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# Use of nematode and oligochaete communities for assessing the effects of toxic pollutants in lake sediments – An exploratory study in Lake Geneva

Utilisation des communautés de nématodes et d'oligochètes pour évaluer les effets de polluants toxiques dans les sédiments lacustres – Une étude exploratoire dans le Léman

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**Vivien R. *et al.*, 2024. Use of nematode and oligochaete communities for assessing the effects of toxic pollutants in lake sediments – An exploratory study in Lake Geneva. *Bulletin de la Société Vaudoise des Sciences Naturelles* 103: 39-59.**

**Abstract**

Sediments represent a habitat for a high number of species and have the property to store pollutants, in particular hydrophobic substances, which can reach concentrations sufficient to induce adverse effects on benthic invertebrates. It is crucial to assess the quality of this environmental compartment to identify whether it can contribute to the degradation of an aquatic ecosystem and disrupt its proper functioning. A complete evaluation of sediment quality requires combining ecotoxicological tests, *in situ* communities' assessment and chemical analyses. Given that nematodes and oligochaetes are abundant in sediments and comprise sensitive and resistant taxa to pollution, indices based on the composition of these communities have been proposed for assessing the biological quality of this compartment. In lakes, nematodes and oligochaetes have been mainly used to assess the effect of eutrophication. The specific effect of sediment contaminants such as trace metal elements, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) on these communities has not or insufficiently been studied in lakes. Here, we determined the oligochaete and nematode indices at six sites of Lake Geneva presenting different degrees of contamination. In parallel, the concentrations of trace metal elements, PAHs and PCBs were measured in the sediments of these sites and compared to sediment quality guidelines. The level of contamination varied from moderate to high, two sites presenting several pollutants with concentrations that largely exceeded the theoretical threshold effect concentrations. We observed some discordances between the results of oligochaete and nematode indices which could be partly explained by the different methods used for classifying the taxa as sensitive to pollution. At some sites, the biological quality inferred from oligochaete and nematode communities was good or very good although the measured contaminant concentrations exceeded previously established effect thresholds on oligochaete and nematode communities. These discordances could be explained by a reduction of the bioavailability of pollutants by Mn/Fe oxides, $CaCO_3$ and organic matter in sediments and by exfiltration of groundwater. The results of this exploratory study should be extended to confirm the pertinence of the use of oligochaete and nematode communities for assessing the effect of chemical pollution in lake sediments.

***Keywords:*** bioindication, lake sediments, sediment quality guidelines, nematode communities, oligochaete communities, NemaSPEAR index, oligochaete metrics.

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**Résumé**

Les sédiments représentent un habitat pour un grand nombre d'espèces et ont la propriété de stocker les polluants, notamment les substances hydrophobes, qui peuvent atteindre des concentrations suffisantes pour induire des effets néfastes sur les invertébrés benthiques. Il est crucial d’évaluer la qualité de ce compartiment environnemental pour identifier s’il peut contribuer à la dégradation d’un écosystème aquatique et perturber son bon fonctionnement. Une évaluation complète de la qualité des sédiments nécessite de combiner des tests écotoxicologiques, une évaluation des communautés *in situ* et des analyses chimiques. Comme les nématodes et oligochètes sont abondants dans les sédiments fins et comprennent des espèces sensibles et résistantes aux pollutions, des indices basés sur la composition de ces communautés ont été proposés pour évaluer la qualité biologique de ce compartiment. Dans les lacs, les nématodes et oligochètes ont été principalement utilisés pour évaluer l’effet de l’eutrophisation. L’effet spécifique de contaminants des sédiments tels que les métaux, les hydrocarbures aromatiques polycycliques (HAP) et les polychlorobiphényles (PCB) sur ces communautés n’a pas ou insuffisamment été étudié dans les lacs. Dans la présente étude, nous avons déterminé les indices oligochètes et nématodes sur six sites du Léman présentant différents degrés de contamination. En parallèle, les concentrations des métaux, HAP et PCB ont été mesurées dans les sédiments de ces sites et comparées à des normes de qualité des sédiments. Le niveau de contamination variait de modéré à élevé, deux sites présentant plusieurs polluants avec des concentrations largement supérieures aux seuils théoriques de concentration avec effet. Nous avons observé quelques discordances entre les résultats des indices oligochètes et de nématodes qui pourraient s'expliquer en partie par les différentes méthodes utilisées pour classer les taxons comme sensibles à la pollution. Sur certains sites, la qualité biologique indiquée par les communautés d'oligochètes et de nématodes était bonne ou très bonne, alors que les concentrations de contaminants mesurées dépassaient les seuils d’effet sur les communautés d'oligochètes et de nématodes précédemment établis. Ces discordances pourraient s'expliquer par une réduction de la biodisponibilité des polluants par les oxydes de Mn/Fe, le $CaCO_3$ et la matière organique dans les sédiments et par l'exfiltration des eaux souterraines. Les résultats de cette étude exploratoire devraient être étendus pour confirmer la pertinence de l’utilisation des communautés d'oligochètes et de nématodes pour évaluer l'effet de la pollution chimique dans les sédiments lacustres.

***Mots-clés:*** bioindication, sédiments lacustres, normes pour la qualité des sédiments, communautés de nématodes, communautés d'oligochètes, Indice NemaSPEAR, métriques oligochètes.

## 1. Introduction

Aquatic ecosystems are regarded as one of the most endangered ecosystems on earth. Among other anthropogenic pressures, surface waters are known to serve as one of the main recipients of chemicals discharged into the environment, resulting in a particular vulnerability of aquatic ecosystems to pollution (IPBES 2019). Within aquatic ecosystems, (soft) sediments are most often the final repository of hydrophobic substances that tend to bind to fine particulate matter. These substances can reach concentrations sufficient to induce adverse effects on benthic organisms and thus disrupt the proper functioning of the ecosystem (Lafont *et al.* 2010, Höss *et al.* 2011, Gurung *et al.* 2018).

