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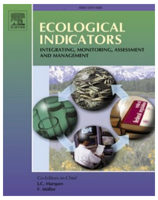

Added value of the NemaSPEAR[%]-index to routinely used macrofauna-based indices for assessing the quality of freshwater sediments

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ABSTRACT

Assessments of the ecological status of freshwater ecosystems, such as mandated by the EU Water Framework Directive, are routinely conducted by monitoring macroinvertebrates. However, for the quality assessment of fine sediments, macroinvertebrates are of limited suitability. In such habitats they show a low species diversity and often low densities, whereas a more diverse meiofauna can be found. Among the meiofaunal groups in benthic habitats, nematodes are one of the most abundant and species-rich. Fine, cohesive sediments considerably contribute to many ecosystem services, but they are often hotspots of chemical contamination as well. In the present study, the added value of the recently developed and validated NemaSPEAR[%]-index was evaluated by directly comparing it to routinely used macrofauna-based indices. Macrofaunal and nematode communities were synchronously monitored at seven sites in six different streams. The results of a chemical analysis of sediment pollutants combined with sediment quality guidelines revealed widely diverging toxic potentials at the seven investigated locations. The seasonal robustness of the NemaSPEAR[%]-index compared with macrofauna-based indices was also determined, by additionally obtaining synchronous samples of macrofauna and nematodes over the course of one year at one of the seven sites, a reference stream with very low toxic potential.

The NemaSPEAR[%] performed robustly despite seasonal variations in the nematode community in the sediment of the unpolluted stream. At the seven sampling sites, representing a pollution gradient, the NemaSPEAR[%]-index correlated well with the toxic potential of the sediments. By contrast, the macrofauna-based indices did not correlate significantly with either the toxic potential of the sediments or with the results of NemaSPEAR[%] at the seven sites. For many non– endobenthic macroinvertebrates, chemical exposure is mostly through the water phase, such that the toxic potential of the sediments will not necessarily be reflected directly by macrofaunal indices. Accordingly, identifying the stressors that contribute to degrading the ecological status of a water body requires the inclusion of methods that examine different types of stressors, targets, and exposure pathways. Our study shows that the NemaSPEAR[%]-index provides added value to routinely used macrofaunal-based indices.

1. Introduction

The European Water Framework Directive (WFD) mandates the good ecological and chemical statuses of all surface waters in Europe (European Community, 2000). Nevertheless, to date, this goal has been achieved only by ~ 40% of European surface water bodies, with rivers generally having a lower status than lakes and coastal waters (European Environment Agency, 2018). Within the WFD, the benthic invertebrate fauna serves as one biological quality element in determinations of the ecological status of rivers. However, the methods routinely used to assess this biological element are based solely on the monitoring of macroinvertebrates (Hering et al., 2004), whereas smaller invertebrates, especially meiofauna, have thus far been neglected. The macrofaunal based indices currently in use mostly detect organic stressors (Hering et al., 2004; Sandin and Hering, 2004). For example, in Germany, the saprobic index, used to identify organic pollution by easily

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biodegradable substances in inland waters, monitors invertebrate species sensitive to a decrease in dissolved oxygen levels (Rolauffs et al., 2004; Sandin and Hering, 2004; von der Ohe et al., 2007). The SPEAR (SPEcies At Risk)-index relies on the specific traits of macroinvertebrate species to identify the stress on freshwater systems posed by anthropogenic organic pollution (von der Ohe et al., 2007). Here a further specification is given with the SPEAR$_{\text{pesticides}}$ which sets the focus on the effect of pesticide toxicants on macrofaunal invertebrate communities (Liess and von der Ohe, 2005; Knillmann et al., 2018). However, to meet the demands of the WFD, the spectrum of the evaluated stressors has been recently extended to include stressors other than organic pollution. The *Perlodes* system, applicable to 31 stream types in Germany, has a modular design that can be used to assess the impact of different stressors on the ecological quality of flowing waters. Via the module saprobic index, it detects organic pollution. The module acidification depends on a metric that shows the relation of acid sensitive to unsensitive species. The module general degradation combines many metrics that set the focus on habitat quality like e.g. the habitat structure, the morphological degradation, or the diversity of undisturbed habitats. The results of the different modules can then be integrated to obtain a final assessment, based on a comparison with a typical reference stream (Meier et al., 2006).

Fine sediments have a high potential to accumulate chemical toxicants and are therefore often hot spots of chemical contamination (den Besten et al., 2003). Moreover, they function not only as sinks but also as sources of contamination, because of the potential remobilization of sediment-bound pollutants, especially after flood events (Hollert et al., 2000). Thus, fine sediments should be considered in assessment as they contribute to the overall quality of surface waters. However, macrofauna-based indices are of limited value in habitats primarily composed of fine sediments, as at these sites the diversity of macroinvertebrates is low (Wolfram et al., 2010). In contrast to macrofaunal organisms, meiofauna (motile invertebrates that pass through a 500-µm mesh, but are retained by a 44-µm mesh; Giere, 2009) reach high numbers in fine sediments and are present throughout the year (Traunspurger, 2000; Beier and Traunspurger, 2001; Traunspurger et al., 2012). Patrício et al. (2012) surveyed the sediment-dwelling fauna of the Mondego River and found that the abundance of meiofauna was almost two orders of magnitude higher than that of macrofauna, with 46.5% of the meiofaunal organisms consisting of nematodes. Indeed, nematodes are often one of the most common meiofaunal groups in river sediments (e.g., Beier and Traunspurger, 2003; Traunspurger et al., 2015; Majdi et al., 2017; Brüchner-Hüttemann and Traunspurger, 2020) and their utility as indicators for river pollution has been demonstrated (e.g., Wilson and Khakouli-Duarte, 2009).

