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Special Series

When and How to Conduct Ecotoxicological Tests Using Natural Field-Collected Sediment

Sara N. Grønlund,[a,b,*] Wing S. Chan,[a] Elettra D'Amico,[a] Mette Flodgaard,[a] Gry Lyngsie,[a] Erin S. McCallum,[b] Annemette Palmqvist,[a] Monica H. Sandgaard,[a] Martina Santobuono,[a] Amalie Thit,[a,c] and Henriette Selck[a]

[a]Department of Science and Environment, Roskilde University, Roskilde, Denmark
[b]Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden
[c]Environmental and Ecotoxicological Laboratory, DHI, Hørsholm, Denmark

Abstract: In recent years, the sediment compartment has gained more attention when performing toxicity tests, with a growing emphasis on gaining more ecological relevance in testing. Though many standard guidelines recommend using artificially formulated sediment, most sediment studies are using natural sediment collected in the field. Although the use of natural field-collected sediment contributes to more environmentally realistic exposure scenarios and higher well-being for sediment-dwelling organisms, it lowers comparability and reproducibility among studies as a result of, for example, differences in the base sediment depending on sampling site, background contamination, particle size distribution, or organic matter content. The aim of this methodology contribution is to present and discuss best practices related to collecting, handling, describing, and applying natural field-collected sediment in ecotoxicological testing. We propose six recommendations: (1) natural sediment should be collected at a well-studied site, historically and by laboratory analysis; (2) larger quantities of sediment should be collected and stored prior to initiation of an experiment to ensure a uniform sediment base; (3) any sediment used in ecotoxicological testing should be characterized, at the very least, for its water content, organic matter content, pH, and particle size distribution; (4) select spiking method, equilibration time, and experimental setup based on the properties of the contaminant and the research question; (5) include control-, treated similarly to the spiked sediment, and solvent control sediment when appropriate; and (6) quantify experimental exposure concentrations in the overlying water, porewater (if applicable), and bulk sediment at least at the beginning and the end of each experiment. *Environ Toxicol Chem* 2024;43:1757–1766. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

For several decades, most environmental risk-assessment frameworks have been focusing on exposure through the water phase when assessing potential impacts of contaminants in the aquatic environment. Consequently, most experimental studies and test guidelines have focused on dissolved chemicals through water exposure. Thus, only a few test guidelines focus on sediment exposure using artificially formulated (Organisation for Economic Co-operation and Development [OECD], 2004, 2007, 2014), natural field-collected (ASTM International, 1999; US Environmental Protection Agency [USEPA], 2001), or contaminated field-collected sediments (OSPAR Commission, 2006; OECD, 2022; International Organization for Standardization, 2005). However, there is increasing awareness of the importance of studying the ecotoxicity of contaminated sediment, predominantly due to aquatic sediments acting as reservoirs accumulating and retaining a large variety of contaminants, for example, nanoparticles (NPs), metals, microplastics (MPs), and organic contaminants (Lehoux et al., 2020; Pavoni et al., 2021; Sandgaard, Palmqvist, et al., 2023). Once accumulated in the sediment, benthic organisms are exposed via ingestion of contaminated sediment and over their surface epithelia by direct contact with the sediment during burrowing, which may lead to contaminant bioaccumulation (Grabicova et al., 2015). Benthic invertebrates constitute a substantial part of the diet of higher organisms (i.e., fish, birds), and thus, it is important to

* Address correspondence to sanigr@ruc.dk
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understand the impacts, bioaccumulation, and subsequent transfer of contaminants in the aquatic food web, in sediment-inhabiting organisms (Grabicova et al., 2015).

A key challenge when working with any sediment system relates to the difficulties in comparing results among studies due to differences in the sediment base (i.e., sediment characteristics including grain size and type, organic matter [OM] content and quality, pH, background contamination) which may cause variations in, for example, compound partitioning and food availability (Picone et al., 2022). Although the majority of laboratory experiments use natural field-collected sediment (Picone et al., 2022), current OECD guidelines recommend artificially formulated sediment (see OECD, 2004, 2007, 2014), most likely due to the theoretically higher homogeneity. Though it has also been recognized that most test organisms do not thrive in artificially formulated sediment—a topic that was recently discussed at the Sediment Interest Group meeting at the Society of Environmental Toxicology and Chemistry, North America Conference, November 2022. Artificially formulated sediment may therefore impact natural behavior, feeding, reproduction, and survival of sediment-dwelling organisms. Furthermore, the abiotic characteristics of artificially formulated sediment likely greatly deviate from those of natural field-collected sediment. For instance, differences in the composition of artificially formulated and natural field-collected sediment may result in variation in contaminant fate, bioavailability, and effects, which could result in either under- or overestimation of contaminant impacts (Landrum et al., 1997).

