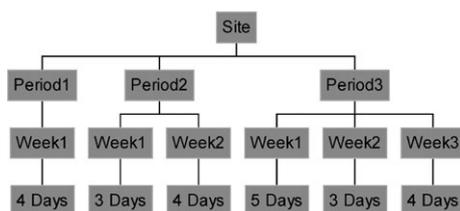


Fig. 1 Pilot sampling design.

and the sampling can take place in several stages, depending upon the levels of strata/clusters. Note that this structure can have many variants depending on the nature of sampling and the total sampling duration. The objective in characterizing the entire sampling duration into a number of clusters or strata (or components) is to identify "sources of variation" in the exposure assessments. For example, how much of the total variation in the data is due to a day-to-day variation, week-to-week, or month-to-month variation, and so on. Once these components of variance are estimated *via* a pilot study, we can redistribute the number of observations to reduce the cost and maximize the precision of our estimated mean. In the study, we have presented the necessary statistical and modeling details of the approach and applied these to one site as an example.

2.2 Statistical modeling and analysis

When a design strategy involves a random selection of days, weeks, and other periods within a nested data structure, an appropriate statistical model for describing exposure measurement X is the random effects model.¹¹

For a given site, we have modeled the exposure measurement following the random effects model as described below:

$$Y_{ijkl} = \log(X_{ijkl}) = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)} \quad (1)$$

where X_{ijkl} is the exposure concentration of the outcomes in consideration (in our example; PM_{2.5}, Fe, Pb, S, and Zn), μ is the overall mean α_i is the period effect, ($i: 1, \dots, p$) $\beta_{j(i)}$ is the week effect (nested in period), ($j: 1, \dots, w$) $\gamma_{k(ij)}$ is the day effect (nested in week), ($k: 1, \dots, d$) and $\varepsilon_{l(ijk)}$ is the Residual component.

It is usually assumed that X_{ijkl} has a log-normal distribution. Further, α_i , $\beta_{j(i)}$, $\gamma_{k(ij)}$, and $\varepsilon_{l(ijk)}$ are assumed to be normally distributed with 0 means and variances σ_α^2 , σ_β^2 , σ_γ^2 , and σ_ε^2 , respectively.

Note that since there is one measurement per day, the day effect is confounded with the error term. Variance component analysis can be performed using any standard statistical software (*e.g.* SAS), and using the restricted maximum likelihood method to estimate the variance components of period, week, and day. One can then employ the relationship between the variance of the overall mean and the estimates of the variance components⁸ as follows:

$$\text{Var}(\bar{y}) = \left(\frac{1}{p} - \frac{1}{P}\right) s_p^2 + \frac{1}{p} \left(\frac{1}{w} - \frac{1}{W}\right) s_w^2 + \frac{1}{pw} \left(\frac{1}{d} - \frac{1}{D}\right) s_d^2 \quad (2)$$

where $s^2(\cdot)$ is the estimate of respective variance components; p , w , and d are respectively the number of periods, weeks within period, and days within weeks, in any proposed sampling design; P , W , and D are the corresponding number of periods, weeks, and days in the entire sampling frame. For example, in our study, we have a year of sampling, and the period is close to a month so $P = 12$, $W = 4$, $D = 7$.

2.3 Measures of "cost", "precision" and "design efficiency"

The precision can obviously be improved by increasing the total number of measurements. Even with a fixed number of total measurements, different combinations of the numbers of periods, weeks, and days for a sampling design (*i.e.*; $p-w-d$), may give different variances of the estimated mean exposure in eqn. (2). Precision in our estimate of the overall exposure mean is defined as the inverse of the standard error of the overall mean. The cost is defined herein as being directly proportional to the total number of measurements ($n = pwd$). The goal is to find a design, which maximizes the precision for a pre-specified cost (n) or minimizes the cost for a pre-specified precision. The estimated variance components, the overall mean exposure and the standard error (se) of the overall mean, are obtained from

the pilot study. In other words, the results of the pilot design become the "point of reference". The performance of other possible environmental sampling designs is evaluated relative to that of the pilot design as follows:

