

**Ecotoxicological risk of trace element mobility in coastal semi-artificial
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**Ecotoxicological risk of trace element mobility in coastal semi-artificial depositional
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Running head: Ecotoxicity of trace elements in coastal depositional areas

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Abstract: Artificial sand replenishments are globally used as innovative coastal protection measures. In these replenishments elevated pore water concentrations of trace elements are found. This study investigated possible ecotoxicological risks at two intertidal depositional sites, the Sand Engine (SE) as a recent innovative Dutch coastal management project, and a semi-artificial tidal flat (TF). Using the Sediment Quality Triad approach, we considered three major lines of evidence (LoE): geochemical characterization, toxicity characterization using bioassays with the estuarine amphipod *Corophium volutator* and ecological field survey. In both depositional areas *C. volutator* is at risk: moderate (SE) and low (TF). For TF, the bioavailability of trace elements differs between the field site and the laboratory. Contamination from arsenic and copper is present, but the low survival rate of *C. volutator* from the bioassay suggests presence of additional contaminations. The highly morphological dynamic environment of SE creates a less favourable habitat for *C. volutator*, where local spots with stagnant water can temporary create hypoxic conditions and sulphate becomes reduced. The dynamic system mobilises especially arsenic, triggering adverse ecotoxic effects at low original sediment concentrations. To conclude, the Sediment Quality Triad approach shows that a semi-artificial tidal flat is preferred over a highly dynamic coastal management project like the Sand Engine. The Sand Engine concept does not provide suitable conditions for macrobenthos species like *C. volutator*, therefore limiting the nature development goal set together with the coastal protection goal. Assessing each LoE from the approach together with additional measurements established more precise and realistic conclusions, showing the need that evaluating contributions of this method is necessary to understand the causes of risk in a site-specific manner. This article is protected by copyright. All rights reserved

Keywords: ecotoxicology, risk assessment, trace metals, intertidal flat, *Corophium volutator*, Sediment Quality Triad

INTRODUCTION

Coastal and estuarine waters mostly receive contaminants via on-shore anthropogenic activities. These, after transport via rivers or air, can induce toxic effects on benthic fauna and contribute to the degradation of ecosystem function (Eggleton and Thomas 2004; Cantwell et al. 2008; Roberts 2012). In coastal areas, sediments are very important in ecosystem functioning, but also act as a sink for anthropogenic contaminants thereby becoming a chemical stressor on benthic fauna and associated species (Burton and Johnston 2010; Roberts 2012). Intertidal flats are usually one of the sedimentary environments that can be distinguished in coastal areas. Estuarine intertidal flats show a high biological activity (Herman et al. 2001). These sites harbour primary sources of organic matter, the food base for benthic biota, and as a result contain considerable biomass (Heip et al. 1995).

Environmental studies of intertidal flats generally focus on chemistry, both organic (Benlahcen et al. 1997; Moreira et al. 2016) and inorganic (Alshahri 2017; Vetrinurugan et al. 2017). From an ecological perspective, toxic substances such as trace elements, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are regarded as an important quality criterion (Speybroeck et al. 2006). Heavy metal binding on solid compounds is known to be a critical factor for mobility and bioavailability (Peijnenburg et al. 1999), and thus toxicity. Concentrations of trace elements in sediments can exceed those of the overlying water by three to five orders of magnitude (Bryan and Langston 1992). Resuspension may result in them becoming toxic, especially to burrowing organisms that are in close contact with pore water. Invertebrates such as amphipods bioaccumulate contaminants through their burrowing and feeding activities (Goodyear and McNeill 1999).

In 2011, a new depositional environment, the Sand Engine, was created along the Dutch coast by an innovative coastal management project (Stive et al. 2013). This mega nourishment

contains 21.5 million m³ of sand and rises up to 6 m above mean sea level. For the Sand Engine, sand was dredged 10 km offshore to a maximum depth of 6 m below the sea floor (Fiselier 2010). The Sand Engine was shaped like a large hook, with an initial length of 2.4 km and a width of 1 km that extends offshore (Figure S1). It is designed to enable sand transport by natural processes to feed coastal parts in a 20 km stretch within 20-30 years that are vulnerable to erosion. Besides coastal protection, the Sand Engine has recreation and nature development as additional goals. Because of its distinctive shape a lagoon is present, sheltered from the waves and with variable tidal velocities. As a result, deposition is taking place and many benthic micro algae are present (Pit and van Egmond, personal observation).

The Rhine flows into the sea approximately 10 km from the Sand Engine and is a source of estuarine and marine contamination (e.g. Laane et al., 2013; Ruff et al., 2015). Numerous physical, chemical and biological processes affect the fate of particles and micropollutants (Schwarzenbach et al. 2017). The sediment grain size distribution affects environmental fate of micropollutants, as the silt-clay fraction increases the sorptive capacity of the contaminants (Villaescusa-Celaya et al. 2000; Cantwell et al. 2008). Sedimentation of silt/clay material in the lagoon of the Sand Engine might therefore reduce the bioavailability of toxic trace elements. Additionally, sorption by organic matter and Fe and Mn oxyhydroxides can lower dissolved metal concentrations in the water column (Cantwell et al. 2008), of which organic matter is correlated with the fine fraction because of its high affinity with clay particles (Oades 1984). However, as the morphological dynamics at the Sand Engine governed by waves and vertical tide are high (De Schipper et al. 2016; Luijendijk et al. 2017), remobilization might occur because of resuspension (Zoumis et al. 2001). A previous study found elevated concentrations of trace elements in pore water at Dutch beach nourishment sites, including the Sand Engine, which had resulted from oxidation processes (Pit, Dekker, et al. 2017). Thusfar

it is unclear whether these elevated trace element concentrations are also present where local deposition occurs, and whether they bear ecotoxicological risks.

The current study aims to assess sediment quality at the intertidal area of the engineered Sand Engine and a semi-artificial depositional area by comparing the ecological, chemical and physical characterizations of the two depositional areas. Both sites show a natural sedimentation, because conditions became favourable after coastal management measures. The intertidal areas are near (the Sand Engine) or at (the semi-artificial depositional area) the mouth of the river Rhine, that brings anthropogenic contaminants into the coastal environment. We will also reflect on the nature development goal of the Sand Engine concept and discuss whether trace elements will hamper its attainment. We used the Sediment Quality Triad approach (Chapman 1990; Linkov et al. 2009), considering three major lines of evidence (LoE): (1) geochemical characterization, (2) toxicity characterization using bioassays and (3) ecological field survey. The focus was on the estuarine amphipod *Corophium volutator* as a relevant macrobenthic species often used for risk assessment of contaminated estuarine and marine sediments in large parts of northern Europe (Peters and Ahlf 2005). In addition, toxic trace elements present in the sediment were examined, as well as the bioaccumulation in *C. volutator* collected from the two field sites.

MATERIAL & METHODS

Site selection and characterization

Site selection. We compared two intertidal areas (Figure 1), the first site was the lagoon of the Sand Engine (SE), located close to Ter Heijde at the Dutch coast (Stive et al. 2013). The Sand Engine is morphological dynamic because of its development by erosion enhanced by wind, waves and currents. The lagoon is on the northern side of the peninsula. Since construction, the entrance to the lagoon has developed enormously, changing the width and depth of the tidal

channels and as a result, the tidal flows entering and leaving the lagoon (Luijendijk et al. 2017). The second site was a semi-artificial depositional area at Oostvoorne (TF), located in the northern part of the estuary of the river Rhine (Noest 1991), which is also known as Slikken van Voorne. Here, natural sedimentation occurs because conditions became favourable after coastal management measures, i.e., shelter from waves and low tidal flow velocities (De Brouwer et al. 2001). Sediment, pore water and *C. volutator* were sampled in August and September 2016. Per site, ten locations were sampled (Figure 1) at low tide, along the low waterline where the intertidal flat was accessible and near-saturated.

Pore water characterization. Rhizons were used to extract pore water from the soil following Seeberg-Elverfeldt et al. (2005). The rhizons are made of a hydrophilic porous polymer tube, with a pore diameter of 0.1 μm , which ensures the extraction of microbial- and colloidal-free, ready-to-analyze solution. The outer diameter of a rhizon is 2.4 mm, and the filter section has a length of 10 cm. Pore water samples were taken at depths of 1.5 cm and 7.5 cm by inserting a plastic platform with two holes at these depths into the sediment. Rhizons inserted through the holes extracted sufficient amounts of pore water horizontally from the sediment allowing minimum disturbance of the sediment. Pore water was stored and taken to the lab in a glass vial for the ion chromatography and a PE vial with 0.2 M HNO_3 (10 μl HNO_3 / 10 mL pore water sample) for the inductively coupled plasma mass spectrometry, which is further described below.