Although artificially formulated sediment has several disadvantages, using natural field-collected sediment poses different challenges. A fundamental part of studying sediment-associated contaminants relates to the procedure and handling of the sediment prior to experimental use. However, only a few standard protocols specifically focus on sediment-handling approaches, including the field collection of natural sediment, pretreatment of sediment (e.g., sieving, freezing), and preparation for spiking and final exposure (ASTM International, 1999; OSPAR Commission, 2006; Simpson et al., 2005; USEPA, 2001). Although a standard sediment test that conforms with ISO Standard 14731:2012 (International Organization for Standardization, 2012) exists, it is very specific and limited to one system. Consequently, the use (e.g., for laboratory rearing and experiments) and preparation of natural field-collected sediment are often developed and evolved locally within research groups. For instance, the ecotoxicology group at Roskilde University has been using natural field-collected sediment when studying sediment-associated contaminants over the past several decades (see Ellegaard-Petersen et al., 2010; Linke-Gamenick et al., 1999; Nielsen et al., 2017; Palmqvist et al., 2003; Sandgaard, Syberg, et al., 2023; Selck et al., 1998, 2003a; Thit et al., 2015).

The goal of this contribution is to present methodologies and discuss best practices related to the collection and handling of natural field-collected sediment for ecotoxicological testing. The goal is addressed by outlining six key steps in conducting ecotoxicity assays with natural field-collected sediment: collecting sediment in the field, preparing the sediment, characterizing the sediment, altering the sediment, spiking the sediment, and finally, using the sediment in an experimental setup.

It is important to point out that any handling of intact natural sediment (i.e., sieving, freezing, homogenizing, spiking, etc.) will change its physical and chemical properties. We propose a stepwise method that provides control and consistency while maintaining sediment properties in as natural a state as possible. The method we propose applies to instances where a uniform sediment is preferred, for example, when rearing cultures or performing ecotoxicological investigations where contaminants are controlled or added. For regulatory compliance monitoring, natural microbial activity, natural sediment layering, or any case where intact natural sediment is preferred, we recommend using the sediment while fresh and minimizing disturbing and manipulating the sediment during collection and preparation (and if spiking a setup with fresh sediment is necessary, we recommend opting for indirect spiking, to disturb the sediment as little as possible).

COLLECTING SEDIMENT IN THE FIELD

Based on the purpose of the planned ecotoxicological test, there are considerations that need to be made prior to choosing a collection site when sampling natural sediment in the field.

Contamination

Due to the numerous compounds that may potentially contaminate a given sediment, it is strongly advised to choose a collection site that is situated away from point sources and is well studied, preferably both historically and by laboratory quantification of sediment-associated contaminants. As a minimum, a nonspiked control sediment should always be included to both serve as an indicator that no extensive contamination is present and demonstrate that the organisms thrive and behave normally.

Water velocity

Flow velocity affects how sediment particles settle and thereby both the particle size distribution and the OM content of a sediment. The aim of the study should be included in deciding on sampling location: For example, a low-flow site should be selected if a small particle size and high OM are preferred, and a high-flow site should be selected when coarser particles and low OM sediment are needed. In general, most setups benefit from having an OM content between 1% and 20% as a low OM could, for example, contribute to low nutritional value and a reduced contaminant sorption potential, whereas a high OM could, for example, lead to oxygen depletion due to extensive microbial activity and increased microbial OM degradation.

Collection

Collecting natural sediment in the field includes sediment collection, presieving, and storing. We recommend collecting

the sediment in shallow waters as it is more accessible, however, the following collection method can also be applied to deeper waters: (1) scraping off and collecting the top few centimeters of natural sediment using either a shovel, a long-handled curved sieve, or a grab; (2) presieving the sediment on site to remove coarse debris and macrofauna (e.g., to a particle size of ≤1000 μm) using water from the site; (3) if possible, saving the water from presieving for later collection of particles suspended during sieving as these particles are in the smaller size range and hence have a high OM content; and (4) transferring the sieved sediment and sieving water to buckets for transportation and storage until further use. To have access to a viable source of natural sediment year-round and to establish consistency among studies, large amounts of natural sediment can be collected and stored (low temperature and limited light preferred).