Calculate the relative cost (RC), relative precision (RP) and design efficiency (DE) of potential design choices, compared to the pilot design.

$$RC = \frac{N(\text{design})}{N(\text{pilot})} \times 100\% \quad (3)$$

$$RP = \frac{\text{precision}(\text{design})}{\text{precision}(\text{pilot})} \times 100\% = \frac{SE(\text{pilot})}{SE(\text{design})} \times 100\% \quad (4)$$

It is possible to have a full study design to be much better than the pilot design in terms of the RP but worse than the pilot design in terms of RC. Therefore, it is useful to evaluate a design in terms of a measure that we define as design efficiency (DE):

$$DE = RP/RC \quad (5)$$

The DE measures gain (or loss) in relative precision of a potential design for one unit change in relative cost of that design. This index allows us to not only evaluate a potential design choice but also compare one design choice to another. Thus, if $DE > 1$, then that design is better than the pilot and the larger the DE, the better the design is relative to the pilot design. Further, since the pilot design is the common reference design, we can compare designs in terms of their design efficiencies.

3. Results and discussion

3.1 Estimates of the variance components

The statistical procedure called MIXED in Statistical Analysis System (SAS) version 8.0 was used to fit the model shown in eqn. (1). In order to meet the normality assumption, logarithmic transformation was applied to each set of measurement data ($PM_{2.5}$ and specific elements). The nested random effects model was utilized to obtain variance components of the $PM_{2.5}$ database collected in the single ambient monitoring site. Table 1 shows the estimates of the variance components for the site obtained for five different exposures, which includes $PM_{2.5}$ and its selected elements (elemental Fe, Pb, S, and Zn). The week-to-week component of variation for each exposure was either almost non-existent ($PM_{2.5}$, Fe, and Pb) or very small (S, Zn). Further, the day-to-day variation was considerably larger than that of the period component (except for Pb, where the two were approximately the same).

The pilot design for this single site included a total of 23 measurements. It is generally preferable to have a "balanced" design (such as three periods, two weeks of sampling for each of the three periods, and four days of sampling for each of the two weeks). However, a slight imbalance (such as in the present pilot design) does not pose problems. In what follows, the results of the variance component analysis are used to calculate various indices of design performance defined earlier.

Table 1 Variance component estimates for the Water Tower site

Variance components	$PM_{2.5}$	Fe	Pb	S	Zn
Period	0.01	0.06	0.29	0.04	0.05
Week (Period)	0.00	0.00	0.00	0.02	0.02
Day (Period, Week)	0.06	0.19	0.23	0.06	0.20
<i>N</i>	23	23	23	23	23
Mean	2.58	4.57	1.26	6.87	2.66
Std. Error	0.06	0.14	0.28	0.12	0.14

3.2 Relative cost (RC)

As indicated above, the cost of a design is proportional to the total number of observations or sampling assessments (n) performed in the study. If a study were designed with environmental sampling in three periods, with two weeks per period and three days per week [a (3-2-3) design] then there would be $18 = 3 \times 2 \times 3$ sampling assessments in this design configuration. With 23 observations performed in the framework of the pilot design, the relative cost can be calculated as follows: $RC = (18/23) \times 100 = 78\%$. Similar calculations can be done for other design choices.

3.3 Relative precision (RP)

One way to compare a proposed sampling design to the pilot design is in terms of the relative precision:

$$\text{relative precision (RP)} = \frac{1/\hat{\sigma}_y(\text{design})}{1/\hat{\sigma}_y(\text{pilot})} \times 100\% \quad (6)$$

where $\hat{\sigma}_y(\text{design})$ is calculated as square root of the estimated variance of the mean exposure using eqn. (2). For example, with $PM_{2.5}$ as an outcome, the estimated standard error of the sample mean of $PM_{2.5}$ obtained by performing a (3-2-3) design choice will be:

$$\begin{aligned} \hat{\sigma}_y(\text{design}) &= \\ &= \sqrt{0.01 \left(\frac{1}{3} - \frac{1}{12} \right) + 0.00 \frac{1}{3} \left(\frac{1}{2} - \frac{1}{4} \right) + 0.06 \frac{1}{3 \times 2} \left(\frac{1}{3} - \frac{1}{7} \right)} \\ &= 0.066 \end{aligned}$$

$$\begin{aligned} \text{Thus, RP} &= \frac{1/\hat{\sigma}_y(\text{proposed})}{1/\hat{\sigma}_y(\text{pilot})} \times 100\% = 0.06/0.066 \\ &= 95.6\%. \end{aligned}$$