Therefore, appropriate methodological approaches are crucial for evaluating sediment quality in order to identify the toxic potential of this complex compartment on aquatic ecosystems. A comprehensive sediment quality assessment requires combining ecotoxicological tools, *in situ* benthic communities’ assessment and physico-chemical analyses (Chapman 2007). Implementation of biological indices is important as they provide information on the *in situ* effects of sediment contaminants on organisms.

In fine sediments, nematodes and oligochaetes are abundant and constitute the majority of biomass (Wolfram *et al.* 2010, Wolfram *et al.* 2012, Vivien *et al.* 2014). Additionally, organisms belonging to these groups are exposed to sediment contaminants over their entire life cycle (Lafont 1989, Giere 2009). Given these characteristics and the fact that both nematodes and oligochaetes include a large number of species encompassing a wide range of pollution sensitivity, *in situ* biological indices based on their species composition were specifically developed to assess the biological quality of sediments in streams and lakes (Prygiel *et al.* 1999, Höss *et al.* 2011, Lafont *et al.* 2012, Vivien *et al.* 2014, Höss *et al.* 2017, Vivien *et al.* 2020a).

Concerning oligochaetes, a methodology was developed to assess both the biological quality and functioning of sediments in lakes (% sensitive taxa, IOBL index) (Lafont *et al.* 2012, AFNOR 2016). The list of sensitive oligochaete species/taxa in lakes was however mainly established based on their sensitivity to eutrophication. Oligochaete community composition has been used for several decades for assessing sediment quality of Swiss lakes (e.g. Lods-Crozet & Reymond 2005), including Lake Geneva (e.g., Lang & Reymond 1995, Lang 1997, Lang 2009, Lods-Crozet 2011, Lang 2016). In this lake, these communities have shown some biological recovery of sediments in the 2000s following the decrease of eutrophication since the 1980s (e.g., Lang 1997, Lang 2016). The sensitivity of oligochaete taxa to pollutants such as trace metal elements, PCBs and PAHs was established in stream sediments (Prygiel *et al.* 1999, Vivien *et al.* 2014, Vivien *et al.* 2020a), but it has never been studied in lake sediments. The nematode-based NemaSPEAR[%]-index was developed to assess the effects of trace metal elements, organic chemicals and pesticides in stream sediments (Höss *et al.* 2011, Höss *et al.* 2017). It has been used to assess the risk posed by these pollutants in various contexts (e.g., Sonne *et al.* 2018, Bighiu *et al.* 2020, Lemaire *et al.* 2022). Nematodes have also proven valuable descriptors of the impact of eutrophication in lakes (Ristau & Trauspurger 2011, Ristau *et al.* 2015).

Here, we present the results of an exploratory study aiming at evaluating the potential use of nematodes and oligochaetes to specifically assess the impact of contaminants such as trace metal elements, PAHs and PCBs in lake sediments. Six sites in Lake Geneva presenting different contamination levels were selected. Surface sediments collected at these sites were chemically analyzed and their toxic potential was assessed by applying widely recognized sediment quality guidelines. Simultaneously, the structure of nematode and oligochaete communities was examined in sediments of these sites and respective biological metrics were calculated. We compared the obtained results of each of these biological metrics between them and to those of the chemical analyses. The results of this study provide a baseline for future developments of oligochaete and nematode community indices for assessing the effect of chemical pollution in lake sediments.

## 2. Material & Methods

### 2.1. Study area and sediment sampling

Six sites (sites 6, 21, 32, 36, 53 and 78) in Lake Geneva were selected from the CIPEL sediment campaign grid to represent different degrees of contamination (Loizeau *et al.* 2017) (table 1). Details of the sampling campaign can be found in Lyautey *et al.* (2021). The level of contamination was expected to be high at site 53 as it directly receives the effluents of the wastewater treatment plant of Lausanne, its outlet being very close to this site, while the other sites were expected to have a low to medium contamination level.

Surface sediments (top layer of 0-10 cm) were collected in October 2017 (sites 32, 53, and 78) and May 2018 (sites 6, 21, and 36), with an Ekman type grab sampler for the assessment of nematode and oligochaete community composition and the physico-chemical analyses of sediments. Two grab samples were collected and combined per sampling site. For the assessment of nematode communities, four sub-samples were randomly collected from the combined grab samples using a corer with an inner diameter of 6 cm, resulting in a sampled sediment surface of 28.27 $cm^2$ for each sub-sample. These sub-samples were preserved in formaldehyde at final concentration of 4%. Sediments were also collected from the two combined grab samples for grain size and chemical analyses. Sub-samples for the analysis of trace metal elements, organic matter (OM), total organic carbon (TOC), $CaCO_3$, PAHs and PCBs were frozen at -20°C, while the sub-samples for granulometry were preserved at 4°C. Three additional grab samples were collected per sampling site (one 5 l container per grab sampler) for the assessment of the oligochaete communities. The sediment samples were preserved in neutral buffered formalin (ThermoFisher Scientific, Ecublens, Switzerland), at a final formaldehyde concentration of 4%.