General characterisation of the pore water was done both directly in the field and in the lab. In the field, we immediately measured O_2 (Hach Intellical LDO10103), Electric Conductivity (EC) (Hach Intellical CDC401), pH (Hach Intellical PHC101), and redox potential (Pt4805- DPA-SC-S8/225). Additionally, pore water was taken to the lab in a glass vial and analysed for Total Organic Carbon (TOC) using a Shimadzu TOC-5050A Analyzer.

Within 24 hours after sampling, alkalinity as HCO_3^- was measured according to Sarazin et al. (1999).

The pore water was analysed for major ions and trace elements, including rare earth elements (REEs) by using ion chromatography (type: 930 Compact IC flex) for (sequenced according to Limits of Detection (LOD), shown in brackets) Cl (0.2 mg/L), PO_4 (0.07 mg/l), NO_3 (0.06 mg/L), SO_4 (0.04 mg/L), F (0.03 mg/L), NO_2 (0.02 mg/L), Br (0.002 mg/L), and inductively coupled plasma mass spectrometry (ICP-MS, type: Agilent 7500cx collision cell) for (sequenced according to LOD): Ca (0.15 mg/L), K (0.1 mg/L), Mg (0.05 mg/L), Fe and P (0.04 mg/L), Al (0.01 mg/L), Mn (5 $\mu\text{g/L}$), Zn (4 $\mu\text{g/L}$), Sr (1.5 $\mu\text{g/L}$), Ag and Ba (1 $\mu\text{g/L}$), Ni (0.8 $\mu\text{g/L}$), Se and Sn (0.6 $\mu\text{g/L}$), Cr and Mo (0.5 $\mu\text{g/L}$) As and Cu (0.4 $\mu\text{g/L}$), Eu, Gd, Tl and V (0.3 $\mu\text{g/L}$), Ce, Co, Cs, Dy, Er, Ga, Ho, In, La, Lu, Nd, Pb, Pr, Rb, Sb, Sm, Tm, U and Yb (0.2 $\mu\text{g/L}$), Be and Cd (0.1 $\mu\text{g/L}$).

To elucidate whether oxidation or reduction processes influenced the pore water, the measured SO_4 concentration was compared with the SO_4 concentration of conservative mixing of fresh water and seawater. First, the fraction of seawater (f_{sea}) was calculated from the Cl concentration:

$$f_{sea} = \frac{R_{Cl,sample} - R_{Cl,fresh}}{R_{Cl,sea} - R_{Cl,fresh}} \quad 1$$

where $R_{Cl,sample}$ is the Cl concentration of the sample, $R_{Cl,fresh}$ the Cl concentration in rainwater, which is assumed to be similar to water from the river Rhine, and $R_{Cl,sea}$ the Cl concentration in seawater. Then, the conservative mixing concentration ($R_{\text{SO}_4,mix}$) is calculated from:

$$R_{\text{SO}_4,mix} = f_{sea} \cdot R_{\text{SO}_4,sea} + (1 - f_{sea}) \cdot R_{\text{SO}_4,fresh} \quad 2$$

where $R_{\text{SO}_4,sea}$ and $R_{\text{SO}_4,fresh}$ are the SO_4 concentrations in seawater and fresh water, respectively. The rainwater composition with 2.4 ppm SO_4 was derived from Stolk (2001) and

that of seawater from Hem (1985) with 2700 ppm SO₄. Finally, the enrichment or depletion of SO₄ (ΔR_{SO_4}) is then obtained by:

$$\Delta R_{SO_4} = R_{SO_4, sample} - R_{SO_4, mix} \quad 3$$

A positive ΔR_{SO_4} indicates pore water is enriched with SO₄ and this enrichment is most likely associated with oxidation of sulphide minerals such as pyrite; a negative ΔR_{SO_4} indicates SO₄ has been depleted, which is normally the result of SO₄ reduction in the environments studied.

Sediment characterization. Approximately 500 g surface sediment was sampled by pooling three subsamples: one taken at the centre of the sample point and two taken 0.5 m from the centre. The sediment was sampled with a plastic scoop and each subsample was taken down to a depth of 10 cm. Sediment samples were oven-dried at 105 °C for at least 24 hours. Grain size distribution was measured with a laser diffraction analyser (Malvern Instruments Ltd., 1998) in accordance with NEN 5753 (1990). The samples were then dry sieved in a plastic sieve with nylon cloth to obtain two fractions < 63 µm (> 250 mesh) and 63-2000 µm (10-100 mesh). The large fraction was ground into particles < 2 µm with a Herzog HP-PA grinding machine. This resulted in contamination with Co and consequently the ground fraction could not be used for interpretation on Co. For quantitative determination of mineral compounds, including organic matter, both fractions were subjected to thermogravimetric analysis (TGA) by heating them from 25 to 1000 °C at a rate of 1 °C/min, using a Leco TGA-601. The method used to estimate organic material and carbonate minerals from the TGA data is described in Pit, Griffioen, et al. (2017). The aqua regia digestion method (Houba et al. 1995) was used to obtain the pseudo-total elemental concentrations of the sediment (Chen and Ma 2001), which were then analysed with inductively coupled plasma optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) to obtain main and trace elements similar to those in the pore water.

Bioassay characterization. Sediment for the bioassay was sampled from two locations at TF and SE. A control was performed with sediment from a control site (CS1), an intertidal mudflat (Oesterput) in the Eastern Scheldt in the southwest Netherlands (Figure 1), which is commonly used as a source of control sediment in toxicity tests (e.g. Schipper et al., 2008; Stronkhorst et al., 2004). As the sampling locations at TF did not significantly differ in terms of numbers of *C. volutator*, sediment from the first and last transects (TF1 and TF10) was chosen for the bioassay. For SE, we used sediment from SE5, which contained the highest number of *C. volutator*, and from SE10, which contained a high clay/silt fraction without presence of *C. volutator*. At each location, a 10 L bucket was filled with sediment and overlying water, closed with a lid and transported to the lab the same or following day. In September 2016 *C. volutator* individuals needed for the bioassay were sampled at CS1 by sieving the first two cm of sediment over a 1 mm sieve and separating *C. volutator* from the other species.

Prior to the experiments the sediment was homogenized and wet sieved over a 500 μm sieve, except for the control sediment which contained finer material and was therefore sieved over a 250 μm sieve. Artificial sea water containing 15‰ salt was used for sieving. In total five sediments (SE5, SE10, TF1, TF10 and the control, CS1) were sieved and included in the bioassay, which was carried out in a climate chamber at 17°C in two phases over a period of seven weeks.

Ecology characterization. The ecological field survey consisted of *C. volutator* field density and the species richness. To estimate *C. volutator* field density, we sampled the population at SE5, SE10, TF1, TF10. Each sample was taken with a stainless steel rectangular corer of 27 by 37 cm (surface area 999 cm^2) up to a depth of 15 cm. Sediment was sieved over a sieve with 1 mm mesh size and the macroinvertebrates that remained were placed in a plastic container containing 70% ethanol. In the laboratory, the *C. volutator* individuals were counted.

The species richness of SE and TF were obtained from parallel studies, of which the species richness for SE was obtained from a study within the same project (van Egmond et al. 2018) and the species richness for TF was obtained from van der Zee et al. (2018).

As a control needed for the LoE output, previously published information on *C. volutator* field density and species richness (Dekker and Waasdorp 2006) at Heringplaat (CS2) was used, because of a similar grain size distribution. CS2 is relatively clean intertidal mudflats with 99% survival rates of *C. volutator* as bioassay result (Van Den Brink and Kater 2006).