Storage

Any material that the sediment comes into contact with will have the potential to release or absorb pollutants (USEPA, 2001). Therefore, it is important to choose a storage material that minimizes the potential of leaching harmful substances to the sediment, especially in the context of further experimental use. Plastic buckets of food grade will often be the preferable choice because they are both lightweight and durable. If the use of new buckets is unavoidable, we recommend soaking these in tap water at least overnight prior to use to reduce chemical transfer from the bucket to the sediment.

PREPARING THE SEDIMENT

Before further use of the natural sediment, it needs to be prepared to fit the experimental requirements (organism preference and the research question) and to make it as uniform as possible. The preparation can be divided into "prefreezing" and "postfreezing."

Prefreezing

We recommend wet-sieving the sediment using a standard stainless-steel-net sand sieve (e.g., Ø = 200 mm) either stacked with decreasing net size top-down for fraction separation or by single sieving only using the net size of the preferred particle size fraction on a mechanical sieve shaker. Alternatively, sieving can be performed manually. Wet sediment is either scooped onto the top sieve while continuously pouring water through the sieve(s) or suspended directly into water and poured onto the sieve. Water used for sieving may be either deionized or experimental test system water (e.g., freshwater or salt water). When preparing bulk sediment for experiments testing different salinities, we recommend using deionized water for sieving as this ensures that the sediment is similar in composition among experimental treatments and allows for subsequent adjustment of salinity. Tap water is unsuitable as it may differ (e.g., in calcium content) over time, potentially affecting calcium-sensitive contaminants (e.g., metals). The preferred sediment particle size fraction and water passing through the final sieve (with the smallest mesh size) are collected in a bucket or something similar. The sediment should be left to settle for 24 to 72 h depending on the size fraction until the overlying water is relatively clear and free from suspended particles. Once settled, the overlying water can be removed by siphoning to avoid loss of OM, and the sediment fractions can be compiled (if sieved in multiple buckets) and should be homogenized (using an immersion blender or by stirring manually) and finally frozen (−20 °C, minimum 24 h) until further use. Sediment may be frozen in suitable containers, that is, buckets, Falcon tubes, or ziplock bags. Freezing is included to avoid unwanted micro- and mesofauna small enough to have passed through the sieving process (i.e., various eggs and neonates).

Postfreezing

After thawing, test water (as used in the experiment) should be added to the sediment to acquire the desired salinity. The process of freezing and subsequently thawing the sediment results in an aggregation/crystallization of sediment particles, which, we have observed, will negatively impact sediment-dwelling organisms. Thus, sediment must be homogenized before any further steps (characterization, spiking, and experimental use) to decrystallize and assure a fully homogenious sediment free of aggregates. Therefore, homogenization after freezing using an immersion blender is a crucial and necessary step, creating a uniform wet sediment with a creamy texture. After settling (24–72 h), the overlying water can be removed by siphoning and discarded. If the sediment is sieved using deionized water and the experimental design requires 31‰ salinity, the sediment needs to be rinsed with test water of the desired salinity, multiple times or with larger water volumes to obtain the desired salinity. Note that the sediment needs to settle to keep the smaller-sized organic-rich particles. The sediment may be stored in the refrigerator (4 °C) if used within a relatively short time frame (weeks) or in the freezer (−20 °C) for longer preservation (months). However, decrystallization procedures must be redone following every freezing event.

CHARACTERIZING THE SEDIMENT

To enhance comparability among sediment exposures, we propose characterizing five essential sediment parameters, which impact the sediment sorption potential, nutritional value, nutrient availability, and compactness. Characterization should be performed on individual subsamples of the sediment when ready for experimental use, which should be discarded after analysis.

Sediment water content

Sediment contaminant concentration should be based on sediment dry weight as the water content depends on grain

size, where smaller grain-sized sediments contain more water compared with larger sand particles. The sediment wet weight to dry weight ratio is used to calculate the amount of contaminant to be spiked into a given quantity of sediment. The contaminant concentration should always be calculated and stated on a dry weight sediment basis. The wet weight to dry weight ratio can be determined by drying (105 °C, 24 h) a subsample (~1–10 g wet sediment) by oven or by rapid drying using a moisture analyzer.