## 2.2. Sediment physico-chemical characterization

Sediment grain-size distribution was determined on wet sediments using a laser diffraction analyzer (Coulter LS-100, Beckman-Coulter, United States). Samples for the other analyses were freeze-dried in a CHRIST BETA 1–8 K freeze drying unit for a minimum of 48 h. Organic matter and $CaCO_3$ contents in sediments were estimated by loss on ignition. Samples were heated to 550°C for 30 min to estimate the OM mass loss and then heated to 1000°C for another 30 min to estimate the $CaCO_3$ content. The $CaCO_3$ content was calculated by multiplying the mass loss at 1000°C by 2.2742, the molar mass ratio of calcite to carbon dioxide. The TOC was determined by Solid Sample Combustion method using SSM-500A (Shimadzu). Mercury (Hg) was measured by atomic absorption spectrophotometry using an Advanced mercury analyser (AMA 254, Altec, Czech Republic). For measurement of the trace metal elements chromium (Cr), zinc (Zn), nickel (Ni), lead (Pb), copper (Cu), cadmium (Cd) and arsenic (As), about 1 g of each sediment sample was dissolved in 2 M nitric acid overnight at 100°C. This method allows the extraction of the bioavailable fraction of trace metal elements. The concentrations of the trace metal elements were determined by a quadrupole based inductively coupled plasma mass spectrometer (ICP-MS, model 7700 series, Agilent). Additionally, the PCB congeners 28, 52, 101, 118, 138, 153, 170 and 180 and the PAHs naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene were measured by gas chromatography-mass spectrometry (GC-MS/MS, Thermo Scientific, TSQ Quantum XLS Ultra, Waltham, MA, United States). More details of the physico-chemical analyses are available in Lyautey *et al.* (2021).

The toxic potential of the measured concentrations of the target substances in the sediments was estimated by applying well-established sediment quality guidelines (SQGs). The SQGs proposed by de Deckere *et al.* (2011) and MacDonald *et al.* (2000) were used. Both SQGs propose a low SQG or threshold effect concentration (Consensus 1 and TEC, respectively) below which the probability of toxicity is low, and a high SQG or probable effect concentration (Consensus 2 and PEC, respectively) above which toxicity is likely. At concentrations between thresholds Consensus1/TEC and Consensus2/PEC, toxicity is possible. Concentrations were compared here to the SQGs proposed by MacDonald *et al.* (2000) which are the most commonly used thresholds. In addition, for each site, two different indices of contamination were calculated: (1) mean PEC-$Q_{met}$: is the sum of the ratios between the concentration of each trace metal element and their respective high SQG (PECs) divided by the total number of trace metal elements (2) mean Consensus2-Q: is the sum of the ratios between the concentration of each trace metal element, PAH compound and PCB compound and their respective high SQG (Consensus 2) divided by the total number of trace metal elements, PAHs and PCBs. These two contamination indices were selected for comparison with results from previous studies: mean PEC-$Q_{met}$ for oligochaetes (Vivien *et al.* 2014) and mean Consensus2-Q for nematodes (Höss *et al.* 2017). The Consensus2-Q calculation corresponds to the "PEC-Q" calculation in Höss *et al.* (2017).

## 2.3. Examination of benthic communities

### 2.3.1. Oligochaete communities

Sediments were sieved through a column of sieves with 5 mm and 0.5 mm mesh size in the laboratory. For each site, the sieved material of each of the three grab samples was combined. The sieved material of each site was transferred into a square sub-sampling box (5 x 5 cells) and the content of randomly selected cells was examined under a stereomicroscope (in Petri dishes). Per site, 100 specimens were extracted (when possible, only 72 specimens extracted at site 78) and the number of cells was used to determine the abundance of oligochaetes per 0.1 $m^2$ of sediments. Sorted oligochaetes were then mounted on slides in a coating solution composed of lactic acid, glycerol and polyvinylic alcohol (Reymond 1994), and identified to the lowest practical level (species if possible) using a compound microscope.

Subsequently, oligochaete-based indices were calculated. The oligochaete index of lake bioindication (IOBL) allows to assess the potential of the sediments to assimilate and recycle nutrients from both water and sediments (Lafont *et al.* 2012, AFNOR, 2016). The IOBL is calculated according to the following formula: $IOBL = S + 3 \times log10\ (D+1)$. The terms "$S$" is the number of taxa identified among 100 oligochaetes and "$D$" is the density of oligochaetes per 0.1 $m^2$. This index gives information on the functioning of the sediments and ranks the metabolic potential into 5 classes as follows: IOBL >15: very strong potential, 15 - 10: strong, 9.9 - 6.1: moderate, 6 - 3.1: low, 3 - 0.1: very low; 0: null.

The examination of indicator taxa completes the diagnosis by providing information, among others, on the trophic state of the environment (oligotrophic or non-oligotrophic) and on the effects of contaminants in sediments or of natural dystrophy (Lafont 2007, Lafont *et al.* 2012). The oligochaete taxa are grouped in four main ecological groups: (1) sensitive taxa to pollution; (2) moderately sensitive taxa to pollution; (3) taxa which characterize a natural dystrophy (due to e.g., presence of peat, coarse vegetal detritus, abundance of Characeae); (4) resistant taxa to pollution. A list of species corresponding to each group is provided in Lafont (2007) and Lafont *et al.* (2012).

The biological quality of the sediments was assessed using the percentages of sensitive taxa to pollution (mainly to eutrophication), group 1. It is classified as follows (AFNOR 2016): percentage >50: very good, 21 - 50: good, 11 - 20: medium, 6 - 10: poor, 0 - 5: bad.