Sediment Quality Triad

The Sediment Quality Triad (from now on referred to as Triad) approach considers three lines of site-specific evidence: chemical characterization (e.g. total concentration, bioavailable concentration, bioaccumulation), toxicology (e.g. bioassay of the sediment, biomarkers), and in situ parameters (e.g. benthic community structure, field observations of vegetation) (Chapman 1990; Jensen and Mesman 2006; Swartjes et al. 2012). To integrate the results of the different LoEs and obtain a Triad value for each site we used the following (Ribé et al. 2012):

$$\text{Triad Effect value} = 1 - ((1 - LoE_{CHEM}) \cdot (1 - LoE_{BIO}) \cdot (1 - LoE_{ECO}))^{\frac{1}{3}} \quad 4$$

The Triad Effect value is a result on an effect scale from zero to one, which corresponds to no effect up to maximum effect (Mesman et al. 2006). For each line of evidence (LoE), a tool is selected as input for the Triad of which the outcome is projected on the effect scale. Here, the LoE_{CHEM} consists of estimating the bioavailable concentration by quantifying the local toxic pressure of a mixture of compounds. This is done by using the Potentially Affected Fraction of species (msPAF) (Posthuma and de Zwart 2012). The msPAF value is estimated using the species sensitivity distribution (SSD) concept. The LoE_{BIO} is a chronic sediment toxicity test

consisting of a 49-day exposure with the *C. volutator*. A chronic bioassay was chosen instead of short-term bioassay because of its higher sensitivity to pollution and its environmentally realistic exposure scenarios (Van den Heuvel-Greve et al. 2007). The LoE_{ECO} consists of two different parameters: the field density of *C. volutator* and the species richness. Two in situ parameters were chosen to have a more realistic overview of the ecosystem where the focus is not just on one species. An elaborated description of each LoE is described below.

LoE_{CHEM}. The first LoE is geochemical characterization (LoE_{CHEM}) using the chemical elements As, Ba, Be, Cd, Co, Cr, Cu, Mo, Ni, Pb, Tl, Sb, Sn, V and Zn. The potentially affected fraction of species (msPAF) was used to quantify the ecotoxicological risk based on pore water concentrations (de Zwart and Posthuma 2005; Posthuma et al. 2016). PAF was calculated using SEDIAS (SEDIment ASsistant, see Hin et al., 2010) from the chronic effect concentrations (EC_{50}), using the following equation (Mesman et al. 2006):

$$PAF = \frac{1}{1 + e^{\frac{\log EC_{50} - \log C_{pw}}{\beta}}} \quad 5$$

EC_{50} is the effect concentration for 50% of the macro benthos species (which includes *C. volutator*), C_{pw} is the pore water concentration (Table S1) and β the standard deviation of the average log effect EC_{50} concentration. Subsequently, msPAF values were derived via the following equation (Mesman et al. 2006):

$$LoE_{CHEM} = msPAF = 1 - ((1 - PAF)_1 \times (1 - PAF)_2 \times (1 - PAF)_n) \quad 6$$

Only PAF values of > 0.03 were included for the calculation of msPAF, because of uncertainties with low PAF values. The input for the Triad was the average msPAF value between both depths. A threshold of 0.05 is often used for deriving environmental quality

standards (e.g. Maltby et al., 2005; van Wezel et al., 2000) at which 5% of the species will exhibit > 50% effects (Posthuma and de Zwart 2012; Posthuma et al. 2016).

LoE_{BIO}. The second LoE consists of a bioassay (*LoE_{BIO}*) to test chronic sediment toxicity with the *C. volutator* (Van den Heuvel-Greve et al. 2007). A total of 800 individuals between 0.5 and 1 mm in size were selected and kept in two 6-L aquaria with a substrate of 4 cm clean silt and artificial seawater (20‰ salt, Instant Ocean) and aerated with compressed air. The aquaria were placed in a climate chamber at 17°C with a light/dark regime (8/16 h) and the animals were fed powdered fish food *ad libitum* every other day. After three weeks, all female *C. volutator* with a brood sac filled with eggs were selected and kept in the aquarium for another week, until juveniles were born. Juveniles aged between 0 and 7 days were used for the bioassay.

In the first phase, 150 randomly selected *C. volutator* individuals were grown in a 6-L aquarium with a 3 cm thick substrate of one of the test sediments overlain by about 10 cm of artificial sea water (20‰ salt, Instant Ocean). The aquaria were covered with transparent plexiglass and aerated with compressed air. After an incubation period of 21 days, mortality was determined. For the second phase, the surviving *C. volutator* individuals were placed in smaller aquaria with fresh sediment that had undergone the same preparation as for the first phase. Approximately 250-300 mL of sediment was divided over four 1000 mL glass beakers and 700-750 mL of artificial sea water (20‰ salt, Instant Ocean) was added. Due to the low survival of *C. volutator* in the first phase for some sediments, there were either two (TF1, TF10 and SE10) or four (CS1 and SE5) replicates, each containing 20 randomly selected surviving juveniles from the first phase. After four weeks, the surviving adults were collected to determine survival percentage, gender, the number of eggs carried by the females and body

length. The value used for the WoE is the growth inhibition (% divided by 100) vis-à-vis the control sample (CS1) (Ribé et al. 2012):

$$LoE_{BIO} = \frac{R_{sample} - R_{control}}{1 - R_{control}} \quad 7$$

After phases 1 and 2, the *C. volutator* were placed on a petri dish for 12 to 24 hours to empty guts and then frozen until further analyses (see paragraph “ LoE_{CHEM} ”).

LoE_{ECO} . The ecological field survey for the third LoE (LoE_{ECO}) consists of determining the field density of *C. volutator* and the species richness at each field site. The ecological field survey was scaled in accordance with Ribé et al. (2012) and with CS2 as $R_{control}$:

$$R_1 = \left| \log \left(\frac{avg(R_{sample})}{avg(R_{control})} \right) \right| \quad 8$$

$$R_2 = 1 - 10^{(-R_1)} \quad 9$$

Where equation 7 and 8 were used for the species richness as well as for the *C. volutator* density. The results were integrated into one LoE using equation 10:

$$LoE_{ECO} = 1 - ((1 - R_{2,density C.vol}) \cdot (1 - R_{2,species richness}))^{1/2} \quad 10$$

Bioaccumulation

Individual specimens of *C. volutator* were collected in the field together with the chemical characterization of the fine (< 63 μ m) fraction to calculate the sediment biota sediment accumulation factor (BSAF) as a field-based measurement endpoint to help reconcile potential differences between the results of the different LoEs. Due to low population density at SE, 20 to 28 adult *C. volutator* individuals were taken per sampling location, compared with 38 to 45 individuals at TF. Individuals were placed in a glass vial and stored in 70% ethanol. Both the individuals stored in ethanol in the field, and the frozen individuals from the bioassay, were placed in a clean glass vial and dried in the oven for 72 h at 70°C. They were then

subjected to aqua regia digestion and analysed by ICP-OES and ICP-MS as described for the sediment samples. Because of a low input weight, the results were corrected for the blanco results in order to prevent any increased concentrations from contamination. When samples contained an input weight of less than 0.0010 g, the results were not used, because of the errors for each variable, which were increased by the high dilution factor. Additionally, when the values were below detection limit, the results were not included in the analyses of BSAF, because of the high uncertainty. The results of the frozen *C. volutator* individuals from the bioassay contained 2 to 4 replicas and were averaged to obtain one value for each sample location (Bio_SE5, Bio_SE10, Bio_TF1, Bio_TF10 and CS1). BSAF was calculated using the following equation:

$$BSAF = \frac{C_b}{C_s} \quad 11$$

Where C_b is the concentration of the trace metals in biota (mg/g), in this case *C. volutator*, and C_s is the concentration in sediment in the fine fraction (mg/g).

Statistical analyses

Statistical analyses were performed using SPSS (version 24) for significant differences between sites or sediment fractions. Prior to the statistical analysis, values below detection limit were set at a value half the detection limit (Reimann et al. 2008). When more than 25% of the values of a variable for the chemical characterization of the sediment were below the detection limit, the variable was excluded from the statistical analysis and presented as < d.l. in the results section. For the chemical characterization of the pore water this was not needed, because of the possibility to exclude the values which were below detection limit to calculate the msPAF.

All chemical variables were transformed using the isometric log-ratio (ilr) transformation (Egozcue et al., 2003), preferred over a normal log-ratio transformation since it generates a robust estimation of the covariance matrix (Filzmoser et al., 2009). The ilr-transformed data was then back-transformed to centred log-ratio (clr) space to maintain the links to the original variables and enable direct interpretation. However, for the Triad, the results of each LoE were not transformed prior to statistical analyses, because of the scaling of the results between 0-1.