Analogous to the SPEAR[%]-index used to assess macrofaunal communities, the NemaSPEAR[%]-index distinguishes between nematode species at risk (NemaSPEAR) and not at risk (NemaSPEnotAR, Höss et al., 2011) to specifically detect pollution-induced disturbances in benthic habitats. However, unlike the SPEAR[%]-index, that uses trait information of the various macroinvertebrate species, the classification of the nematode species are based on a co-occurrence approach using empirical data (Höss et al., 2011, 2017).

The NemaSPEAR[%]-index is relatively robust in identifying polluted sediments (Wolfram et al., 2012; Höss et al., 2017; Sonne et al., 2018). Moreover, defined thresholds are useful to classify sediments in terms of their ecological status (Höss et al., 2017). Application of the NemaSPEAR[%]-index to genus-level data (Höss et al., 2017) and the inclusion of a DNA-based taxonomy (Schenk et al., 2020) will extend the index's application to a broader range of user groups, including those with less taxonomic expertise. However, whether the NemaSPEAR [%]-index can provide added value in assessments of the ecological status of river and stream sediments requires a direct comparison of its results with those of routinely used macrofauna-based indices, including the SPEAR[%]-index. Therefore, in this study we synchronously monitored the macrofaunal and nematode communities at seven locations at six different streams characterized by a gradient of chemical contamination. To obtain a better comparability of chemical and biological data, the contaminant concentrations measured in the sediments were transformed into toxic potentials by dividing them by effect-based sediment quality guidelines (de Deckere et al., 2011). Additionally, macrofauna and nematodes were synchronously monitored over the course of one year at a single site within a reference stream where chemical contamination is very low. The two sets of measurements allowed a determination of the robustness of the NemaSPEAR[%]-index along a chemical pollution gradient and against seasonal variations as well as with respect to the results of macrofauna-based indices. Specifically, we sought to answer the following questions:

(i) Do the NemaSPEAR[%]-index and macrofaunal indices reflect the toxic potential of fine sediments?
(ii) Is the NemaSPEAR[%]-index affected by seasonal variations in the nematode community?

2. Material and methods

2.1. Sampling sites

Sampling for this study took place at seven sites of six different streams in Germany (Fig. 1, Table 1). The sampling sites were chosen based on chemical and nematode data, showing a large gradient in terms of chemical (lowly to highly polluted) and ecological status (NemaSPEAR[%]-index, Höss et al., 2017). Samples were taken at the Furlbach (FB), the Saale at Rischmühlenschleuse (RM), the Luppe (LU), the Veerse (VE), the Örtze (ÖR) and at two sites of the Elbe, Hitzacker (HI) and Cumlosen (CU). All sites were permanent flowing waters. FB represents a reference stream as defined by the German Federal Environment Agency for type 14 (sand-bottomed lowland stream, Pottgießer and Sommerhäuser, 1999) and was selected for monthly sampling from June 2016 to May 2017. Sampling at the other six locations took place in April and May 2018. The data obtained from FB in May 2017 were included for the analysis of the indices along a pollution gradient.

2.2. Physico-chemical analysis

The physico-chemical data of the water at all sampling sites, including temperature (°C), dissolved oxygen (mg l^{-1}), conductivity (µS cm^{-1}) and pH (Table 1), were collected in situ using a multi-probe (Multi 3430, WTW, Weilheim, Germany). Chemical contamination of the sediment at the sampling sites was assessed in 5–10 subsamples of sediment collected with a stainless-steel grab sampler and then pooled in a stainless-steel tub to avoid the effects of local variability. After the removal of large debris, the sediment material was homogenized, and 1 kg was transferred to glass containers.

Chemical analyses were conducted for 33 compounds: arsenic (As), 7 metals (Cd, Cu, Pb, Cr, Hg, Ni, Zn), 16 polycyclic aromatic hydrocarbons (PAHs, according to the US EPA), 7 polychlorinated biphenols (PCBs 28, 52, 101, 118, 138, 153, and 180), *p,p*'-DDD and *p,p*'-DDE. Sediment quality guidelines were applied to the chemicals measured in the sediment samples. For each chemical, the measured sediment concentration was divided by the consensus-based probable effect concentration (PEC) according to de Deckere et al. (2011), resulting in a PEC-quotient (PEC-Q) for each chemical. As a measure of the toxic potential, the mean of all PEC-Q values was calculated for each sample (mean PEC-Q). According to MacDonald et al. (2000), a mean PEC-Q < 0.5 indicates a very low probability of toxicity and a PEC-Q > 0.5 a proportionally higher probability of toxicity and thus a proportionally higher toxic potential. Thus, the toxic potential of the sampling sites could be ranked based on the calculated mean PEC-Q values.