In other words, with respect to the $PM_{2.5}$, the estimated relative precision of the proposed design will be decreased by 4.4% in comparison to the pilot design used.

3.4 Design efficiency (DE)

On the basis of eqn. (5), DE for $PM_{2.5}$ can be calculated for any specific design using the estimates of RC and RP. For a chosen design, we will have as many DEs' as the number of different outcomes considered in the study. For example, in this study, we are looking at five different outcomes ($PM_{2.5}$ and four elements). A reasonable solution would be to obtain a total (or average) of all the DEs' per design choice. While in theory, there can be a large number of design choices for a given situation, we recommend restricting possible choices of the number of levels for different strata to be close to the number of levels used in the pilot design. For example, the pilot design discussed above (Fig. 1) had three periods, three different choices of weeks (1, 2, or 3) and three choices of days per week (3, 4, or 5). In order to stay close to the pilot design scenario, we will consider a total of 27 potential design choices resulting from 3 choices for periods {2, 3, or 4}, three choices for weeks {1, 2, or 3}, and three choices for the number of days per week {3, 4, or 5}. Table 2 lists estimates of RC, RP, and DE for each outcome ($PM_{2.5}$ and 4 elements) as well as the sum of the individual DEs for some design choices. We only consider and list those designs whose relative precisions are at least 90%. This is proposed since there are designs, which have large savings (in terms of RC) but, at the same time, are characterized by even larger decrease in RP, thus leading to high DE values. The latter may often be counter-productive because the design efficiency would occur at the expense of substantially reduced precision, and such a full-scale study design would

Table 2 Measures of relative cost, relative precision and design efficiency for some design choices^a

Design (p-w-d)	N (Design)	RC (%)	RP (%)					DE					Total	Rank
			PM _{2.5}	Fe	Pb	S	Zn	PM _{2.5}	Fe	Pb	S	Zn		
4-1-3	12	52.2	93.3	102.6	114.9	98.0	93.3	1.79	1.97	2.20	1.88	1.79	9.62	1
4-1-4	16	69.6	110.5	115.0	119.9	102.6	103.8	1.59	1.65	1.72	1.47	1.49	7.93	2
4-1-5	20	87.0	126.9	125.0	123.3	105.7	112.2	1.46	1.44	1.42	1.22	1.29	6.82	3
3-2-3	18	78.3	95.6	97.3	99.1	97.5	94.1	1.22	1.24	1.27	1.25	1.20	6.18	4
4-2-3	24	104.3	113.8	117.2	120.7	117.4	112.8	1.09	1.12	1.16	1.13	1.08	5.58	5
3-2-4	24	104.3	107.0	103.9	101.2	100.4	101.0	1.03	1.00	0.97	0.96	0.97	4.92	6
4-2-4	32	139.1	128.4	125.9	123.5	121.3	121.7	0.92	0.90	0.89	0.87	0.87	4.46	7
3-3-3	27	117.4	103.9	102.2	100.7	104.7	101.7	0.88	0.87	0.86	0.89	0.87	4.37	8
3-2-5	30	130.4	116.1	108.6	102.5	102.3	105.9	0.89	0.83	0.79	0.78	0.81	4.10	9
4-3-3	36	156.5	124.4	123.6	122.8	127.0	122.6	0.80	0.79	0.78	0.81	0.78	3.96	10
4-2-5	40	173.9	140.4	132.1	125.3	123.8	128.1	0.81	0.76	0.72	0.71	0.74	3.74	11
3-3-4	36	156.5	113.3	107.2	102.1	107.1	107.3	0.72	0.68	0.65	0.68	0.69	3.43	12
4-3-4	48	208.7	136.7	130.2	124.8	130.2	130.0	0.65	0.62	0.60	0.62	0.62	3.12	13
3-3-5	45	195.7	120.3	110.5	103.0	108.6	111.1	0.61	0.56	0.53	0.56	0.57	2.83	14
4-3-5	60	260.9	146.0	134.7	126.0	132.3	135.1	0.56	0.52	0.48	0.51	0.52	2.58	15