Total OM or total carbon and nitrogen

Being an essential food source for most benthic organisms, the level of sediment OM (measured as loss on ignition: 550 °C, 2 h) provides an indication of whether organisms will be food-limited. Further, OM content influences the sorption capacity of sediment: With increasing OM follows increasing sorption potential; however, contaminants have different affinity toward OM, further affecting sediment sorption (Chin, 2003). Total C and N can be determined via dry combustion. The molar C to N ratio provides an indication of the OM source and quality: A high C to N ratio (>20) suggests a terrestrial source (mean 36, range 7.5–225), and a C to N ratio <15 implies an aquatic source, with a C to N ratio mean of 10.2 (range 2–24) and 7.7 (range 4.5–10) for lake and marine sediment, respectively (Sterner & Elser, 2003). Sediment-feeding invertebrates usually prefer a C to N ratio <10 (Liess & Hillebrand, 2005) because they have a high nutritional demand (Cebrian, 1999). Organic matter, C, and N should be determined on dry homogenous (e.g., ground on a ball mill) sediment.

pH of the sediment

Sediment pH should be determined as it controls nutrient availability but also speciation of ions and compounds. pH may be determined by measuring sediment porewater pH directly, based on the natural moisture content of the collected sediment, by inserting a spear-headed pH probe directly into the sediment. Alternatively, sediment pH can be measured potentiometrically in 0.01 M $CaCl_2$, that is, on a disposable subsample, using a solid to solution ratio of, for example, 1:5 (International Organization for Standardization, 2022).

Particle size distribution

The particle size distribution may greatly influence the packing of sediment grains (Figure 1) and thereby the environment of the experimental setup. For instance, a well-sorted sediment with the particle size skewed toward large particle sizes will have larger pores than a bimodal or small–particle size sediment (Figure 1). Further, sediment-feeding organisms have particle size preferences, and these should be known when selecting a suitable sediment base. Smaller particles tend to have a higher OM load and thus a higher contaminant sorption capacity relative to larger particles. The preferred particle size depends on whether the sediment is used for ecotoxicity testing or for rearing of cultures in the laboratory. For example, in cases where feeding rate is determined by measuring fecal pellet production, it is recommended to select a sediment particle size less than the produced pellets (e.g., <63 µm for *Capitella teleta*, *Potamopyrgus antipodarum*). The benefit is that the pellets may be sieved easily and fast from the sediment. Because it is very time-consuming to sieve and collect the 63-µm size fraction, it is recommended to use larger particle size fractions, for example, 125 or 250 µm, for rearing cultures and for other experimental setups. Other considerations include the organism of choice: Some require coarser sediment, for example, for building tubes, whereas others rely on finer-sized, organic-rich particles due to a high food demand. The particle size distribution can be measured by, for example, the hydrometer method or various laser-based methods (Ferro & Mirabile, 2009).

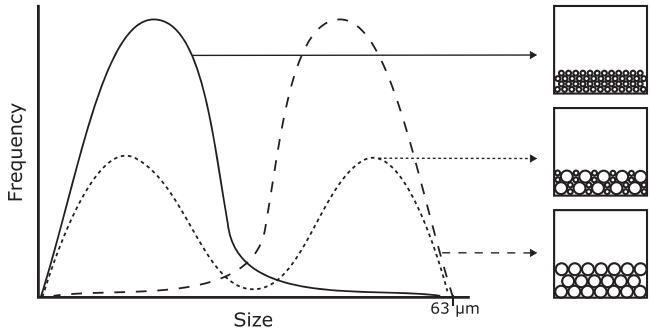

FIGURE 1: Schematic representation of different particle size distributions in a ≤63-µm sediment fraction and their grain packing. Smaller particles will pack more tightly, resulting in less space between particles compared with larger particles.

Background contamination of natural sediment

Contamination is, in general, unavoidable and should be characterized as much as possible prior to using the sediment. However, it is not possible to screen for all contaminants. Therefore, it is important to determine which contaminants could be problematic (e.g., having elevated concentration of or interfering with the compound of interest or affecting the test organism). Background contamination can be quantified using methods like liquid/gas chromatography and mass spectrometry; however, the specific detection methods and quantification of background contaminants depend on the type of contaminant of concern.

ALTERING THE SEDIMENT

It can be necessary to alter the sediment OM to fit the requirements for the setup regarding, for example, food requirements and sorption capacity of the contaminant prior to spiking. Due to seasonal variation of sediment OM, it may be necessary to either add or remove OM from the sediment. There exist several methods to either increase or decrease sediment OM levels. However, the simplest method to increase the OM level is by adding a portion of a smaller-sized (i.e.,

particles ≤63 μm) sediment as more OM is associated with smaller-sized particles. When a lower OM level is required, the sediment OM level of a subsample of the sediment can be reduced by oxidizing the OM with hydrogen peroxide (with a subsequent wash to remove residual peroxide), thus reducing the OM content to approximately zero (Selck et al., 1999). The original sediment can then be mixed with the oxidized sediment to reach the desired OM content. This method has formerly been used to test the impact of OM level for contaminant effects (Selck & Forbes, 2004; Selck et al., 1999, 2003a). Our experience is that sediment-dwelling organisms (e.g., polychaetes) do not thrive in sediment where the OM level has been reduced by combustion (i.e., heating at 550 °C), and we recommend avoiding this method. The sediment should be recharacterized after any type of alteration is made, making sure the final characteristics are determined before spiking.