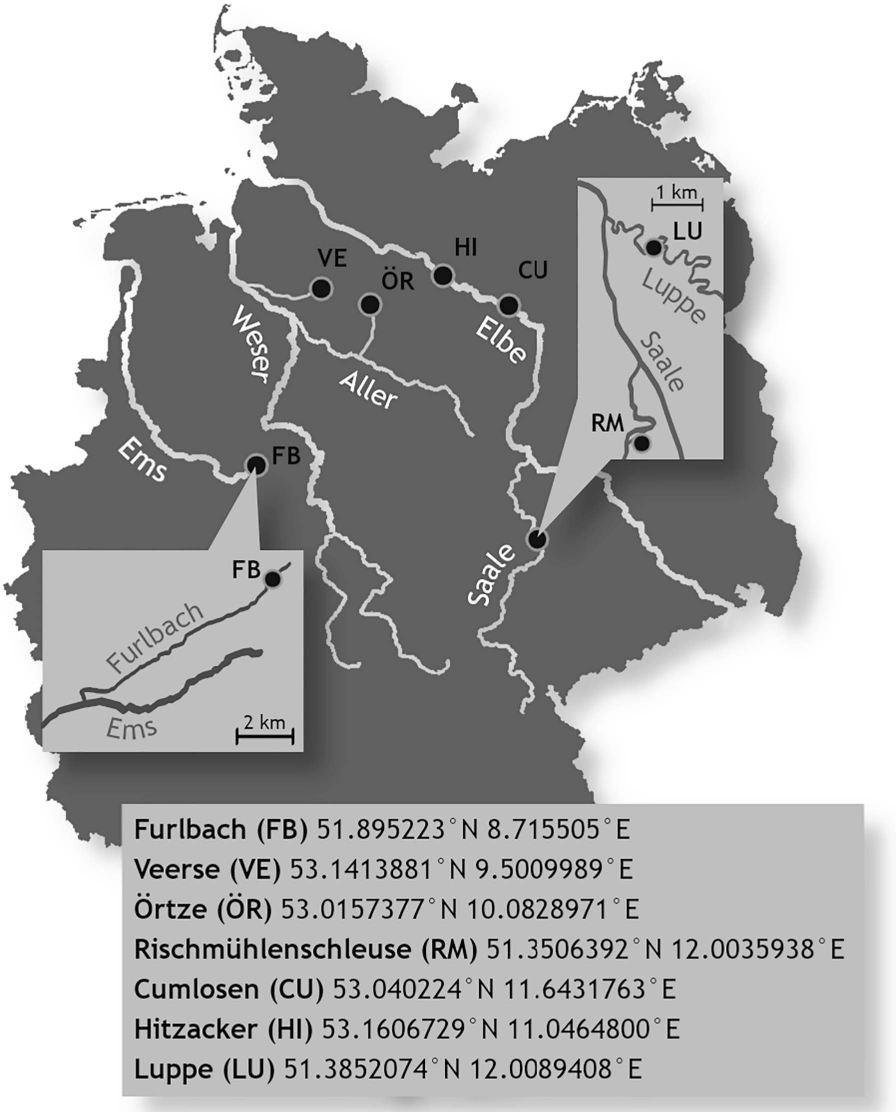


Fig. 1. Map showing the positions of the seven sampling sites and the respective coordinates.

Table 1
Stream type, and physico-chemical values of the seven sampling sites. For FB mean (±sd) is given for 12 months (June 2016 – May 2017). FB = Furlbach, VE = Veerse, ÖR = Örtze, RM = Rischmühlenschleuse, CU = Cumlosen, HI = Hitzacker, LU = Luppe. Stream types: 9.2 = Large rivers in low mountain range, 14 = Sand-bottomed lowland stream, 16 = Gravel-bottomed lowland stream, 19 = Small streams in floodplains, 20 = Sandy-bottomed rivers.

Site	Stream type	Temperature (°C)	pH	O_2 (mg l^{-1})	Conductivity (µS cm^{-1})
FB	14	8.7 (±1.7)	7.4 (±0.1)	8.1 (±0.5)	386 (±6.9)
VE	14	18.2	7.6	8.3	436
ÖR	16	16.6	6.8	8.3	135
RM	9.2	16.0	8.2	8.8	1631
CU	20	16.3	8.2	9.9	853
HI	20	15.5	8.2	10.3	860
LU	19	14.6	7.7	5.4	1776

2.3. *Sampling of macrofauna and nematodes*

The macrofaunal community at each of the seven stream sites was sampled by multi-habitat sampling, as described in Meier et al. (2006). At each site, 1.25 m^2 substrate was sampled in total, divided in 20 subsamples (~0.06 m^2 each). All substrate types were sampled whereby the number of subsamples per substrate type was determined proportionate to their occurrence at the site. Sampling was performed with a landing net (500 µm mesh size, 25 × 25 cm edge length) by kick-sampling. Alternatively, macrobenthos was collected from removed stones, wood or macrophytes, replacing single subsamplings with the net. As FB, VE and ÖR are wadable waters, subsamples were distributed over the whole stream section. RM, LU, CU and HI are not wadable, so samples were taken from the riverbank. All subsamples were sieved in the field through a filter tower (1 cm, 2 mm, 0.5 mm) and retained macrofaunal organisms were pre-sorted and counted. A relevant number of individuals per taxon was fixed with 75% ethanol. For taxa that have been determined in the field to the required level of the "operational taxa list" (to be found at www.gewaesser-bewertung.de) three individuals were fixed, for taxa that have not been clearly identified in the field a specific number of individuals per taxon was fixed, given in Appendix III in Meier et al. (2006). For the analysis of the nematode communities at the seven sites four (FB, VE) or five (ÖR, RM, HI, CU, LU) replicate sediment samples were taken using a corer (FB: diameter 3.3 cm, depth 2 cm; all other sites: diameter 2.5 cm, depth 5 cm). For each replicate, three randomly located, adjacent cores were pooled in 250-ml

PET bottles (final volume of sediment ~ 74 ml, for FB ~ 51 ml) to take account of any small-scale heterogeneity. Average distance between pooled cores was ~ 20 cm, average distance between the replicates was ~ 5 m. Immediately after sampling, all replicates were fixed with formalin (final concentration 4%). Macrofaunal and nematode samples were brought to Bielefeld University and stored at room temperature until further analysis.

In the laboratory, all macrofaunal invertebrates from each of the sampling sites were observed at 40-fold magnification using an Olympus SZ40 stereomicroscope (Shinjuku, Tokio, Japan) and identified to the lowest taxonomic level using the standard literature descriptions recommended in Meier et al. (2006). The number of organisms was expressed as individuals (ind.) m^{-2}.