For significance tests comparing two datasets, the Mann-Whitney U test or independent t-test were used, depending on whether or not the data are normally distributed. When multiple groups of normally distributed data were compared, an independent ANOVA was performed with a post hoc test. The homogeneity of variances varied significantly per variable and therefore a Games Howell post hoc test was chosen (Field, 2009). When a normal distribution was not found, an independent non-parametric Kruskal Wallis test was performed.

Before integrating the LoE results of the chemical characterization per site (msPAF) with the sediment toxicity test (growth inhibition of *C. volutator*) and ecological field survey (density determination of *C. volutator* and species richness), a bootstrap was performed (Efron and Tibshirani 1993). Bootstrapping estimates the properties of the sampling distribution from the sample data, resulting in a more realistic standard deviation when the original sample data comprises a small amount. The sample data are treated as a population (Field 2009), where a total of 100 samples had been randomly drawn from LoE results. However, bootstrapping is only possible when the LoE results are $n > 1$ with non-equal values. From the bootstrapping, a mean and standard deviation are calculated and used for Triad input.

RESULTS

General characterization

First, the chemical characteristics of the sediment for the total sediment and the fine fraction, as well as the grain size distribution are shown of the two intertidal areas at SE and TF (Table 1). The Intervention Value is given to compare it with the chemical characterisation of the sand. To do this, the Intervention Value is corrected from a standard soil with 10% organic material and 25% clay (soil particles $< 2 \mu\text{m}$) to a soil most comparable to sand, with 2% organic material and 2% clay (VROM 2013). The sediment contents are below the Intervention Value, especially the concentrations in the coarse fraction. The differences between TF and SE are large, both for properties like CaCO_3 , S and D50 as for the trace metals. Differences are even more pronounced for the fine fraction.

Information on the pore water quality is presented in Table 2, with averages on field measurements and TOC. Differences between the two intertidal areas are significant for TOC, EC and pH (Mann-Whitney U, $p < 0.05$). Overall, pore water at TF is more mixed with fresh water, the pH is slightly lower and TOC higher compared to SE. Redox conditions are comparable, but the highest mean value is at SE for 1.5 cm depth and the same trend is seen for the alkalinity. Oxygen concentrations are relatively low both at 1.5 cm and 7.5 cm depth, with SE having an average of $1.3 \pm 0.5 \text{ mg/L}$ and TF $1.7 \pm 0.4 \text{ mg/L}$ at 1.5 cm depth, and at 7.5 cm depth it is a bit higher with SE having $1.6 \pm 0.4 \text{ mg/L}$ and TF $1.9 \pm 0.6 \text{ mg/L}$. From two locations at both SE and TF, the oxygen concentrations of the surface water was measured, showing an average of $9.3 \pm 0.4 \text{ mg/L}$ and thus close to saturation with air at equilibrium (Stumm and Morgan 1996), illustrating the right accuracy of the measurements.

Figure 2 confirms that TF is more fresh compared to SE, where the concentrations of SO_4 were plotted against Cl together with a mixing line of rainwater and seawater. TF shows

an average of 6904 ± 758 mg Cl /L, compared to 13077 ± 1840 mg Cl /L at SE. The SO_4 concentrations are mainly below the mixing line which indicate reduction processes. However, the relative distance from the mixing line is small and with ΔR_{SO_4} showing an average of -90 mg/L for SE and -44 mg/L for TF, reduction may be dominant, but oxidation processes are likely to play a role as well. To get more information on the dominance of anoxic or oxic processes present at the two study sites, Fe and Mn in pore water as well as those of the sediment are plotted in Figure 3. Iron concentrations at TF are on average higher compared to SE for 1.5 cm depth: 4.3 ± 3.4 mg/L versus 2.0 ± 2.0 mg/L, although the difference is not significant (Mann-Whitney U test, $p < 0.05$). For 7.5 cm depth, Fe concentrations are a bit higher at SE compared to TF (3.5 ± 2.4 mg/L versus 3.1 ± 1.9 mg/L) and not significant. Manganese concentrations in the pore water are for both 1.5 cm depth as well as 7.5 cm depth higher at TF (1.1 ± 0.8 mg/L and 1.1 ± 0.6 mg/L) compared to SE (0.7 ± 0.6 mg/L and 0.6 ± 0.4 mg/L) and not significant. The mutual occurrence of oxygen and dissolved Fe and Mn indicate that the sediments are not in redox equilibrium at the sample scale suggesting on-going redox reactions. Iron sediment contents at SE with an average of 3926 ± 841 mg/kg is significantly lower compared to TF with an average of 4907 ± 1851 mg/kg (Mann-Whitney U test, $p < 0.05$). For Mn, TF has significantly higher concentrations as well, with an average of 179 ± 97 mg/kg compared to 84 ± 24 mg/kg at SE (Mann-Whitney U test, $p < 0.05$). Both the Fe and Mn sediment contents for TF are increasing with sample location number, coinciding with distance inland (Figure 1).

Sediment Quality Triad

The LoE_{CHEM} with the chemical characterization of the pore water at depths of 1.5 cm and 7.5 cm (Table 3), show that overall, the msPAF values for these depths were higher at SE than TF (the actual pore water concentrations are presented in Table S1). The difference between both sites is significant for 1.5 cm depth (independent t-test, $p < 0.05$), but not for the

7.5 cm depth. Arsenic is the main contributor for each msPAF, followed by Cu, Ni, Zn and Cr (Table 2). The msPAF per study site averaged over both depths is 0.22 ± 0.03 for SE and 0.18 ± 0.04 for TF, of which SE is significantly higher compared to TF (independent t-test, $p < 0.05$). These averaged msPAF values were used as input values for the Triad.

The results of the bioassay (LoE_{BIO}) are given in Table 4 and Table S2. In the first part of the bioassay, 21 d population growth inhibition is shown for *C. volutator*. Subsequently for the 2nd generation, growth of the individuals and population growth inhibition are given. For CS1 and SE5 enough individuals were present after phase 1 for four replicas in phase 2, for the other sites two replicas were possible. CS1 shows no population growth inhibition and the highest individual growth in length, followed by SE and then TF. The differences for the population growth inhibition between the two phases are large. As input for the Triad the location averages of population growth inhibition of phase 2 were used reflecting a long exposure period. The average values in phase 2 are 0.00 ± 0.00 (SE), 0.58 ± 0.18 (TF) and significantly differ (Games Howell, $p < 0.05$).

The results of the ecological field survey (LoE_{ECO}), including numbers of *C. volutator* per m² and species richness, are presented in Table 5. The average number of *C. volutator* is lower for SE (215 ± 304), which significantly differs from the high numbers for CS2 (12535 ± 2496) (Games Howell, $p < 0.05$). For the species richness, TF and SE are more similar (3.3 ± 1.1 versus 3.6 ± 3.4) and CS2 has the greatest species richness (17 ± 1.0), with CS2 being significantly different from TF (Games Howell, $p < 0.05$). Both input values were first scaled vis-à-vis CS2, which gives 0.98 (SE) and 0.29 (TF) for the number of *C. volutator* and 0.75 (SE) and 0.82 (TF) for the species richness. Then, the two values for each site were integrated as one value for the Triad, the LoE_{ECO} .

Integrated results for the Triad Effect value are given in Table 6. Bootstrapping was done for LoE_{CHEM} and LoE_{ECO} , but could not be performed for LoE_{BIO} because SE input values

equalled zero and bootstrapping of the LoE_{BIO} of TF would create spurious comparisons between the two sites. The calculated Triad Effect value is significantly higher for SE (0.60 ± 0.17) compared to TF (0.50 ± 0.12) (Games Howell, $p < 0.001$).

Bioaccumulation

To understand whether *C. volutator* is affected by the fine fraction, BSAF values are calculated (Table 7). For Se, Sb, Sn and Tl BSAF was not calculated, because the majority of the values from both sediment and *C. volutator* were below detection limit. Also, for TF10 the *C. volutator* numbers were too low to obtain accurate results and for SE, only 5 locations are visible, as no *C. volutator* was found at the other locations. For many elements BSAF values are higher at TF compared to SE, indicating higher bioavailability for uptake of the trace elements at TF. However for As, which drives msPAF, BSAF is higher at SE. BSAF from the bioassays are for several elements (As, Ba, Ni, Zn) higher compared to the field sites, but certainly not for all chemicals.