Unlike those of group 1, the percentages of taxa of groups 2, 3 and 4 cannot be determined accurately because several species of tubificid belonging to these groups cannot be identified when the individuals are in an immature state. The Tubificinae with hair setae not recognizable in an immature state can belong to groups 3 or 4 and the Tubificinae without hair setae not recognizable in an immature state to groups 2 or 4. All species of Tubificinae without hair setae belong to group 4, except *Limnodrilus profundicola* (group 2). Therefore, low percentages of the ecological groups 2, 3 and 4 cannot be used for interpretation of results (as they could be actually higher). Only high percentages of these three groups are informative.

#### 2.3.2. Nematode communities

The four replicate sub-samples per site were analyzed separately. The methodology described in Heininger *et al.* (2007), Höss *et al.* (2011, 2017) and Traunspurger *et al.* (2012) was followed. Approx. 100 g of sediment of each replicate sub-sample was rinsed into centrifugation tubes and centrifuged for 5 min at 800 g. After discarding the supernatant, the sediment was mixed with a colloidal silica suspension (Ludox TM 50;8/17, Sigma Aldrich, Munich, Germany) that was adjusted to a density of 1.13 g/ml with deionized water (Pfannkuche & Thiel 1988). After centrifugation for 15 min at 800 g, the supernatant was filtered through a 10-µm gaze that retained all meiofaunal organisms. The extraction steps were repeated three times. The meiofauna gained from these three extraction steps were fixed with 4% formaldehyde (stained with Rose Bengal) and stored until further analysis. After removing the formaldehyde by filtering through a 10-µm gaze, the retained organisms were rinsed into Petri dishes, where they were counted using a dissecting microscope (25-40-fold magnification). From each replicate sub-sample, approximately 50-60 nematodes (except for one replicate sub-sample of site 53, where only 14 nematodes were found) were transferred and prepared in glycerol and were identified using a compound microscope to species level when possible (1250-fold magnification; Leitz, Dialux), following literature (Bongers 1988, Andrássy 2005, Andrássy 2007, Andrássy 2009).

Subsequently, the NemaSPEAR[%] index was calculated according to the following formula (Höss *et al.* 2011, 2017): *NemaSPEAR[%] = 100 × log % [NemaSPEAR] / log % [All Species]*. The relative abundance (%) of nematode taxa classified as sensitive (NemaSPEAR) was calculated with respect to the relative abundance of all species or taxa (All Species). Relative abundances were log (x+1)-transformed to relativize the abundances of very dominant species before they were included in the calculations. At each site, the average NemaSPEAR[%] index value (± standard deviation) of the four replicates were calculated. Biological quality of sediments was classified as follows: NemaSPEAR[%] >54: high, 30-54: good, 20-29: medium, 10-19: poor, <10: bad.

Moreover, nematode taxa were grouped according to their feeding habit following the classification of Traunspurger (1997): deposit feeders (BF; mainly bacterial feeders), epistrate feeders (AF; mainly algae feeders), suction feeders (SF; feeding on plants and fungi), and chewers (Pre/Om; predators and omnivores).

## 3. Results

### 3.1. Sediment properties and comparison with SQGs

The surface sediments from the study sites showed distinct physico-chemical characteristics (table 2). The sediments were mainly fine-grained with median grain sizes between 7.7 µm and 34.8 µm. The highest OM and TOC percentages were obtained at site 53 (13.6% OM, 7% TOC), and the lowest at site 78 (2.7% OM, 0.7% TOC). The other sites had relatively high % of OM/TOC, between 4.8 and 9% (OM) and between 2.1 and 4.6% (TOC). Sites 6, 21, 32 and 36 were characterized by high proportions of $CaCO_3$, between 36.6 and 48.9%, while sediments from sites 53 and 78 contained less than 20% $CaCO_3$. The concentration of Mn was the highest at site 78 (552 µg/g d.w.), and between 264 and 352 µg/g d.w. at the other sites. The concentration of Fe was also the highest at site 78 (23371 µg/g d.w.), and between 10 720 and 20 475 µg/g d.w. at the other sites.

As expected, the studied sites showed different contamination levels and could be classified from moderately to highly polluted (table 2). The examination of the degree of SQGs exceedances indicates that toxicity to benthic organisms cannot be excluded for none of the sediments, with at least two of the target substances above the corresponding threshold effect level. Site 53 stands out among the rest of sites. It showed the highest measured concentrations for most of the substances analyzed, with the trace metal elements Cu, Hg, Zn, the PAHs fluorene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(a)pyrene and the sum of PAHs above the probable effect concentrations (toxicity is likely), and Cd, Pb, Cr, Ni, the PAHs naphthalene and anthracene and the sum of PCBs above the threshold effect concentrations.

The concentrations of trace metal elements and PAHs at sites 36 and 32 were also at levels of potential toxicity to benthic invertebrates, but the incidence of SQGs exceedances at these sites was lower than at site 53. At site 36, concentrations of the PAHs anthracene, pyrene, benzo(a)anthracene and chrysene exceeded the probable effect concentrations and additionally concentrations of fluorene, phenanthrene, fluoranthene, benzo(a)pyrene, the sum of PAHs and the trace metal elements Ni and Hg were above the threshold effect concentrations. At site 32, the PAHs pyrene, benzo(a)anthracene, chrysene, benzo(a)pyrene, the sum of PAHs and the trace metal elements Cu, Ni and Hg were above the threshold effect concentrations. Sites 78, 21 and 6 showed the lowest toxic potential, although two to three exceedances of the threshold effect concentrations were reported at each site. The metal Ni was above the probable effect concentration at these sites, but this could be due to the relatively high background concentrations of this metal in the region (Casado-Martinez *et al.* 2016).