Nematodes from the sediment samples were extracted by density centrifugation using LudoxTM50 (Sigma–Aldrich, Munich, Germany; 1.14 g cm^{-3}, mesh size 10 μm) as described in detail in Heininger et al. (2007). From the extracted material, the nematodes were observed at 40-fold magnification using an Olympus SZ40 stereomicroscope (Shinjuku, Tokio, Japan) and then individually isolated. From the FB samples, either the first 50 nematodes per replicate or, if < 50 nematodes per replicate were available, all nematodes were isolated. For all other sampling sites, 100 nematodes per replicate were isolated until a total of ~ 500 nematodes per sampling site was achieved. The nematodes were prepared as described by Seinhorst (1959) and identified to the species level using a Leica Dialux microscope (1250-fold magnification).

2.4. Indices

A detailed list of the nematode species of all seven sampling sites that form the basis for the calculation of the NemaSPEAR[%]-index is provided in the supplementary information (Table S2). Nematodes were classified as species at risk (NemaSPEAR) and species not at risk ($\text{NemaSPE}_{\text{not}}\text{AR}$) according to Höss et al. (2017). Species present in the samples but not included in the reference literature were classified as $\text{NemaSPE}_{\text{not}}\text{AR}$. The NemaSPEAR[%]-index was then calculated for all replicates as

$$NemaSPEAR[\%] = \frac{\sum \log[NemaSPEAR]_{relAb}}{\sum \log[AllSpecies]_{relAb}} \times 100$$

where $\log[\text{NemaSPEAR}]_{\text{relAb}}$ and $\log[\text{AllSpecies}]_{\text{relAb}}$ are the log(x + 1)-transformed relative abundances of NemaSPEAR and all species, respectively. Class boundaries used in the assessment of ecological status were also taken from Höss et al. (2017).

A detailed list of the macrofaunal species of all seven sampling sites that form the basis for the calculation of the macrofaunal-based indices is provided in the supplementary information (Table S3).

The ecological status of all sampling sites was assessed via *Perlodes*, using the ASTERICS software (version 4.04).

The SPEAR[%]-index was calculated as:

$$SPEAR = \frac{\sum_{i=1}^{n} x_i \times t_i}{\sum_{i=1}^{n} x_i}$$

where x_i = abundance of taxon i, and t_i = SPEAR classification of taxon i (1 for sensitive taxon i, otherwise 0). Invertebrate species were classified according to their risk of being affected by organic toxicants, based on their physiological sensitivity (von der Ohe and Liess, 2004); a sensitivity > -0.36 was classified as SPEAR and otherwise as $\text{SP}_{\text{not}}\text{AR}$.

The $\text{SPEAR}_{\text{pesticide}}$ was calculated using the software Indicate (version 1.0.0) as follows:

$$SPEAR_{pest} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \times t_i}{\sum_{i=1}^{n} \log(x_i + 1)}$$

where n = number of taxa, x_i = abundance of taxon i, t_i = SPEAR classification of taxon i (1 for sensitive taxon i, otherwise 0). Invertebrates were classified as SPEAR and $\text{SP}_{\text{not}}\text{AR}$ according to the software. Invertebrate taxa not included in the software were downgraded manually to the next higher taxon or to the most similar taxon found in the software.

2.5. Data processing and statistical analysis

For the assessment of seasonal variation of the nematode species composition and NemaSPEAR[%]-index at FB, data of the four spatial replicates were pooled and relative abundance of each species and the NemaSPEAR[%]-index was calculated for each month (June 2016 to May 2017). Three months were used as temporal replicates for each season: June, July and August representing summer; September, October and November representing autumn; December, January and February representing winter; March, April and May representing spring.

Non-metric multidimensional scaling (nMDS) based on the Bray-Curtis similarity index calculated from the untransformed nematode percentage data was used to determine the differences of nematode and macrofauna species composition of the various sites along the pollution gradient and seasonal differences in the nematode species composition of FB. The resulting stress value served as an indicator of the reliability of the nMDS plot. A stress value < 0.2 is considered acceptable (Clarke and Warwick, 2001). Significance of the differences in nematode species composition between the various sites along the pollution gradient and between seasons were tested with a one-way analysis of similarity (ANOSIM, number of permutations: 999). The resulting R value ranges between 0 and 1; a value > 0.5 is considered to indicate a difference between groups (Clarke and Warwick, 2001). For the macrofauna data, no ANOSIM could be performed, as there existed no replicates. A hierarchical cluster analysis with SIMPROF (similarity profile analysis) was performed based on the Bray-Curtis similarities (see nMDS analysis) for all data to identify significantly different cluster. The nMDS, ANOSIM and SIMPROF determinations were performed using PRIMER (version 6.1.5, PRIMER-E, Plymouth, UK). The mean NemaSPEAR[%], calculated for the various sites, was plotted against the toxic potential of the respective sediments (mean PEC-Q) and the data were fitted using a sigmoidal logistic model. Spearman's rank correlation test was used to determine whether the toxic potential of the sites (mean PEC-Q) correlated with the SPEAR[%] and $\text{SPEAR}_{\text{pesticides}}$, respectively, as well as whether the two SPEAR-indices correlated with each other or with the NemaSPEAR[%]. For testing for significant differences between NemaSPEAR[%] values between sites or seasons, one-way ANOVA was used with Tukey post-hoc test, after testing for homogeneity of variance (Levene's test) and normal distribution (Shapiro-Wilk test). The latter three analysis were performed using SigmaPlot (Systat software version 11).