DISCUSSION

This research investigated trace element contamination and ecotoxicological risks at two semi-artificial depositional sites along the Dutch coast, a recent artificial nourishment (Sand Engine (SE) and a semi-artificial tidal flat (TF)). Using the Sediment Quality Triad approach (Chapman 1990; Linkov et al. 2009) we considered three major lines of evidence (LoE): (1) chemical characterization of pore water, (2) toxicity characterization of the sediment using bioassays and (3) ecological field survey. The results showed a higher Triad Effect value at SE (0.60 ± 0.17) than at TF (0.50 ± 0.12), and can be quantified as a moderate risk and a low risk, respectively (Jensen and Mesman, 2006). Combining different LoEs in one number might

destroy distinct information (Suter and Cormier 2011). Therefore, each LoE will be discussed in detail below.

For LoE_{CHEM}, the msPAF with averages of 0.22 ± 0.03 for SE and 0.18 ± 0.04 exceed 0.05, the threshold. For both SE and TF, the main and often only contributor to the msPAF values is arsenic (As) in the pore water. If instead of using directly measured pore water as input for msPAF, msPAF was based on measured sediment contents. The msPAF based on measured sediment contents were standardized and translated to pore water concentrations assuming equilibrium partitioning using K_d and this would result in lower msPAF values of 0.04 ± 0.01 for SE and 0.05 ± 0.01 for TF. This points to the relevance that equilibrium partitioning is not a precautionous assumption in this case. A physical process that would enhance mobilization is resuspension, which results in the oxidation of the sediment and therefore remobilization of the trace elements (Cantwell et al. 2008). Besides aerobic conditions, reducing conditions were observed based on the SO_4 measurements compared to the composition of conservative mixing of fresh water and seawater and also the dissolved Fe and Mn concentrations. The presence of anoxic microniches with sulphidic conditions within suboxic surface sediment may explain the presence of both aerobic and anaerobic conditions (Van Cappellen and Wang 1996; Stockdale et al. 2010), which can be a consequence of bioturbation (Bertics and Ziebis 2010). As a result, the msPAF values show the mobilized trace elements at the time of sampling, which are influenced by bioturbation and probably resuspension. Redox conditions are an important factor to control the bio-availability of trace elements. A low redox potential leads to a low mobilization of cationic metals like Cd, Cu, Ni and Zn, but promotes the mobility of oxyanion As (Frohne et al. 2011), which is the case for both sites, especially at SE. Increased As can be attributed to the reduction of Fe oxyhydroxides and the reduction of arsenate to arsenite (Mitsunobu et al. 2006; Frohne et al. 2011).

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It can be questioned whether influences of the Rhine are apparent, when marine sediments containing sulphide minerals like pyrite are common (Johnston et al. 2010). To estimate whether Rhine water influences occur at SE and TF, REE were analysed (Supplementary Material and Table S3), because the Rhine has high concentrations of Lanthanum (La) and Gadolinium (Gd) (Moermond et al. 2001; Kulaksiz and Bau 2011). It is clear that the sediment along the Dutch coast and in the Wadden-Sea show influences from La and Gd (Moermond et al. 2001; Van Den Brink and Kater 2006). However, it is not known whether the influence of the Rhine has increased after the construction of the Sand Engine, because of similar concentrations 10 km out of shore (Table S3), of which the location is equal to the distance from where the sand from the Sand Engine was collected out of a sand pit (Pit, Griffioen, et al. 2017). Further research and correction to background values of La and Gd (Klaver et al. 2014) is needed to estimate the influence from the suspended solids and fresh water originated from the Rhine. Additionally, organic pollutants in sediments from two locations (one in front and one behind the low waterline) at both SE and TF were all found to be below their respective detection limits (Table S4). As a result, pollution originating from the river Rhine seems apparent at both sites, but the intensity at the time of sampling was most likely low.

The LoE_{BIO}, the bioassay results, yielded results contradicting those of the third LoE, the ecological field survey. Although the field-estimated number of *C. volutator* at TF is relatively high, the bioassay results indicate the environment for this species is contaminated, because survival is low. The opposite is the case for SE, where low numbers of *C. volutator* were found in the field, but the bioassay results were similar to those for the control site. Therefore, we compared differences of the lab and field environmental settings: the salinity of the water used in the lab (15 ‰ and 20 ‰) coincides with an EC of 21 – 27 mS/cm and similar EC values were found at TF (~22 mS/cm), but not at SE (~40.5 mS/cm). Salinities between

15-20 ‰ are preferred for optimum growth and moulting (Mclusky 1967). This factor might explain why SE sediment in the lab was more favourable than sediment in the field, but does not explain why TF sediment resulted in such low survival rates during the bioassay. Temperature can be a factor as well (Kater et al. 2001), but during sampling in September the field conditions and the lab environment were similar and between the 15-20 °C, a range that does not affect the growth rate of *C. volutator* (Kater et al. 2008). The discrepancy between the bioassay and ecological field survey is likely due to differences in bioavailability between the laboratory and the field, as also shown by the different BSAF for elements driving msPAF (Table 7). A possible explanation for the disparity between the field and laboratory is the sulphide oxidation environment in the laboratory, where absence of binding phases may encourage mobilization of trace elements (Cantwell et al. 2008). The oxidative environment in the lab and the preparations prior to the bioassay, like sieving, may have mobilized specific trace elements in the sediment for TF causing a low survival rate of *C. volutator*.

The reason that field abundance of *C. volutator* was greater at TF than at SE, which is included in LoE_{ECO}, might be the different history of the sites. Although both field sites are semi-artificial depositional areas, TF has developed more gradually during several decades and the intertidal flat comprises a larger area. Given this, plus the overall eroding conditions at SE (De Schipper et al. 2016; Luijendijk et al. 2017), it can be assumed that at TF the environment is more stable, favouring higher production by the early summer broods (Gratto and Thomas 1983). The eroding conditions at SE are desired: the mega nourishment has to be implemented into the beach and dunes within 20-30 years (Stive et al. 2013). Consequently, the dynamic environment at SE creates spots where refreshing of seawater is absent and stagnant water may occur. Although *C. volutator* might not respond to stagnant water (Kater et al. 2001), it is likely that this species reacts to an hypoxic environment, where the survival rate of *C. volutator* drastically decreases with time, especially when sulphidic conditions occur (Meadows et al.

1981; Gamenick et al. 1996; Marsden and Rainbow 2004). Nonetheless, *C. volutator* is an opportunistic species and recovery can be as fast as two weeks, although responses are successively greater at larger spatial scales (Norkko et al. 2006). However, with the intertidal flat at the Sand Engine having many benthic micro algae (Pit and van Egmond, personal observation), hypoxic conditions are more likely to occur and the recovery of *C. volutator* might be slow (Gamenick et al. 1996). Therefore, the stability of the field situation and the large spatial scale at TF may account for the differences in numbers of *C. volutator* between SE and TF. However, compared to the control site (CS2), TF shows lower numbers of *C. volutator*, which, in light of the low survival rates from the bioassay results may be because of another potential contamination (Suter and Cormier 2011).

Besides the number of *C. volutator*, species richness is also included in the third LoE. For both SE and TF, the species richness was much lower than for CS2, resulting in a high (SE) or moderately high (TF) value for LoE_{ECO}. Given the very dynamic physical conditions and possibly temporary hypoxic conditions at the SE site, there might not have been enough time for a high species richness to develop. When the Sand Engine was created in 2011, time was needed to develop mud-flat like conditions and create a habitat for species like *C. volutator*. This situation occurred many years ago for TF and the low numbers for species richness might be caused by another factor as seen from the bioassay results. Less opportunistic species may not recover as quickly as *C. volutator*, potentially resulting in a lower macrobenthos diversity (Norkko et al. 2006).

In view of the foregoing, it seems that the sediment quality can be evaluated with the Triad Effect value, but it gives more precise and realistic conclusions when assessing each LoE individually. Additional measurements can be included more easily and a more flexible evaluation is possible. The Triad Effect value reveals that at the SE field site the ecotoxicological risk of the sediment to *C. volutator* is higher than at TF. In more detail, it

seems that for both field sites another variable is involved, the dynamic environment at SE and a potentially different contamination at TF, which were not included in the present Triad.