SPIKING THE SEDIMENT

It is important to consider the chemical properties of the contaminant. Especially, properties affecting the contaminant sorption ability to the sediment are essential to consider and include the following.

Water solubility

The water solubility of a contaminant affects both its bioavailability and environmental fate (Birch et al., 2019). The less water-soluble a compound is, the more likely it is to accumulate in the sediment compartment by binding to OM, resulting in decreased susceptibility to photolysis and hydrolysis (Peterson & Batley, 1993).

Hydrophobicity

The partition constant between octanol and water (log K_{OW}) describes the hydrophobicity of a compound. High hydrophobicity combined with low water solubility makes a contaminant more likely to bioaccumulate and persist in the sediment compartment (Connell, 1994; Jafvert et al., 1990; Peterson & Batley, 1993). Such properties may also affect the choice of contaminant solvent. For contaminants of low water solubility, water is seldom a viable choice (Picone et al., 2022). Organic compounds with low water solubility and high hydrophobicity (high log K_{OW}; i.e., polychlorinated biphenyls, polyaromatic hydrocarbons, and some phosphatidylcholines) often requires an organic solvent (e.g., acetone, methanol, hexane, or dimethyl sulfoxide) to be dissolved (see Méndez et al., 2001; Picone et al., 2022; Selck et al., 2003a). The choice of organic solvent depends on the solubility of the contaminant and the potential solvent impact on the test organisms (Picone et al., 2022). Metals (i.e., Cd, Cu, Pb, and Zn) can be dissolved in weak acid (e.g., HCl) and spiked into the sediment. However, this method requires the acid to be neutralized with base in a corresponding molarity (Selck et al., 1998; Thit et al., 2020, 2021) or that the volume added is small enough to avoid impacting pH or the test organism. No matter the choice of solvent, we recommended always using as little solvent as possible.

Volatility

Volatility is often described as the vapor pressure of the contaminant at a given temperature (often 25 °C). The higher vapor pressure a compound has, the more likely it is to evaporate rather than stay in solution. Volatile compounds often require very well-ventilated space (fume hood) and spiking methods that limit contaminant evaporation and increase the likelihood of remaining in the sediment (Table 1). We recommend to add volatile compounds by inserting the pipette tip into the wet sediment (i.e., not apply on the sediment surface).

Dissociation potential

The dissociation potential describes a compound's potential to dissociate reversibly into smaller components and describes how likely a compound is to ionize at its current conditions depending on solvent and pH (Jafvert et al., 1990). The strength of an acid is described by the acid dissociation constant (pK_a), where a strong acid will have a low pK_a value and be more likely to donate its protons, thereby having greater ionization potential (Patel et al., 2019). The strength of a base is described by the base dissociation constant (pK_b) and is interpreted in a similar way as pK_a. If a contaminant is likely to dissociate under the experimental conditions, the setup will be influenced by the effects of the deconjugated contaminant, which would change the premises of the exposure. In such cases it is important to monitor pH in the overlying water during exposure because changes in pH can affect the dissociation potential and partitioning of the contaminant between water and sediment.

Degradation

As with the dissociation potential, degradation (e.g., photolysis, hydrolysis, microbial degradation) may change the exposure scenario by decreasing the concentration of the contaminant with the formation of metabolites. If the contaminant is likely to be photolyzed, it is important to perform the sediment spiking in the dark or with light as limited as possible (e.g., by wrapping the beakers in foil).

Spiking

In general, sediment spiking can be conducted by either direct or indirect addition of the contaminant (Table 1 and Figure 2). By direct addition, the contaminant is added directly to wet sediment and mixed thoroughly by homogenization into the sediment (Table 1 and Figure 2). By indirect addition, the contaminant is added to the overlying water of a water–sediment setup and left to reach equilibrium (or steady state) between water and sediment (Table 1 and Figure 2). However, indirect addition of a contaminant to the overlying water will result in an uneven vertical contaminant distribution in the sediment column,