3. Results

3.1. Toxic potential of the sites

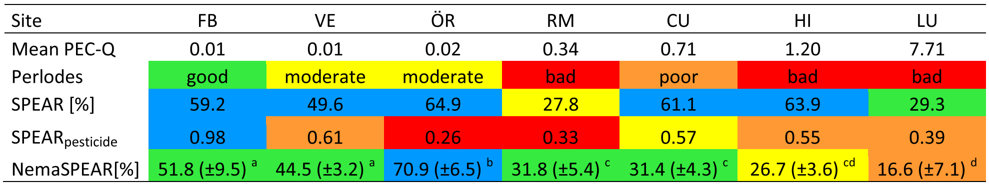
The toxic potential of FB and VE (mean PEC-Q of 0.01) and of ÖR (mean PEC-Q of 0.02) was very low (Table 2), so that no toxic effects were expected. For the other sampling sites the probability of toxic effects increased along the order RM (mean PEC-Q 0.34), CU (mean PEC-Q 0.71), HI (mean PEC-Q 1.20) and LU (mean PEC-Q 7.71). The results of the chemical analysis that form the basis for the calculation of the mean PEC-Q values are reported in detail in the supplementary information (Table S1).

3.2. Nematode and macrofauna communities along a pollution gradient

Non-metric MDS plot revealed typical nematode species compositions for each of the seven sites, which was supported by hierarchical cluster analysis that significantly clustered the replicates of the various

Table 2

Toxic potentials (mean PEC-Q), NemaSPEAR[%], *Perlodes*, SPEAR[%] and $\text{SPEAR}_{\text{pesticides}}$ values of the seven sampling sites. The results of the different assessment methods are color-coded according to the determined ecological status (blue = high, green = good, yellow = moderate, orange = poor, red = bad). Class boundaries for NemaSPEAR[%] were set according to Höss et al. (2017), and for SPEAR[%] according to von der Ohe et al. (2007). Class boundaries for *Perlodes* were those included in the software Asterics and for $\text{SPEAR}_{\text{pesticides}}$ in the software Indicate. For the NemaSPEAR[%] mean (±sd, FB and VE n = 4, for all other sites n = 5) is given with superscripted small letters indicating significant differences ($p < 0.05$; one-way ANOVA, post-hoc Tukey). FB = Furlbach, VE = Veerse, ÖR = Örtze, RM = Rischmühlenschleuse, CU = Cumlosen, HI = Hitzacker, LU = Luppe.



Site	FB	VE	ÖR	RM	CU	HI	LU
Mean PEC-Q	0.01	0.01	0.02	0.34	0.71	1.20	7.71
Perlodes	good	moderate	moderate	bad	poor	bad	bad
SPEAR [%]	59.2	49.6	64.9	27.8	61.1	63.9	29.3
$\text{SPEAR}_{\text{pesticide}}$	0.98	0.61	0.26	0.33	0.57	0.55	0.39
NemaSPEAR[%]	51.8 (±9.5) ^{a}	44.5 (±3.2) ^{a}	70.9 (±6.5) ^{b}	31.8 (±5.4) ^{c}	31.4 (±4.3) ^{c}	26.7 (±3.6) ^{cd}	16.6 (±7.1) ^{d}

sites (Fig. 2A). ANOSIM revealed significant differences in nematode species composition at the sites (global R: 0.974; p = 0.001), with binary comparisons showing significant differences between each pair of sites ($p < 0.05$; number of actual permutations: 126). Cluster analysis (SIMPROF) revealed four significant clusters on a 20%-similarity level (Fig. 2A), containing the replicates of FB (cluster 1), VE and ÖR (cluster 2), HI and CU (cluster 3) and RM and LU (cluster 4). On a higher similarity level (45%), significant differences could be shown between the replicates of ÖR and VE within cluster 2, and between the replicates of RM and LU within cluster 4, which agrees with the ANOSIM results. However, in contrast to the ANOSIM analysis, the replicates of the two sites from the river Elbe (HI and CU) could not be separated by the cluster analysis. Moreover, nMDS separated the sites according to their toxic potential, with the lowly contaminated sites (FB, ÖR, VE) being displayed on the left side of the nMDS plot, while the moderate to highly polluted sites were plotted on the right side (Fig. 2A).

The nMDS plot for macrofauna species composition showed three clear clusters, with following sites closely clustering together: (1) FB, VE and ÖR, (2) RM, HI and CU, (3) LU alone (Fig. 2B). Due to the lack of replicate data for the macrofauna communities, only cluster analysis via SIMPROF was performed, which confirmed the obvious clusters on a 20% similarity-level that appeared at the nMDS plot (Fig. 2B). Similar to the nematode communities, the macrofauna species composition was clearly related to the toxic potential of the sites.

3.3. The NemaSPEAR[%]-index and macrofauna-based indices along a pollution gradient

Based on the class boundaries defined by Höss et al. (2017), the NemaSPEAR[%]-index indicated a good (>30) or high (>56) ecological status for five sampling sites (FB, VE, ÖR, RM, CU) and a moderate (<30) or poor (<20) ecological status for two sampling sites (HI, LU; Table 2). However, it has to be noted that the values for RM and CU were scarcely above the threshold of 30 (Table 2). The mean NemaSPEAR[%]-index differed significantly between the sampling sites (one-way ANOVA: F = 45.573, df = 6, p = <0.001). The multiple comparisons revealed significant differences between ÖR and all other sites (Tukey test all tested pairs: p = ≤0.001) and between FB as well as VE and RM, CU, HI and LU (Tukey test all tested pairs: p = <0.05). Moreover, differences between LU and RM and CU, respectively, were significant (Tukey test both tested pairs: $p < 0.05$). The decrease of the NemaSPEAR[%]-index could be explained by the increasing mean PEC-Q values at the sites, as the fitted dose–response curve showed a significant regression by using a logistic function ($\text{NemaSPEAR}[\%] = 79.0/1+(x/0.181)^{0.360}$; $r^2 = 0.80$, $p = 0.041$, Fig. 3).