The value of the contamination index mean PEC-$Q_{met}$ (considered for comparison of oligochaete results with those of previous studies) was high at site 53 (=0.96) and lower at all the other sites (between 0.22 and 0.33). The highest value of the contamination index mean Consensus2-Q (considered for comparison of nematode results with those of previous studies) was also obtained at site 53 (4.41). The mean Consensus2-Q value was also high at site 36 (0.9), characterized by a high level of PAH contamination, while the other sites had much lower values (0.17 to 0.26).

## 3.2. Oligochaete communities

### 3.2.1. Community composition and biological indication

Twenty-three oligochaete taxa belonging to the subfamilies Tubificinae (16 taxa) and Naidinae (4 taxa) and the family Lumbriculidae (3 taxa) were found (table S1). All encountered species are common in Lake Geneva and in Switzerland except *Quistadrilus multisetosus*. The presence of this species was newly recorded in Lake Geneva (present sampling) and had only been mentioned in Switzerland in Lake Bienne (one specimen on the shore), in Lake Lucerne (one specimen on the shore) and in Lake Constance (two locations) before (Vivien *et al.* 2020b). This species is highly tolerant to pollution and its presence at site 53 seems to be explained by the proximity of this location to the outlet of the wastewater treatment plant (Vivien *et al.* 2020b).

Results of oligochaete communities indicated at all sites a very strong potential of the sediments to recycle and assimilate organic matter (table 3). The density of oligochaetes was very high at site 53 (>45 000 per 0.1 $m^2$) and much lower at the other sites (<520 per 0.1 $m^2$). The biological quality of sediments (indicated by the percentage of sensitive taxa to pollution) was bad at site 53 (with 0% of sensitive taxa), medium at sites 6 and 32 and good at the other sites. The sensitive taxa included only or mainly *Embolocephalus velutinus* and

Lumbriculidae at sites 21 and 36, and *Psammoryctides barbatus* and *Vejdovskyella intermedia* at site 78. At site 78, we observed in addition a high percentage (32%) of moderately sensitive species to pollution.

#### 3.2.2. Biological results vs chemical contamination

The bad biological quality at site 53 was completely expected given the high toxic potential of trace metal elements, PAHs and PCBs measured at this site. At this site, the enrichment of organic matter was reflected in the large proliferation of resistant taxa to pollution. At sites 6 and 32, where some pollutants were above the thresholds for toxic effects, the biological quality indicated by oligochaete communities was medium. However, even if some alteration of the biological quality was expected at the other sites (21, 36 and 78), oligochaete communities indicated a good biological quality. The most discordant case was obtained at site 36 (characterized by high concentrations of PAHs).

Vivien *et al.* (2014) proposed for stream sediments a threshold of PEC-Q = 0.18 above which effects are clearly visible on oligochaete communities. Above this threshold, 80% of the sites in Vivien *et al.* (2014) showed a medium, poor or bad biological quality. The results obtained at sites 6, 32 and 53, where the mean $PEC\text{-}Q_{met}$ was higher than 0.18 and the biological quality insufficient, agreed with this effect threshold. Site 53 showed a particularly high mean $PEC\text{-}Q_{met}$ value (0.96) and a bad biological quality. However, the results at the three other sites, presenting a good biological quality and mean $PEC\text{-}Q_{met}$ values between 0.22 and 0.33, did not agree with the previous results from stream sediments.

More recently, and also for stream sediments, a threshold of the metal contamination index $mPEL_{oligo}\text{-}Q$ = 0.92 (PEL= probable effect level) above which effects on oligochaete communities were likely to occur, was proposed (Vivien *et al.* 2020a). This index is based on the combination of concentrations of Cr, Zn, Ni, Pb, Cu, Cd, Hg and As and uses the probable effects levels PELs on oligochaete communities provided in Vivien *et al.* (2020a). Again, this threshold was exceeded at all sites, so that only results obtained at sites 6, 32 and 53 showed concordances with the previous results from stream sediments.

### 3.3. Nematode communities

#### 3.3.1. Community composition and biological indication

Nematodes occurred in low to very high abundances, with mean density values ranging from 24 to 1004 individuals per 10 $cm^2$ (table 4), which is comparable to findings from other European lakes (Traunspurger 1996, Michiels & Traunspurger 2004, Traunspurger *et al.* 2012). However, the number of species (19 to 25; number of identified individuals per site: 166 – 206) was relatively low compared to other lakes (Traunspurger 1996, Michiels & Traunspurger 2004, Traunspurger *et al.* 2020). At all sites, deposit feeding nematodes (mainly bacterial feeders) dominated the communities (55 – 92%). Algae feeders occurred in relatively high abundances at sites 78, 32 and 36 (9 – 19%), while predatory and omnivorous nematodes were abundant at sites 6, 21, 32 and 36 (13 – 25%). At site 53, pollution-tolerant species of the families Rhabditidae (*Diploscapter coronatus*, *Pelodera punctata*) and Diplogastridae (*Diplogasteritus nudicapitatus*, *Diplogaster rivalis*) dominated the community, while at the other sites a completely different set of species was dominant (*Daptonema dubium* and *Hoffmaenneria brachystoma* [Xylidae], *Eumonhystera andrassy*, *E. barbata*, *E. vulgaris*, *Monhystera paludicola*, *Monhystrella paramacrura* [Monhysteridae] and *Epitobrilus medius* [Tobrilidae]) (table S2).

In terms of NemaSPEAR[%] index values, site 53 showed a poor biological quality (NemaSPEAR < 20%), while all the other sites showed a good (site 6) or very good biological quality (sites 21, 32, 36, 78).

#### 3.3.2. Biological results vs chemical contamination

The poor biological quality (NemaSPEAR[%] value of 14) obtained at site 53 was expected given its high toxic potential. This site also exhibited the lowest nematode densities compared to the other sites, which is, however, not necessarily related to contamination. The biological quality obtained at the other sites was good or very good, although several trace metal elements and PAH compounds exceeded the SQGs at these sites.