TABLE 1: Different spiking methods

	Method	References
Direct spiking		
Solvent evaporation	Contaminant fully dissolved into a solvent is added to an empty glass container and left until complete evaporation of the solvent (e.g., on a shaking table) before adding wet sediment. *Application*: Compounds with low water solubility, high hydrophobicity, and low volatility, likely to adhere to the glass container after solvent evaporation and partition to the OM of the sediment when added.	Granberg and Selck (2007), Selck et al. (2003a, 2003b, 2005)
Addition to wet sediment	Contaminants fully dissolved into a solvent is added directly into wet sediment by placing the pipette tip below the sediment surface. The suspension is released during a slow movement through the sediment. *Application*: Compounds with high volatility and metals in suspension.	Dai et al. (2012), Palmqvist et al. (2003), Ramskov et al. (2014), Selck et al. (1999) Thit et al. (2020, 2021)
Addition of powder	Powdered contaminants either mixed directly into an aliquot of sediment or as an aqueous suspension that is subsequently mixed with the bulk sediment to a homogenous slur. *Application*: Powdered contaminants unable to (fully) suspend into a solvent (e.g., MPs, metal NPs).	Sandgaard, Syberg, et al. (2023)
Stock sediment	Applying either of the previously described direct spiking methods to make a stock sediment with a high contaminant concentration. The stock sediment is then diluted by mixing with uncontaminated sediment to reach the desired concentrations. *Application*: Allows a concentration–response setup using the same bulk of stock sediment as the base.	Selck et al. (1999)
Indirect spiking		
Addition to overlying water	Contaminant either in suspension or as powder is added to the overlying water of a sediment test system and left to reach steady state. *Application*: Simulation of conditions where contaminants are released into the overlying water (e.g., outlet from wastewater-treatment plants or runoff from fields).	Buffet et al. (2013)
Control		
Uncontaminated control	Irrespective of the type of spiking and to assure that the sediment itself is not affecting the experiment (e.g., background contamination), an uncontaminated control should always be included. If wet spiking is applied, the same volume of water per sediment weight as used in spiking should be added to the control sediment to allow similar conditions (e.g., dry wt to wet wt ratio).	Buffet et al. (2013); Dai et al. (2012); Granberg and Selck (2007); Ramskov et al. (2014); Sandgaard, Syberg, et al. (2023); Selck et al. (1999); Thit et al. (2020, 2021)
Solvent control	To ensure that the application of a solvent is not affecting the test results (e.g., adversely affecting the test organism or allowing excessive microbial growth), a setup must contain a control with the same volume of solvent per sediment weight as the spiked sediment and further treated the same as the contaminated sediment.	Palmqvist et al. (2003), Selck et al. (2005)

All types of direct spiking, independently of the method, are followed by homogenization (e.g., on a shaking table) and equilibration for an appropriate amount of time. Sediment (regardless of being uncontaminated or spiked) should undergo the same treatment (i.e., spiking method, quantity of solvent, placed on shaking table, etc.). Note that the solvent volume added to sediment should be as small as possible to avoid impact of the solvent itself. The references provide examples of the various spiking methods.
OM = organic matter; MPs = microplastics; NPs = nanoparticles.

with the highest concentration at the top sediment layer, whereas direct addition of a contaminant to wet sediment will result in a more homogeneous distribution of the contaminant in the sediment column due to homogenization.

There is a general lack of consistency among equilibration time after sediment spiking, ranging from hours or days (see Palmqvist et al., 2003; Sandgaard, Syberg, et al., 2023; Selck et al., 1999; Thit et al., 2020) to weeks or months (see Brumbaugh et al., 2013; Hutchins et al., 2007; Nielsen et al., 2017; Northcott & Jones, 2000), without consistency among similar contaminants. We recommend choosing the spiking method and equilibration time that allow a relatively homogeneous distribution of the contaminant based on both contaminant properties and the research (regulatory or scientific) question. As an example, we have good experience using equilibration times between 24 and 72 h, which results in a homogeneous contaminant distribution in the sediment (see Selck et al., 2003a, 2003b).