The overall evaluation of *Perlodes* rated one sites as good (FB), whereas all other sites (VE, ÖR, RM, CU, HI, LU) were assessed as moderate to bad by this method (Table 2). The SPEAR[%]-index indicated a good (>29) or high (>43) ecological status for six sampling sites (FB, VE, ÖR, HI, CU, LU; Table 2) and a moderate ecological status for the remaining site (RM). The $\text{SPEAR}_{\text{pesticides}}$ attested only for FB a high ecological status, whereas all other sites are rated as moderate to bad. The SPEAR-indices neither correlated with the mean PEC-Q of the sites (Spearman's rank test; SPEAR[%]: r = -0.0357, p = 0.905, $\text{SPEAR}_{\text{pesticides}}$: r = -0.393, p = 0.341) nor with the NemaSPEAR[%]-index (Spearman's rank test; SPEAR[%]: r = 0.321, p = 0.438; $\text{SPEAR}_{\text{pesticides}}$: r = 0.0357, p = 0.905).

3.4. Robustness of the NemaSPEAR[%]-index against seasonal variations

The ANOSIM of the nematode communities showed clear differences in species composition between sampling dates (global R = 0.716, p = 0.002), however pairwise comparisons revealed no significant differences between the seasons (p = 0.1). Cluster analysis could define three

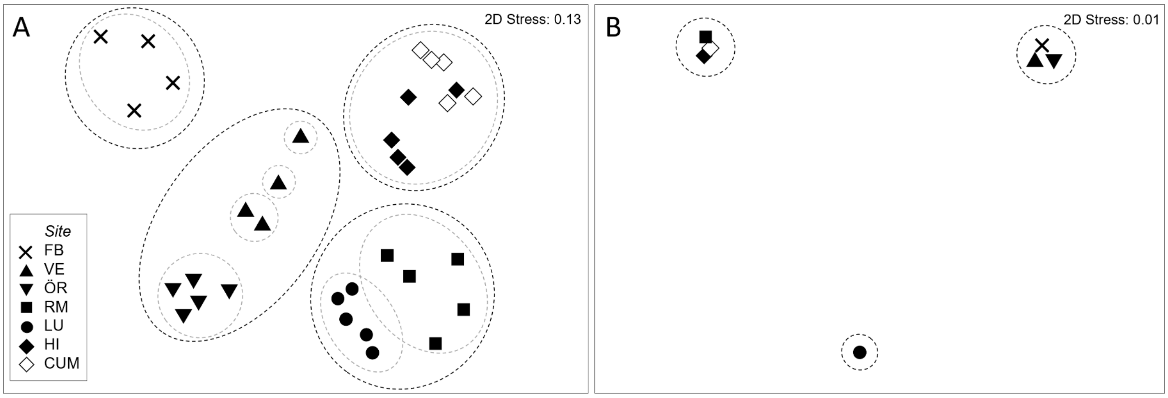


Fig. 2. nMDS plots for non-transformed relative abundances of (A) nematode and (B) macrofauna species at seven sites (FB = Furlbach, ÖR = Örtze, VE = Veerse, RM = Rischmühlenschleuse, CU = Cumlosen, HI = Hitzacker, LU = Luppe), based on Bray-Curtis similarities; superimposed dotted circles separate significantly different clusters (SIMPROF; $p < 0.05$) on a 20% (black dotted lines) and 45% (grey dotted lines) similarity-level as revealed by hierarchical cluster analysis.

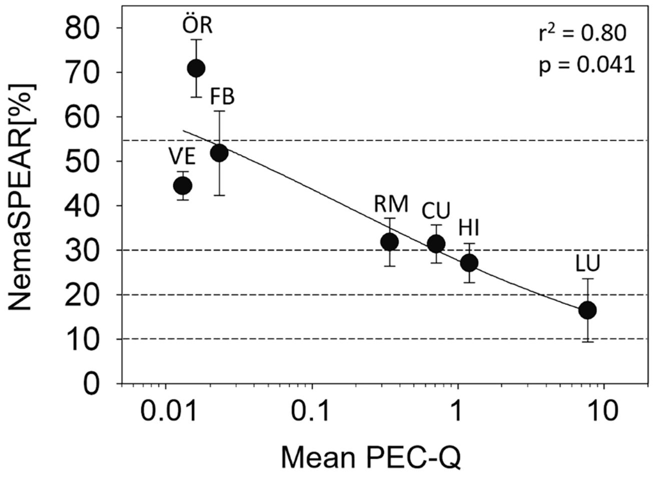


Fig. 3. Non-linear regression of the NemaSPEAR[%]-index (mean ± sd; FB and VE n = 4, all other sites n = 5) with the toxic potential of the sediment samples (mean PEC-Qs based on the sediment quality guidelines according to de Deckere et al., 2011). PEC-Q = quotient of a measured sediment concentration and the respective probable effect concentration of a specific substance (PEC). The data were fitted using a sigmoidal logistic model ($y = a/1+(x/x_0)^b$); the dotted lines represent the borders of the ecological quality classes (high ≥ 54; good 30 – 56; moderate 20 – 30; poor 10 – 20; bad < 10).

different clusters of months that were significantly different to each other (SIMPROF: $p < 0.05$), however, not related to specific seasons: (1) June and July 2016; (2) August to December 2016; (3) January to May 2016 (Fig. 4A).

The calculated NemaSPEAR[%] for the nematode communities of FB varied between 46.6 in August 2016 and 66.9 in June 2016 (Fig. 4B). Mean NemaSPEAR[%] calculated for the seasons varied only slightly, ranging from 50.1% (±3.2) in Autumn to 59.7% (±11.4) in Summer (Fig. 4B). The differences between the seasons were not significant (one-way ANOVA: F = 1.760, df = 3, p = 0.232).