For stream sediments, Höss *et al.* (2017) observed that above a threshold of mean Consensus2-Q of 0.17, 80% of the sediment samples showed a moderate, poor or bad biological quality. Results agreed with this threshold only at site 53 (poor biological quality and mean Consensus2-Q=4.41). The biological quality obtained at the other sites was good or very good despite an exceedance of this threshold at these sites (slightly at sites 6, 21, 32 and 78 and clearly at site 36). Höss *et al.* (2017) also calculated a threshold of mean Consensus2-Q = 0.45 below which NemaSPEAR[%] values ≥30 (good or very good biological quality) can be expected. The results of nematode communities agreed with this threshold at all sites, except site 36.

## 4. Discussion

### 4.1. Ecological diagnoses provided by the biological indices

For the first time, nematode and oligochaete communities were analyzed together to assess the biological quality of lake sediments. In this first comparative study with lake sediments, the results of both communities were in good agreement for four sites (21, 36, 53, and 78) and discordant for two sites (6 and 32), where the oligochaetes indicated a medium biological quality and the nematodes a good or very good biological quality. Previous results of the oligochaete index IOBS and the NemaSPEAR index at three artificial canals of the Rhône River (Valais, Switzerland) also showed a tendency of the oligochaete index to provide a slightly more pessimistic ecological diagnosis than the NemaSPEAR index (Beauvais *et al.* 2020).

We observed that for stream sediments, a calibration of the limits of medium / good quality classes among the indices could allow to increase the concordance rate between oligochaete and nematode results. Indeed, in Beauvais *et al.* (2020), results showed a better concordance between both indices when the limit of good quality class was fixed at NemaSPEAR=40% (instead of 30%). Idem using the data of Höss *et al.* (2017): 80% of sites with a value of the mean PEC-$Q_{met}$ index above the threshold for effects on oligochaete communities of 0.18 had NemaSPEAR values <40%, while 80% of sites with NemaSPEAR values <30% was obtained above a threshold of mean PEC-$Q_{met}$ = 0.55.

But in the present study, an adaptation of the limits of quality classes could not be proposed to delete the discordances between the biological index results. Indeed, at sites 6 and 32, the percentages of sensitive oligochaete and nematode taxa were very different. Furthermore, at the other sites except site 53, the percentages of sensitive nematode taxa were much higher than the percentages of oligochaete sensitive taxa. These results suggest a different pollution sensitivity of the indicator taxa selected for the oligochaete and nematode community indices, and therefore a complementarity of these two taxonomic groups. These discordances may also be caused by the different methodologies used to define the pollution-sensitive taxa. For oligochaetes, sensitive taxa were defined as those dominant in pristine or very slightly impacted sites such as in sources of streams or in oligotrophic mountain lakes and taxa that tended to proliferate in impacted areas such as streams in agricultural zones or meso-eutrophic lakes were designated as resistant to pollution (Lafont 1989, Vivien *et al.* 2014, Vivien *et al.* 2020a). The NemaSPEAR index used multivariate statistical analyses to determine nematode taxa occurring at sites with low toxic potential (defined according to porewater-based toxic units using *Daphnia magna* lethal concentrations 50 -LC50-), i.e., the nematode species at risk (NemaSPEAR; pollution sensitive species). All the other species were defined as nematode species not at risk (NemaSPE$_{not}$AR; ubiquitous or pollution tolerant species) (Höss *et al.* 2011).

Each method of classification has its advantages and disadvantages. The list of sensitive oligochaete taxa could be too restrictive; therefore, the oligochaete indices could tend to provide overall pessimistic ecological diagnoses because they indicate departures from a highly protective reference condition. On the other side, the classification of taxa inferred from the statistical distribution approach used in the development of the NemaSPEAR index is dependent on the statistical method applied, on the approach used to identify sites with low toxic potential (which used effect concentrations for daphnids) and on the underlying dataset. Due to the limited range of targeted chemical analyses, sediment classified as having low toxic potential might actually contain some unmeasured contaminants at significant concentrations. We cannot exclude that some nematode taxa are incorrectly classified as sensitive, which could lead in some cases to an overestimation of the biological quality of sediments.

## 4.2. Biology vs chemistry

Effects of trace metal elements, PAHs and PCBs in lake sediments are newly assessed using oligochaete and nematode communities. Eutrophication could impact both oligochaete and nematode results (cf. introduction) and could mask the effects of toxic pollution. Nematodes showed in the present study a good or very good biological quality at all sites except one site presenting a high degree of chemical contamination, and so did not seem to be influenced by the eutrophication factor. However, we cannot exclude that oligochaete results obtained at sites 6 and 32 are influenced by some deficit of oxygenation in sediments.

Some discrepancies between the results of the biological indices and the chemical analyses were noted. Specifically, at site 36, the high concentrations of PAHs did not appear to impact the nematode and oligochaete communities. It is widely recognized that bioavailability plays a crucial role in sediment toxicity, meaning that the concentrations in sediments do not necessarily reflect the concentration of the available fraction for benthic organisms (Tessier & Campbell 1987, National Research Council 2002, ICCM 2007). PAHs are known to strongly interact with dissolved organic carbon (Akkanen *et al.* 2012) and sediment organic matter (Thorsen *et al.* 2004) which was present at relatively high concentration at site 36. A reduction of toxicity of PAHs by the binding of these compounds to organic matter can be expected at this site.