With the Sand Engine having recreation and nature development as additional goals as to coastal protection, this research reveals that nature development at the intertidal flat of this mega beach nourishment is restricted because of its dynamic environment. Stagnant water can occur temporarily, where reducing conditions will create an hypoxic environment and macrobenthos species like *C. volutator* may avoid sediments, especially when a sulphidic redox state is present (Meadows et al. 1981). The dominant reducing conditions are causing cationic metals to become less mobilized in the pore water, but the opposite is seen for the oxyanion As. Even though this trace element may create a decreasing water quality, external and internal factors affect bioaccumulation, for example adaptation, uptake via pore water or sediment, short and long-term exposure and the behaviour of *C. volutator* (Bat et al. 1998; Marsden and Rainbow 2004). To obtain three different goals as a new coastal management technique, the Sand Engine concept does not provide suitable conditions thus far for macrobenthos species like *C. volutator* in order to establish a stable population. However, when this mega nourishment is compared to a traditional nourishment that needs to be replenished periodically, the Sand Engine does create more opportunities, for example mud-flat conditions, which would otherwise not be a question as traditional beach nourishments show generally a coarse grain size distribution (Pit, Dekker, et al. 2017) and not contain intertidal flats as sedimentary subenvironments. One may, however, wonder whether a beach that must be replenished for coastal protection reasons should be partially transformed into an intertidal flat by means of these sand replenishments: is there a need or a benefit for landscape diversification at the small scale in addition to a desire?

CONCLUSIONS

The Sediment Quality Triad method, considering three major lines of evidence (LoE), was used to investigate trace element contamination and possible ecotoxicological effects for the macrobenthos species *C. volutator* at two depositional sites along the Dutch coast: a recent innovative Dutch coastal management project, the Sand Engine, and a semi-artificial tidal flat at Oostvoorne. The sediment quality was evaluated with the Triad Effect value, but shows to be more precise and realistic when assessing each LoE individually. Additional measurements can be included easily and a more flexible evaluation is possible. The Triad Effect value reveals that the ecotoxicological risk of the sediment to *C. volutator* is higher at the SE field site than at TF. Evaluation of each LoE from the Triad method together with additional measurements established that at both sites arsenic are bioavailable. For the semi-artificial tidal flat (TF), the Sediment Quality Triad method shows that the bioavailability of trace elements seems to differ between the field site and the laboratory. Contamination from the trace elements arsenic and copper is present, but the extremely low survival rate of *C. volutator* from the bioassay suggests that additional potential contaminations are present at this tidal flat, which may be the reason why the sediment biota sediment accumulation factor (BSAF) was higher here than at the Sand Engine. The highly diverse and dynamic environment of the Sand Engine (SE) creates a less favourable habitat for *C. volutator*, where stagnant water can temporary create hypoxic conditions and sulphate becomes reduced. Due to the fact that SE has been designed to erode because of its shape and location, it is unlikely that the intertidal flat at SE will become stable in time. The dynamic system brings along the mobilisation of especially arsenic, which can trigger adverse ecotoxicological effects at low original sediment contents. The SE does not provide suitable conditions for macrobenthos species like *C. volutator* to create a stable population. Besides coastal protection, the Sand Engine concept also has recreation and nature

development as additional goals. We conclude that the dynamics at the intertidal flat of SE creates ecotoxicological risks and as a result, limits the nature development goal of the Sand Engine concept. However, when a beach must be replenished for coastal protection reasons it is questionable whether there is a need or a benefit for landscape diversification at the small scale.

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Data availability - Data are available on request from the corresponding author (i.r.pit@uu.nl).

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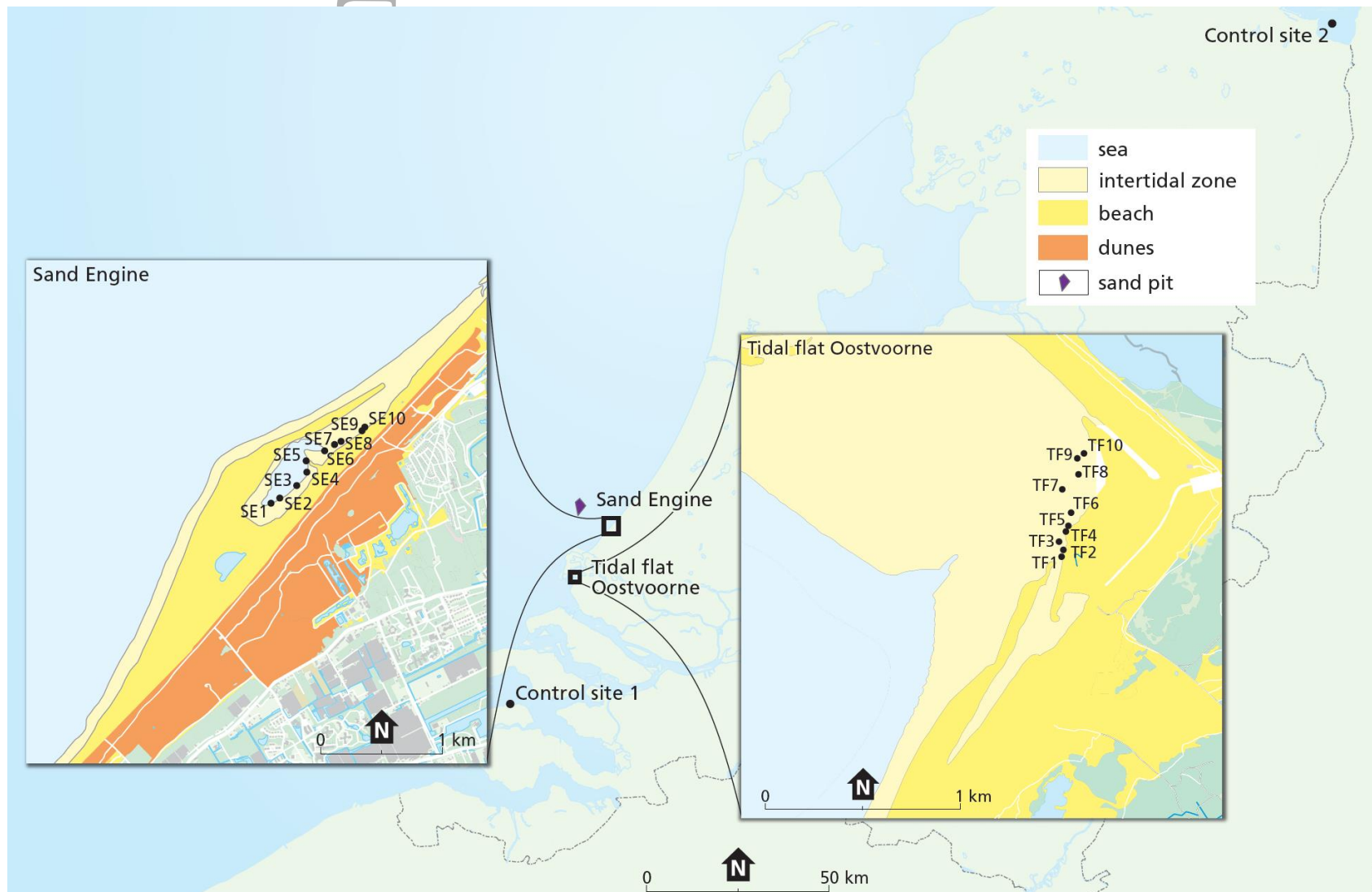


Figure 1. The locations of the field sites including sample locations. The dynamic artificial sand replenishment (Sand Engine), and the semi-natural tidal flat at Oostvoorne, control site 1 and control site 2.