Many contaminants (i.e., metals) have the potential to affect the pH of the sediment following spiking. When monitoring pH changes of such contaminants (e.g., during equilibration time), pH should be measured directly in the porewater by inserting a

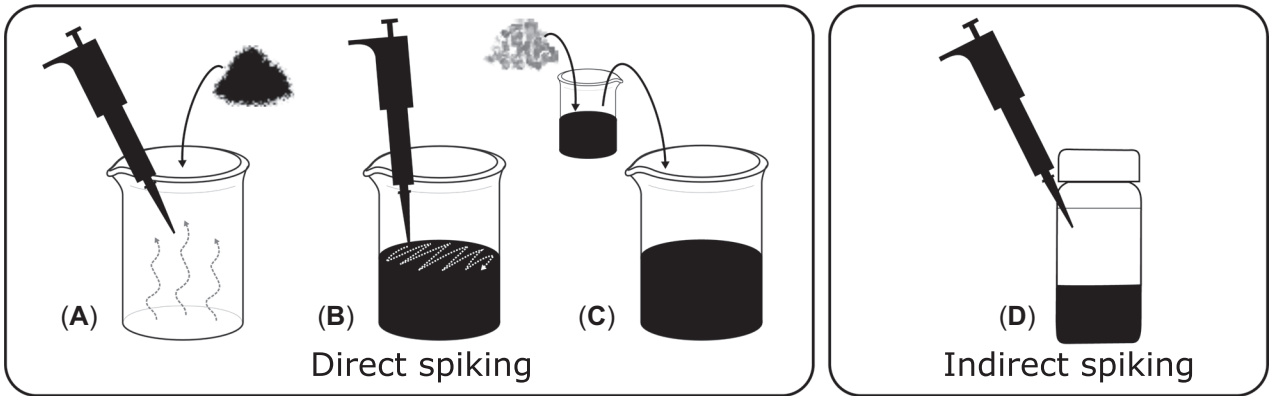

FIGURE 2: Schematic representations of different spiking methods. Direct spiking: (**A**) solvent evaporation, (**B**) addition to wet sediment, and (**C**) addition of powder. Indirect spiking: (**D**) addition to overlying water. Direct spiking will result in a more homogeneous contaminant distribution in the sediment compared with indirect spiking that may result in an uneven vertical contaminant distribution in the sediment column with the highest concentration at the top sediment layer. All spiking methods are described in Table 1.

pH probe directly into the sediment. One way to try to compensate for this is to avoid redox by working in a fully oxygenated setup using a thin layer of sediment (Turner, 1995).

Spiking efficiency

To assess spiking efficiency, when relevant, sediment samples should be collected and analyzed immediately after spiking and equilibration (i.e., before exposure initiation).

Exposure concentration

To assess the exposure concentration, the actual concentration of the contaminant in the sediment should, as a minimum, be measured at the experiment's start and end (i.e., to determine if the contaminant concentration changes over time). We recommend measuring the contaminant concentration in overlying water, porewater, and sediment at experimental termination. Yet, porewater can be difficult to quantify and may, alternatively, be estimated using the equilibrium partitioning equation between water and sediment (see Van Der Kooij et al., 1991). For indirect spiking we recommend measuring the concentration throughout the vertical profile by collecting and analyzing sediment sampled from several depths, if possible.

USING THE SEDIMENT IN AN EXPERIMENTAL SETUP

Sediment transfer

When setting up an experiment we recommend transferring wet sediment to the test containers at least 1 day prior to exposure initiation (i.e., for directly spiked sediment). However, some guidelines require transfer up to 2 weeks prior to experimental start, to allow sedimentation of suspended matter and equilibration of test substance between sediment, porewater, and overlying water (e.g., for some NPs and via indirect spiking; see OECD, 2004, 2022). The amount of wet sediment added to the test vials depends on the experimental setup, for example, organism requirement (allowing burrowing and ventilation, i.e., a relatively thick layer depending on organism size, food level) or contaminant properties (e.g., requirement for oxidized sediment for metals, i.e., a thin layer). Wet sediment may either be transferred using a plastic Pasteur pipette (e.g., with the tip cut off for easier flow for both types of pipettes) or plastic syringe, by spoon, or by the piping bag method using a ziplock bag with a hole cut in one corner. The precise wet weight sediment can be assured by either transferring a given volume (pipetting) or weight (e.g., spoon). The relation between volume and wet weight needs to be established when the volume method is applied. Independent of the transfer method, it is pivotal that the sediment remains homogeneous (i.e., in a consistency where water and sediment do not separate) to ensure consistency among replicates and treatments. This can be achieved by vigorously stirring the sediment prior to every sediment transfer. When using the piping bag method, the sediment can be "massaged" between additions to each test vial. In any case, the most reliable addition to the test vial requires that the sediment has a consistency that is neither too wet nor too dry. If the sediment is too wet, the particles will more rapidly settle out of the water or will settle in a particle size–related manner. This will result in a difference in the sediment composition among test replicates, which may have implications for contaminant concentration and food availability. However, if the sediment is too dry it will be impossible to mix homogenously or transfer by anything but a spoon because it will be too viscous. For high-OM sediment, approximately 80% moisture provides a good homogenous sediment.