We observed a slight misfit between the results of the oligochaete and nematode indices in lake sediments and previously proposed thresholds for effects for each of these communities in streams. For oligochaetes, sensitive taxa were abundant at sites 21, 36 and 78, where the threshold for effects on oligochaete communities in streams set at a mean PEC-Q$_{met}$ value of 0.18 was exceeded. For nematodes, the threshold of mean Consensus2-Q of 0.17 was exceeded at sites 6, 21, 32, 78 (threshold values between 0.17 and 0.25) and 36 (threshold value = 0.90) and presented a good or very good biological quality. In fact, between values of mean Consensus2-Q of 0.17 and 0.45, obtained NemaSPEAR values are very diverse and can indicate biological qualities from bad to very good (Höss *et al.* 2017). So, the threshold of mean Consensus2-Q = 0.17 can be considered as a first threshold above which toxic effects are possible. In Beauvais *et al.* (2020), out of 10 sites with Consensus2-Q values between 0.18 and 0.31, the NemaSPEAR index indicated for 3 sites a poor biological quality, for 4 sites a medium biological quality and for 3 sites a good biological quality. Only the NemaSPEAR value obtained at site 36 did not agree with the upper threshold of mean Consensus2-Q of 0.45. Brüchner-Hüttemann *et al.* (2021) also obtained a NemaSPEAR[%] value indicating a sufficient biological quality at a site with a mean Consensus2-Q largely exceeding 0.45 (0.71), however, with a NemaSPEAR value close to the limit of moderate quality class (31.4).

The good/very good biological quality inferred from oligochaete and nematode communities at sites exceeding thresholds for effects for these communities could be explained by several factors. First, it is possible that pollutants are less bioavailable due to increased levels of $CaCO_3$, OM (sites 6, 21, 32 and 36) and Mn/Fe oxides (site 78) (Tessier & Campbell 1987). Two previous studies in Lake Geneva already suggested a reduction of bioavailability in association with increased levels of Mn/Fe. Benejam (2016) studied oligochaete communities along a transect (five sites) affected by a WWTP and a combined sewage overflow in the Vidy Bay and reported increased percentages of sensitive oligochaete taxa (good biological quality) at the two sites farthest from the WWTP with high mean $PEC\text{-}Q_{met}$ values (0.56 and 0.58) but also high concentrations of Mn and Fe, similar to those measured at site 78. The three other sites of the transect had poor biological quality with high mean $PEC\text{-}Q_{met}$ values (between 0.47 and 0.50) but lower concentrations of Mn and Fe. Lang & Lang-Dobler (1979) showed an association between Mn and the sensitive oligochaete species *Embolocephalus velutinus* and *Stylodrilus lemani* at impacted sites in Lake Geneva. Even if Lang & Lang-Dobler (1979) did not formulate this hypothesis, we could presume that a reduction of bioavailability of the trace metal elements by Mn oxides could explain the significant presence of the sensitive oligochaete species at these sites.

A higher tolerance of sensitive taxa to chemical contamination in lakes compared to stream sediments due to an adaptation of the "sensitive" species to such contaminants is also possible. The organisms could have acquired mechanisms of resistance to these contaminants (Reinecke *et al.* 1999, Lopes *et al.* 2005) in lake sediments, where their concentrations tend to be stable (no or weak sediment remobilization). The acquisition of mechanisms of resistance to trace metal elements and other toxic compounds has for example been demonstrated in the nematode *Caenorhabditis elegans* (e.g. Broeks *et al.* 1995, Broeks *et al.* 1996, Swain *et al.* 2004).

Another factor that could locally influence oligochaete and nematode communities in lake sediments is groundwater exfiltration, by constantly providing specimens of sensitive taxa and well oxygenated water in surface sediments. The sensitive oligochaete taxa *Embolocephalus velutinus* and Lumbriculidae, abundant at sites 21 and 36 as well as at the two furthest sites of Benejam (2016) transect are indicators of exfiltration of groundwater in stream sediments. Results of a study conducted in the 19$^{th}$ century (Piguet 1889) in the area of Ouchy in Lake Geneva (quite close to the sites studied in Benejam (2016)) show a similar pattern of oligochaetes taxa along a transect from 6-30 m deep to 120 m deep, the sensitive species *E. velutinus* (indicator of exfiltration) being absent or not abundant at depths lower than 50 m, abundant between 50-60 m deep and dominant at sites at 90, 100 and 120 m deep. At depths of 90-120 m, the sensitive species *Stylodrilus lemani* and *Stylodrilus heringianus* (also indicators of exfiltration) were also well represented. These results show the existence of a pattern in oligochaete community composition in this zone (from Vidy Bay to Ouchy) persistent over time, refuting the hypothesis formulated in Benejam (2016) of a reduction of the effect of organic matter input from the WWTP outlet/overflow (due to the distance). Although exfiltration of groundwater was suggested at some spots in Lake Geneva (Juget 1967a, Juget 1967b), to our knowledge it has not been formally demonstrated yet. If the existence of these exfiltrations were confirmed, such results could indicate the higher sensitivity of these species to the level of oxygenation of sediments than to the chemical pollution in sediments and overall, the important influence of exfiltration on oligochaete community composition in the surface sediments of Lake Geneva.

A difference between degrees of resistance of the oligochaete species *Psammoryctides barbatus* to eutrophication and to trace metal elements could also explain the discordant result obtained at site 78. This species, dominant at this site, was classified as sensitive in lakes as never or rarely found in deep zones (Lafont 1989), which are generally less oxygenated than the shallow zones, but as resistant to pollution (eutrophication and toxic type) in streams. All the other sensitive oligochaete taxa in lakes are considered as sensitive in streams. We cannot therefore exclude that *P. barbatus* is also tolerant to trace metal elements in lake sediments.