4. Discussion

Nematode communities which were sampled from the seven sites clearly differed in terms of their species composition, whereas lowly contaminated sites (FB, VE, ÖR) could be distinguished from sites with moderate to high chemical pollution (RM, CU, HI, LU). This is in agreement with other studies that showed a relation between the pollution status of sediments, and the species composition of the inhabiting nematode communities (e.g. Zullini, 1976; Beier and Traunspurger, 2001; Heininger et al., 2007; Wolfram et al., 2012; Höss et al., 2011).

In line with the multivariate analysis of the nematode species composition in our study, the corresponding values for the NemaSPEAR [%]-index at lowly contaminated sites (mean PEC-Q 0.01 and 0.02) were significantly lower compared to sites with higher mean PEC-Q values (≥0.34). The NemaSPEAR[%] classification reflected the toxic potential of the sediments very well, indicating a good or high ecological status at sites with low toxic potential and worse results for higher toxic potentials. This finding is in accordance with former investigations (Höss et al., 2011, 2017). Although the NemaSPEAR[%] values at RM (31.8) and CU (31.4) indicated a good ecological status, it has to be noted that the values were close to the threshold of 30, marking the border to the moderate status. The curve fitting the NemaSPEAR[%] to the toxic potential (Fig. 3) yielded a mean PEC-Q of 0.71 as the critical threshold, corresponding to a NemaSPEAR[%]-index below 30, which is in good agreement with that reported by Höss et al. (2017). In the latter study a critical mean PEC-Q of 0.45 was determined. Moreover, MacDonald et al. (2000) defined the critical threshold at a mean PEC-Q of 0.5, above which a toxic effect can be expected. Thus, the NemaSPEAR[%] at RM and CU (mean PEC-Q of 0.34 and 0.71, respectively) indicated that these sites were at the threshold between a good and a moderate ecological status (31.8 and 31.4, respectively). This result further supports a mean PEC-Q of 0.5 as a robust endpoint for indicating toxic effects.

Additionally, the macrofauna species composition in the nMDS followed the pollution-related gradient, with macrofauna community structure of lowly contaminated sites (FB, VE, ÖR) being significantly different to sites with moderate (CU, RM, HI) and strong contamination (LU) (Fig. 2B). Despite the clear relation of the macrofauna taxa composition with the toxic potential (i.e. mean PEC-Q), none of the macrofauna based indices reflected the potential stress arising from the sediments at the various sites. Moreover, the different assessment methods applied to the different sites yielded highly discrepant results. Thus, neither a correlation between the macrofauna indices among each other nor with the result of the NemaSPEAR[%] was evident.

For different European rivers Wolfram et al. (2012) showed that the SPEAR[%] generally showed lower values than the NemaSPEAR[%] for reference sites as well as contaminated sites. In the present study the SPEAR[%]-index indicated a good or high ecological status at all sites, independent of the chemical pollution gradient. The saprobic index

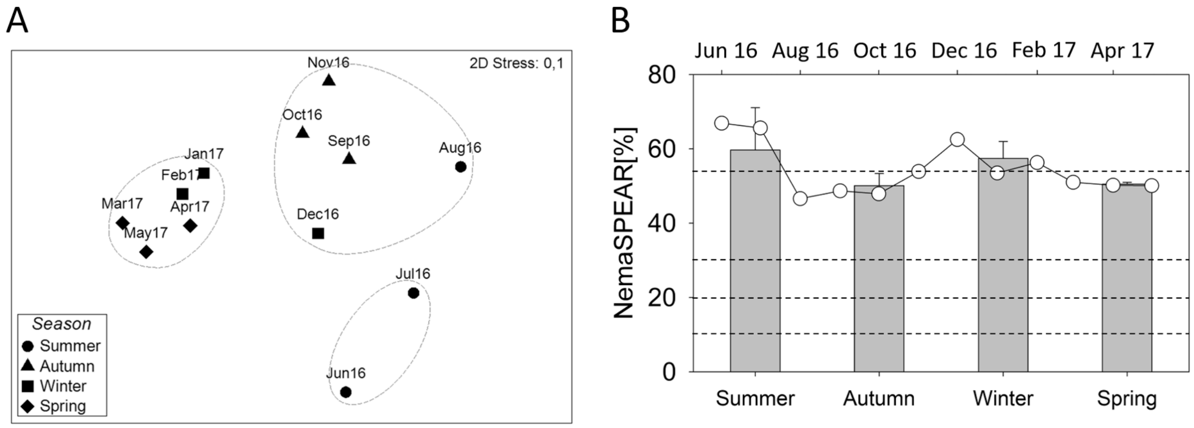


Fig. 4. (A) nMDS plot of nematode species composition based on the Bray-Curtis similarity and using the non-transformed relative species abundance data from the Furlbach for 12 months (June 2016 – May 2017). Superimposed dotted circles separate significantly different clusters (SIMPROF; $p < 0.05$) on a 50% similarity-level as revealed by hierarchical cluster analysis. (B) Calculated NemaSPEAR[%]-index in the course of one year (June 2016 – May 2017) and mean NemaSPEAR[%]-index (bar chart, ±SD, n = 3) calculated for the four seasons (Summer = June – August 2016; Autumn = September – November 2016, Winter = December 2016, January and February 2017, Spring = March 2017 – May 2017) based on the nematode species of the Furlbach. The dotted lines represent the borders of the ecological quality classes (high ≥ 54, good 30–54, moderate 20–30, poor 10–20, bad < 10).