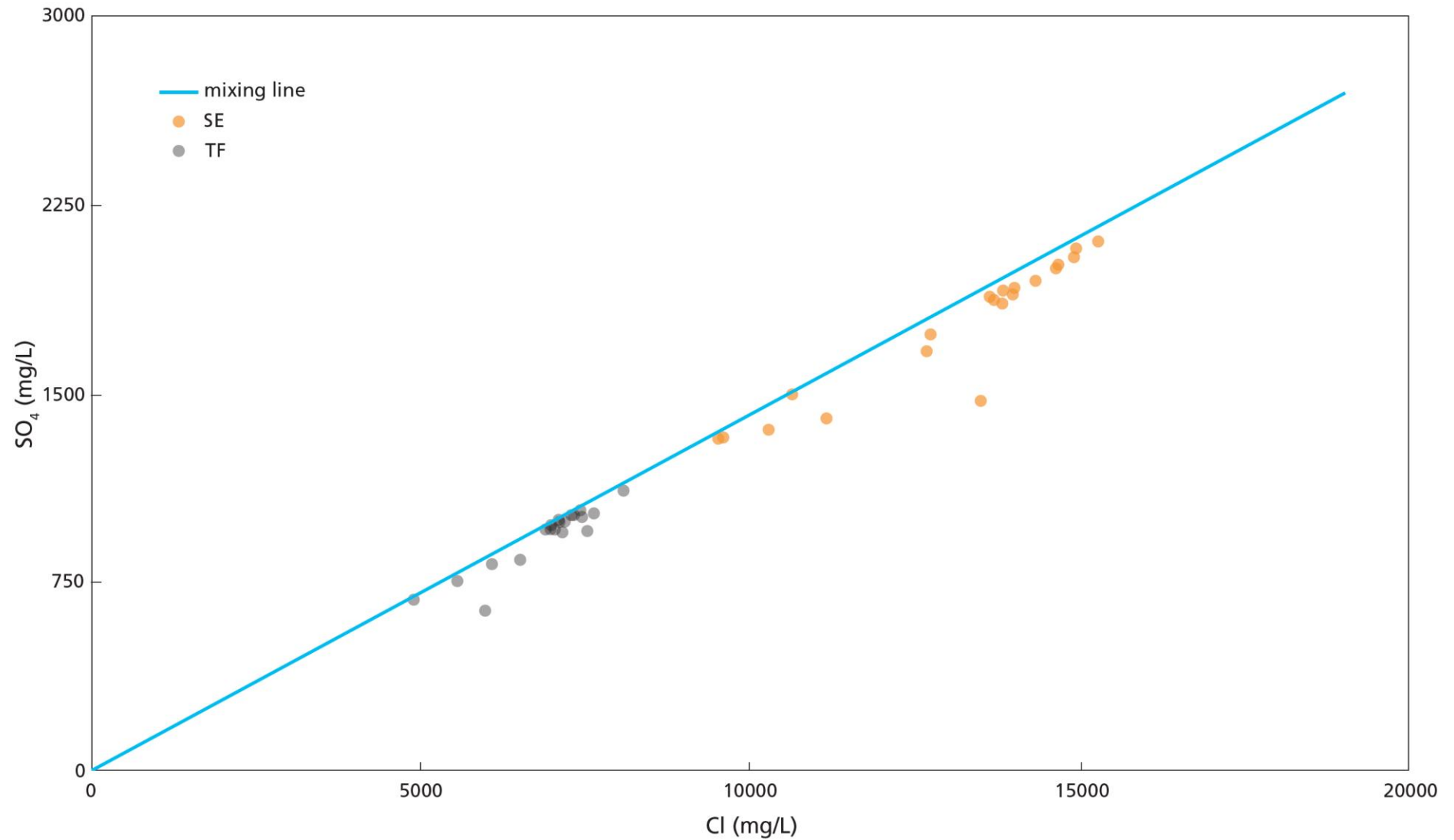


Figure 2: Concentrations of SO₄ versus Cl for the pore water samples, with the mixing line of rainwater and seawater.

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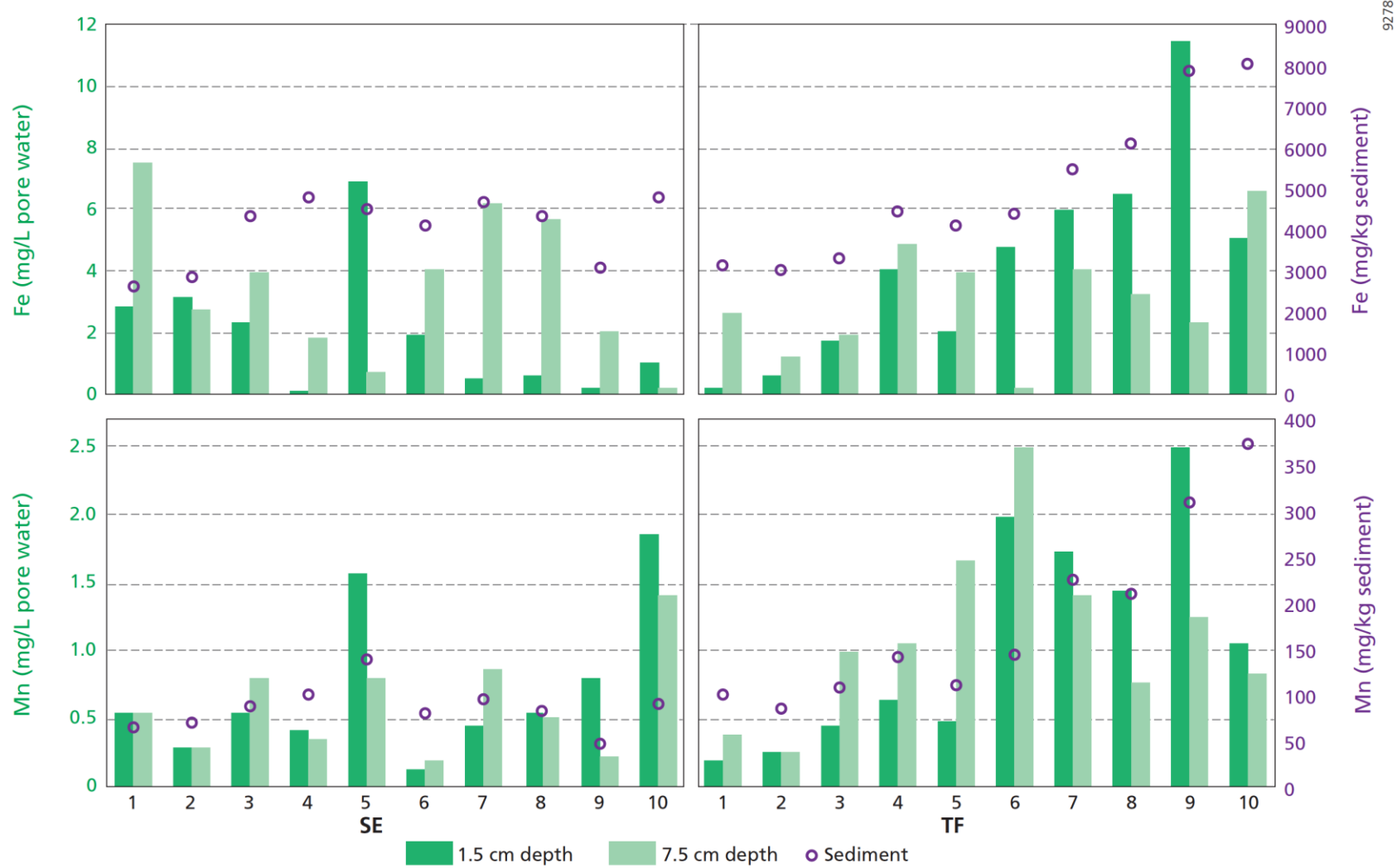


Figure 3. Concentrations of Fe (top graphs) and Mn (bottom graphs) with bars for the pore water concentrations and coloured dark for the 1.5 cm depth concentration and light grey for 7.5 cm depth concentration. The numbers represent the different sample locations. Sediment concentrations are shown by open circles.

Table 1. Chemical characteristics of the sediment at SE and TF for total sediment and the < 63 µm fraction. The concentrations of the trace elements are in mg/kg and D50 represents the median diameter of particle size distribution.