^a The designs with (RP < 90%) are not included.

have undesirable policy or poor applicability to health outcomes. Thus, setting a cut-off limit for an acceptable loss in RP is highly recommended. The designs are ranked based on total DE in Table 2. The results show that (3-2-4) and (4-2-3) are “similar” to the pilot design in terms of RC and total DE. Recall that the pilot design had 23 observations and was somewhat “unbalanced”. (3-2-4) and (4-2-3) designs, both have 24 observations each and both are “balanced” designs. Designs which ranked above those two are considered “better” than the pilot design, while those below are “worse” than the pilot design. Design (4-1-3) ranks number 1. This design (*i.e.*, exposure sampling in four periods, with one week per period and three days per week) would save sampling costs by 48%, relative to the pilot design. The relative precisions for PM_{2.5} and Zn would be reduced by 7%. While the RP for S will be down by 2%, RPs for Fe and Pb show some increase. If any loss in precision would be deemed as unacceptable, then the (4-1-4) design can be selected, which saves approximately 30% in sampling cost and increases precision for each outcome relative to the pilot design. If we wish to keep the sampling cost for the full-scale study close to the pilot design, then (4-2-3) design may be chosen. This design provides a gain in relative precisions of 13% to 21%. Fig. 2 graphically depicts DEs for all the 27 design choices presented for each outcome. The graphs demonstrate remarkable similarity of the designs with respect to the DEs for the five outcomes. This further ensures that the total DE (as the sum of the five individual DEs, one for each outcome) provides a good index for choosing a design for the specified outcomes measured at a specific site. The entire process of arriving at the potential

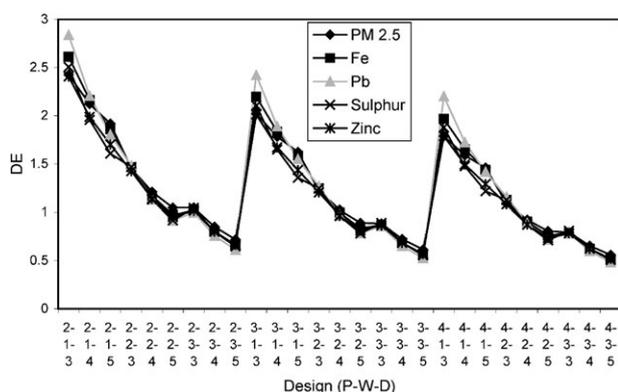


Fig. 2 Design efficiency by design choices for the selected site.

design choices can be summarized in the following sequence of steps:

Step 1: Conduct a pilot study with a feasible environmental sampling design choice.

Step 2: Using a random effects model, obtain estimates of the appropriate variance components from the pilot database.

Step 3: Identify a reasonable number of potential design choices to be evaluated for the full-scale study.

Step 4: Using eqns. (2)–(5) along with the appropriate quantities in them, obtain RP, RC, and DE.

Step 5: Choose an acceptable cut-off for RP (*e.g.*: 90%). Discard designs with RP below the cut-off level. Then, rank-order the other designs with respect to DE (or total DE if more than one outcome is involved).

Step 6: Based on the study parameters, select the design ranking among the top two or three.

We used this step-by-step approach to identify potential design choices for PM monitoring for other sites involved in our study. As expected, the top ranking designs were not always the same from one site to another. This variability occurred primarily because different sites reflected differing sources of temporal and spatial ambient exposure variations resulting in different relative magnitudes of variance components and thereby, arriving at somewhat different top design choices.