module of *Perlodes* similarly determined a good ecological status at all sites, indicating that easily biodegradable organic substances and organic chemicals in general did not influence the macrofaunal communities. In contrast, the $\text{SPEAR}_{\text{pesticides}}$ assessed a moderate to bad ecological status at all sites, except FB. Since the latter index intends to identify the effects of pesticides on invertebrate communities (Liess and von der Ohe, 2005) it indicated the contribution by specific chemicals to the overall stress. However, the substances that induced a decrease of the $\text{SPEAR}_{\text{pesticides}}$ were apparently not part of the list of priority substances that had been measured in the sediment, as the index was not related to the mean PEC-Q values. Even at two of the lowly contaminated sites a poor (VE) or even bad (ÖR) ecological status was indicated (Table 2). Indeed, the percentages of detected macrofaunal taxa classified as species at risk according to $\text{SPEAR}_{\text{pesticides}}$ was very low, especially at ÖR. In the latter, only the Limnephilidae *Chaetopteryx fusca/villosa*, accounting for 5% of species and only 2% of individuals, was at risk.

In line with the results of the $\text{SPEAR}_{\text{pesticides}}$ the overall result of *Perlodes* indicated a moderate to bad ecological status for all sampling sites except FB, which, however, can be attributed to the general degradation module, downgrading the overall results of the *Perlodes* at those sites. Although a few metrics in this module accounted for the impact of chemical pollution to some extent, the metrics combined to the module-result reveal that major stressors were likely the degradation of habitat diversity and structure.

For many macroinvertebrates that are not endobenthic the most important route of chemical exposure is through the water phase. This means that the toxic potential of the sediments is not necessarily directly reflected by macrofauna indices. Thus, the NemaSPEAR[%], which is based on nematodes as exclusively endobenthic organisms, can provide important additional information to the routinely used indices. In the present study, contamination of the sediments at HI and LU was indicated by the NemaSPEAR[%] in accordance with the toxic potential of those sites, whereas at all other sites the major stressors obviously do not arise from toxic potentials of the sediments. Since the results of macrofauna-based indices are frequently compromised by low invertebrate numbers, especially for habitats with large contributions of finer sediments the additional use of meiofauna-based indices such as the NemaSPEAR[%] can be advantageous. Fine particles (<0.63 mm) made up $\geq 60\%$ of the sediment composition at all of the chosen sampling sites in this study, and between 19 and 60 nematode species were detected (supplementary information Table S1 and S4). This species numbers can be regarded as typical in stream sediments (Traunspurger, 2000; Hodda, 2006 and references therein). In addition, at most of the studied sites (6 of 7) the number of macrofaunal species was outweighed by the number of nematode species with, on average, twice as much nematode compared to macrofauna species (supplementary information Table S4). This was already shown in former studies examined fine-sediment habitats (e.g., Lòpez-Doval et al., 2010; Wolfram et al., 2010; Patrício et al., 2012).

However, methodological aspects that may have influenced our results have to be discussed. The number of species that can be identified often depends on the number of individuals sampled and in turn, on the number, size, and spatial arrangement of the taken samples (Gotelli and Colwell 2011). Thus, sampling directly influences the results of most macrobenthic indices because they use species density to quantify ecological quality as discussed in detail by Gislason et al. (2017). To meet this issue rarefaction can be performed that overcome the bias of underestimated species richness. Nevertheless, in our study we used the observed number of species without rarefaction. We performed the sampling and data processing according to the guidelines of the WFD described in Meier et al. (2006) for assessment via *Perlodes* and as it is set as a standard in Germany. We performed data analysis similar for all indices to enable direct comparison of the results of all used assessment methods.

Our study is the first to evaluate potential seasonal variations in the NemaSPEAR[%] results. The NemaSPEAR[%] showed some variation over the year, sometimes also reflecting the results of the nMDS (e.g. higher values in June/July). Nevertheless, it performed robustly throughout the year, always indicating good to high ecological status with values $\geq 47\%$, whereby the mean values for the various seasons did not differ from each other significantly. This was in good agreement with the low toxic potential of the sediment-associated chemicals at FB (mean PEC-Q $= 0.01$). Thus, the NemaSPEAR[%]-index can be applied without seasonal restrictions, wherefore methodically it might be at an advantage over the macrofauna-based indices. For example, sampling of the macrofaunal community for the *Perlodes* index should be carried out between February and August (Meier et al., 2006), as hatching periods can influence the appearance of certain macrofaunal species (Stead et al., 2003).

5. Conclusion

Identification of the stressors impacting freshwater communities and thus contributing to a less than good ecological status can benefit from the use of more than one assessment method, such that different kinds of stressors, targets and exposure pathways are considered. The integration of more than one benthic group is advantageous especially for habitats dominated by fine sediments, where nematodes are usually a highly abundant and diverse component of the benthic invertebrate fauna. The NemaSPEAR[%]-index represents a sensitive indicator for contaminated sediments specifically - a stressor that is not detected by any macrofauna index, thus providing an added value to the routinely used macrofauna-based indices. Consequently, it helps to identify the need of taking measures to improve the sediment quality and the overall ecological status of the investigated water body of interest, as indirect effects of various stressors on higher trophic levels are likely to occur via the food web. Moreover, since the NemaSPEAR[%]-index performed robustly against seasonal variations in the investigated nematode community, it represents a seasonally independent tool for the assessment of sediments.

CRediT authorship contribution statement

Henrike Brüchner-Hüttemann: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Sebastian Höss:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Christoph Ptatscheck:** Conceptualization, Investigation, Writing - review & editing. **Marvin Brinke:** Conceptualization, Writing - original draft, Writing - review & editing. **Janina Schenk:** Conceptualization, Writing - review & editing. **Walter Traunspurger:** Conceptualization, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107015.

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