	Intervention value ¹	Total sediment				Sig. ²	< 63 µm				Sig. ²
		SE		TF			SE		TF		
		mean (st. dev)	max	mean (st. dev)	max		mean (st. dev)	max	mean (st. dev)	max	
organic C (%)		0.9 (0.3)	1.6	1.8 (0.56)	2.8		7.7 (1.1)	9.21	5.4 (1)	6.5	**
CaCO ₃ (%)		4.1 (1.5)	7.3	5.6 (1.7)	9.5	*	12 (3.1)	20.76	7.6 (1.6)	9.1	*
S (%)		0.07 (0.03)	0.1	0.06 (0.02)	0.1	*	0.9 (0.06)	1.0	0.3 (0.1)	0.5	**
Fe (%)		0.4 (0.08)	0.5	0.5 (0.2)	0.8	*	1.7 (0.2)	1.9	1.5 (0.1)	1.7	**
fraction < 63 µm (%)		1.4 (1.1)	2.8	4.4 (3.1)	9.9						
D50		349 (31)	389	210 (40)	259	**					
As (mg/kg)	44	2.8 (0.8)	4.2	5.0 (1.1)	7.6		11 (2.1)	14	15 (4.0)	21	**
Ba	237	22 (2.2)	25	24 (3.1)	31	**	35 (3.5)	38	49 (7.4)	61	**
Be	10	0.17 (0.04)	0.25	0.2 (0.07)	0.3	*	0.6 (0.08)	0.7	0.6 (0.05)	0.7	
Cd	8	0.02 (8.9)	0.04	0.08 (0.04)	0.1		0.2 (0.07)	0.3	0.3 (0.03)	0.3	**
Co	54	-	-	-	-		7.3 (1.4)	9.8	5.0 (0.5)	5.7	**
Cr	42	9.1 (1.8)	11.8	10.6 (3.7)	17	**	34 (2.3)	37.8	29 (1.7)	32	
Cu	92	2.6 (1.0)	4.2	3.3 (1.0)	5.4	*	11 (1.7)	14	10 (2.9)	18	
Mo	190	0.12 (0.08)	0.29	0.12 (0.02)	0.15	*	1.1 (0.36)	1.7	0.4 (0.2)	0.7	**
Ni	34	5.6 (0.8)	7.1	4.7 (1.2)	6.5	**	20 (2.7)	23	13 (2.0)	16	**
Pb	337	3.1 (0.9)	4.9	7.5 (2.0)	11		20 (2.4)	23	23 (4.3)	29	**
Tl	15	0.04 (0.07)	0.21	0.2 (0.05)	0.2	*	< d.l.		< d.l.		
Sb	22	0.03 (0.04)	0.14	0.08 (0.03)	0.1		< d.l.		< d.l.		
Sn	246	0.1 (0.2)	0.44	0.4 (0.1)	0.6		< d.l.		< d.l.		
V	86	6.9 (2.1)	10	11 (4.0)	17	*	37 (5.4)	43	31 (2.3)	35	**
Zn	303	10 (2.9)	17	27 (6.7)	40		65 (11)	84	81 (11)	98	**

¹ The Intervention Value is corrected from a standard soil (10% organic material and 25% lutum) to a soil most comparable to sand (2% organic material and 2% clay)

² Significance estimated for differences between SE and TF with a Mann-Whitney U: * indicates p < 0.05, ** indicates p < 0.001

Table 2. Means and standard deviation (st.dev) of different variables measured in the field (O₂, EC, pH and redox) and in the lab (TOC and alkalinity). Significance (Sig.) was tested using Mann-Whitney U, $p < 0.05$.

	TOC (mg/L)	O ₂ (mg/L)		EC (ms/cm)		pH		redox (mV)		alkalinity (mg/L)	
	0-10 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm
	mean (st. dev.)										
SE	4.4 (1.2)	1.3 (0.5)	1.6 (0.4)	42 (5.8)	39 (5.1)	7.7 (0.6)	7.9 (0.3)	-153 (88)	-128 (22)	251 (121)	233 (62)
TF	5.9 (2.0)	1.7 (0.4)	1.9 (0.6)	22 (3.3)	22 (3.0)	7.6 (0.1)	7.6 (0.3)	-133 (24)	-122 (41)	173 (15)	197 (44)
Sig.	*			*	*	*	*				

Table 3. Chemical characterization of the pore water at 1.5 cm and 7.5 cm depth, and potentially affected fraction of species per trace element (PAF) and for multiple substances (msPAF) values.

	PAF										msPAF		msPAF		
	As		Cu		Ni		Zn		Cr		Be	V	1.5 cm	7.5 cm	Average
	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm	1.5 cm	7.5 cm					
SE1	0.25	0.26											0.25	0.26	0.26
SE2	0.23	0.22											0.23	0.22	0.23
SE3	0.19	0.20		0.04									0.19	0.23	0.21
SE4	0.16	0.19											0.16	0.19	0.18
SE5	0.20	0.11	0.04	0.03		0.05							0.23	0.20	0.22
SE6	0.16	0.18					0.07						0.22	0.18	0.20
SE7	0.17	0.20	0.05	0.05									0.21	0.24	0.23
SE8	0.19	0.21			0.12								0.29	0.21	0.25
SE9	0.21	0.16											0.21	0.16	0.19
SE10	0.29	0.19	0.05						0.03				0.35	0.19	0.27
TF1	0.12	0.21	0.08										0.19	0.21	0.20
TF2	0.11	0.13											0.11	0.13	0.12
TF3	0.14	0.17											0.14	0.17	0.16
TF4	0.18	0.18	0.06	0.07									0.23	0.23	0.23
TF5	0.17	0.18		0.07									0.17	0.23	0.20
TF6	0.16	0.10											0.16	0.10	0.13
TF7	0.18	0.16											0.18	0.16	0.17
TF8	0.17	0.13											0.17	0.13	0.15
TF9	0.23	0.15	0.07	0.04									0.29	0.19	0.24
TF10	0.20	0.22											0.20	0.22	0.21

Table 4. Results of the bioassay with the population growth inhibition for phase 1 and 2 and individual growth in length for *C. volutator* for phase 2.

		Population growth inhibition % (st. dev)	Growth in length <i>C. volutator</i> including antennae in mm (st. dev)
phase 1 (21 days)	SE5	19.3	
	SE10	58.7	
	TF1	70.7	
	TF10	67.3	
	CS1	9.3	
phase 2 (49 days)	SE5 (n=4)	0 (0)	5.4 (1.4)
	SE10 (n=2)	0 (0)	9.6 (1.9)
	TF1 (n=2)	70 (5)	2.8 (1.2)
	TF10 (n=2)	45 (0)	2.8 (0.4)
	CS1 (n=4)	0 (0)	13.1 (2.8)

Table 5. Results of the ecological field study, with means and standard deviations (st.dev). Sites that show a significant difference (Sig.) are listed with a different letter (Games Howell, $p < 0.05$).

	# <i>C. volutator</i> per m ²			Literature	Species richness per m ²			Literature
	<i>n</i>	mean (st.dev)	Sig.		<i>n</i>	mean (st.dev)	Sig.	
SE	2	215 (304)	b		4	4.1 (3.9)	ab	van Egmond et al. (2018)
TF	2	8960 (2517)	ab		2	3.0 (1.0)	a	van der Zee et al. (2018)
CS2	3	12535 (2496)	a	Dekker & Waasdorp (2006)	0			
					3	17 (1.0)	b	Dekker & Waasdorp (2006)

Table 6. Results of the three Lines of Evidence (LoE) for SE and TF.

Scaled/combined LoE	SE	TF
LoE _{CHEM}	0.22 (0.03)	0.19 (0.04)
LoE _{BIO}	0.00 (0.00)	0.58 (0.15)
LoE _{ECO}	0.87 (0.11)	0.56 (0.27)
Triad Effect value	0.60 (0.17)	0.50 (0.12)

Table 7. The BSAF values for trace elements, with the means and standard deviations (st.dev) for SE, TF and bioassay. Significance was tested using Games Howell post hoc, $p < 0.05$, except when $n = 1$ and no standard deviation is visible.

<i>C. volutator</i> & sediment	As	Ba	Be	Cd	Cr	Co	Cu	Mo	Ni	Pb	V	Zn
	average BSAF (st.dev)											
SE	0.3 (0.1) a	0.6 (0.3) a	0.1 (0.1) ab	0.3 (0.1) a	0.1 (0.1) a	0.1 (0.02) a	3 (0.1) a	0.3 (0.05) a	0.1 (0.04) a	0.1 (0.04) a	0.1 (0.04) a	1.0 (0.1) a
TF	0.2 (0.03) a	1.1 (0.2) b	0.2 (0.1) b	1.4 (0.5) b	0.2 (0.04) b	0.2 (0.05) b	5.7 (1.3) b	1.5 (0.6) b	0.2 (0.1) b	0.1 (0.1) a	0.2 (0.05) b	0.9 (0.1) a
Bioassay	1.1 (0.2) b	7.7 (3.4) ab	0.05 (0.04) a	0.7 (0.6) a	0.02 (-)	0.1 (0.1) ab	1.1 (-)	0.3 (-)	0.4 (0.4) ab	0.1 (0.1) a	0.1 (0.03) a	1.5 (0.4) a