Instead of adopting the proposed multi-stage sub-sampling scheme, what if the investigator decided to have a simple random sample of the same number of observations over the entire period of sampling? This situation is akin to one where stratified sampling is preferred over simple random sampling. It is well known that stratified sampling leads to increased precision of the mean as long as the within strata (in our case within period) variation is considerably lower than the between-strata variation. In our study, it is clear that there was a sizeable variation between periods, almost no week-to-week variation within a period, and again sizeable day-to-day variation within a week. Each period in the study consisted of a month of sampling and the months were chosen to broadly conform to the seasons. Thus, a large variation between periods reflects large seasonal variation, which appears to be consistent with the notion, that environmental pollutants in ambient air such as particulate matter and its constituents generally have considerable seasonal variation. Further a sizeable day-to-day variation may be reflecting such short-term local conditions as daily meteorological conditions, daily traffic intensity and industrial emissions including an interaction between them on a daily basis. Consequently, it is reasonable to expect that a design, which accounts for the seasonal

variation by creating strata for seasons (periods) to be more precise than a simple random sample of same number of sampling assessments. One can well imagine that a simple random sample of days can easily fail to represent all possible seasons and therefore underestimate the seasonal component.

In this paper, we primarily focused on obtaining efficient designs for sampling over time. The same generic approach, however, can readily be applied for spatial sampling. For example, in an exposure monitoring application, we could incorporate site-to-site variation in the random-effects modeling and analysis. This approach could lead to a comprehensive sampling design, providing guidelines for both temporal as well as spatial dimensions of environmental sampling. One such application of our approach has been recently described with respect to the temporal and spatial redistribution of monitoring stations in a large metropolitan area.¹²

4. Conclusion

It is often the case in environmental exposure assessment that sites, days, *etc.*, are not actually selected randomly from a larger population but are what become available to the investigators at the time of the measurements. Usually it is safe to assume their availability is the result of a process that is sufficiently haphazard to assure that no bias is involved. If the representativeness is in question, however, then these measures should be used cautiously for estimation of the population characteristics as proposed in this paper.

The methodology is primarily driven by the pilot study results. It is implicit in the approach that the results of the pilot study are indeed applicable to the future full-scale study. In that, we also assume the pilot study not only captures most of the important sources of variability but also that it is representative of the characteristics of the full-scale study. In addition to the temporal factors, different sampling scenarios (be it ambient pollution assessment, water contaminant measures, sediment/soil sampling, personal exposure assessments, *etc.*) require different relevant co-factors to be included in the model. For example, we may include certain meteorological factors such as wind-direction and wind-velocity in the model and “parcel out” the variability due to such co-factors in the exposure assessments from that due to the temporal factors. Inclusion of such factors may or may not effect our final choice of the optimum design. While it is true that the variability found in the pilot study is analyzed and parameterized, our approach to obtaining optimum designs is directly dependent upon the relative sizes of the variance components involved and not on their absolute magnitudes. Therefore, as long as the co-factor’s variance component follows similar general pattern of relative variation as the temporal factors, there will be a minimal impact on the relative sizes of the variance components and so a minimal impact on the choice of the optimum design with or without such cofactors in the model. Nevertheless, it is recommended that the pilot study consider incorporating relevant factors (besides the temporal ones) into the model-building phase of this methodology.

The objective of the study must be clearly delineated in advance so that a clear model specification (both in terms of the number of stages (strata) over time as well as number of sampling sites plus any relevant cofactors) can be made without confusion. In this paper, we are proposing a cost effective and efficient approach to environmental sampling design that is grounded in sound principles of survey sampling. The implications of having a design choice that is objective and data driven and that can be applied to a large full-scale sampling study, are far reaching. Quite often, considerable saving in costs can be realized with little to no loss (not to mention a gain) in

precision of the estimated population quantities. These savings are equally plausible for both environmental monitoring studies^{14,15–18,20,23,25} as well as epidemiological studies of research in environmental health.^{13,19,21,22,24}

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