Overlying water

Once the sediment has been transferred to the test vials, aerated water should be carefully added to the vial to limit resuspension of the sediment (e.g., by adding it slowly down the side of the test container). In our experience, we have found that there is a risk the organisms will be negatively impacted if the overlying water is not renewed prior to adding the

organisms to the system. We speculate that this is potentially due to impurities or waste products being released from the sediment after spiking. To limit these, we recommend letting the system settle for a minimum of 2 h, followed by replacing approximately two-thirds of the overlying water prior to adding organisms. In cases of indirect spiking, we recommend renewing the water prior to adding the contaminant. In setups using direct spiking, the removed water should be saved and analyzed to determine potential loss of contaminant from the system.

Organisms

After renewing the water, the time before adding test organisms depends on the system (dimension, water to sediment ratio, freshwater to salt water ratio) and how carefully the water has been added (high salinity will lead to faster sedimentation). We recommend preparing test organisms 1 day prior to the experimental initiation and letting the organism depurate overnight in uncontaminated water to let the test organisms empty their gut prior to the exposure because a full gut will both delay the internal exposure of the organism and add fecal matter produced during cultivation to the exposure.

Aeration

The amount of water added to the system affects the need for aeration. If the water level is shallow enough for oxygen to easily penetrate from the surface to the sediment, there is no need for aeration of the test system (and the air will likely disrupt the sediment surface). However, most setups require a water column of several centimeters; and in these cases, aeration may be needed, for example, aerating the water by constantly bubbling the surface water (avoiding disturbing the sediment) or by changing the overlying water with new aerated water, say, every second day. The oxygen supply should be monitored every second day as a minimum because oxygen depletion can have fatal consequences for the test organisms. To limit evaporation and avoid potential airborne contamination, test vials should be covered, and evaporated water should be replaced with aerated deionized water to sustain the salt balance in the system.

Food requirements

To avoid food depletion during exposure, the amount of sediment used in the exposure should be determined according to the food requirement of the test organism, exposure duration, and sediment OM content. For standard test organisms such requirements can be found in the standard guidelines (e.g., OECD, 2004), and for other organisms their daily food requirements may be found experimentally or through a literature search. To ensure a constant exposure concentration and to avoid food limitation in long-term setups, organisms can be transferred to new test vials at, for example, weekly census times. In cases where the contaminant concentration decreases over time, changing the system at each census time will result in the contaminant being added in pulses, and the concentration of the contaminant metabolites will be removed at each census time. Thus, for some setups it is better to use the same sediment throughout the exposure period. Alternatively for sediment-associated organisms that are non-deposit-feeders, additional food may be added to the system; however, note that this may lead to other challenges including dilution of the contaminant in the system.

Ambient factors

Ambient factors such as temperature and light may also affect the compound (e.g., decay, metabolism) and the behavior and well-being of the test organism. Therefore, it is important to consider temperature and light cycle, that is, if the test organism needs to be acclimated to a more suitable temperature for the sake of the compound or the environment that is simulated or if the experiment requires either dark or a fixed light cycle.

Monitoring effect

Besides applying standard effect modeling (i.e., concentration–response) to assess the effects of an exposure, another useful tool when monitoring a setup can be to apply cumulative sum (CUSUM) due to it being sensitive to small and moderate changes (Newman, 1994).

SUMMARY OF RECOMMENDATIONS

- When collecting natural sediment, the site should be well studied, both historically and by laboratory analyses.
- Larger quantities of sediment can be collected and stored to secure a uniform experimental sediment base. Note that the storage containers should have no or low potential to contaminate the sediment.
- All sediment should, at the very least, be characterized by determining water content, OM (total organic carbon or C to N ratio), pH, and particle-size distribution, as these factors influence nutritional value, nutrient availability, chemical sorption potential, and packing of the sediment.
- Selection of spiking method (direct, indirect), equilibration time, and experimental setup (e.g., thickness of sediment layer) should be determined based on the properties of the contaminant and the research question.
- Control sediment should always be treated similarly to spiked sediment (i.e., adding the same volume of solvent per weight unit). If using a carrier solvent during spiking, the experimental setup should include solvent controls to determine the toxicity of the solvent.
- Experimental concentration should be quantified in overlying water, porewater (if possible), and bulk sediment at least at the beginning and end of an experiment.

IMPLICATIONS

Following the recommendations will ensure more environmentally realistic sediment exposure scenarios; a higher degree of comparison between experiments using different base sediments; and higher reproducibility among sediment exposures.

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