## 5. Conclusion

Here, we presented the results of an exploratory study implemented to evaluate the potential use of nematodes and oligochaetes for assessing the effects of contaminants in lake sediments. This study considered six sites in Lake Geneva presenting different levels of contamination. In general, there was good agreement between the oligochaete and nematode communities although some discordances between the ecological diagnoses inferred from oligochaete and nematode indices were observed. These discordances could partly be explained by different methods of classification of sensitive taxa. Even if further calibration of the methods could be considered in the future, such discordances could also show that results of each taxonomic group are complementary and that both taxonomic groups should be considered for a comprehensive biological assessment of sediments.

Given the limited number of sites assessed, the results of this study are preliminary, and more information should be gathered to confirm the suitability of these communities for specifically assessing the effects of trace metal elements, PAHs and PCBs in lake sediments. As in lake sediments, an effect of lack of oxygenation can mask those of hydrophobic pollutants (and inversely), future investigations should include lakes over a range of eutrophication conditions, from oligotrophic to oligo-mesotrophic (or mesotrophic) and sites outside pits where organic matter tends to accumulate (without a pit effect). In addition to physico-chemical and biological information, investigations on the exfiltration of groundwater at some sites of Lake Geneva (e.g., sites 21 and 36) could help explaining the presence of sensitive taxa at increased levels of contamination.

Improvement of the biological indices is hampered by the fact that only a fraction of the oligochaete and nematode specimens present in a sample can be identified to the species level (e.g. Vivien *et al.* 2017, Schenk *et al.* 2020, Vivien *et al.* 2023). The use of the genetic approach called “high-throughput DNA barcoding”, which allows to determine the exact percentage of each lineage (species) present in a sample, already developed for oligochaetes (Vivien *et al.* 2020c, Vivien *et al.* 2023), could allow to improve the precision of species classification according to degrees of resistance to the pollutants, and therefore the refinement of quality classes.

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**Table 1.** Details of the sampling: location, sampling date, coordinates (Swiss coordinate system (1903) and sampling depth.

| Site | Location | Sampling date | Coordinates | | Depth (m) |
|---|---|---|---|---|---|
| | | | x | y | |
| 32 | Buchillon | 26/10/2017 | 2521999 | 1146600 | 20-25 |
| 53 | Vidy Bay | 26/10/2017 | 2534721 | 1151336 | 42-44 |
| 78 | Grangettes | 26/10/2017 | 2558140 | 1139994 | 70 |
| 6 | Mies | 22/05/2018 | 2503999 | 1127985 | 54 |
| 21 | Yvoire | 22/05/2018 | 2514799 | 1137100 | 52 |
| 36 | Thonon | 22/05/2018 | 2524002 | 1135995 | 32 |

| | 6 | 21 | 32 | 36 | 53 | 78 | TEC | PEC |
|---|---|---|---|---|---|---|---|---|
| Median grain size | 7.73 | 7.98 | 14.95 | 19.07 | 34.82 | 11.12 | | |
| Organic matter | 8.95 ± 0.1 | 7.18 ± 0.3 | 5.27 ± 0.2 | 4.79 ± 0.04 | 13.65 ± 0.09 | 2.73 ± 0.3 | | |
| TOC | 4.59 | 2.93 | 2.08 | 2.67 | 6.96 | 0.68 | | |
| $CaCO_3$ | 44.3 ± 0.3 | 42.1 ± 0.8 | 36.6 ± 0.4 | 48.9 ± 0.4 | 19.7 ± 0.2 | 14.4 ± 0.3 | | |
| As | 3.22 ± 0.05 | 3.32 ± 0.08 | 2.73 ± 0.03 | 2.64 ± 0.12 | 6.80 ± 0.27 | 9.43 ± 0.18 | 9.79 | 33 |
| Pb | 24.2 ± 2.5 | 21.7 ± 3.1 | 16.5 ± 2.0 | 16.2 ± 1.4 | 123.3 ± 5.6 | 21.3 ± 2.8 | 35.8 | 128 |
| Cd | 0.44 ± 0.05 | 0.37 ± 0.07 | 0.28 ± 0.04 | 0.33 ± 0.04 | 1.54 ± 0.06 | 0.25 ± 0.03 | 0.99 | 4.98 |
| Cr | 38.6 ± 1.0 | 40.5 ± 0.9 | 35.6 ± 0.9 | 22.7 ± 0.9 | 75.8 ± 2.5 | 64.7 ± 1.54 | 43.4 | 111 |
| Cu | 44.7 ± 1.6 | 40.2 ± 0.6 | 31.7 ± 0.5 | 22.5 ± 0.8 | 280.5 ± 9.5 | 29.4 ± 0.5 | 31.6 | 149 |
| Ni | 57.6 ± 13.3 | 50.1 ± 1.34 | 37.4 ± 0.68 | 37.1 ± 4.5 | 42.5 ± 1.97 | 52.8 ± 0.92 | 22.7 | 48.6 |
| Hg | 0.193 ± 0.002 | 0.251 ± 0.005 | 0.206 ± 0.028 | 0.216 ± 0.014 | 1.468 ± 0.028 | 0.028 ± 0.0003 | 0.18 | 1.06 |
| Zn | 114.6 ± 3.6 | 99.9 ± 1.0 | 79.2 ± 0.8 | 60.9 ± 2.4 | 645.2 ± 68.3 | 99.7 ± 2.3 | 121 | 459 |
| Mn | 352.2 ± 4.2 | 327 ± 10.1 | 302.6 ± 5.6 | 283.1 ± 4.5 | 264.3 ± 4.7 | 551.8 ± 3.9 | | |
| Fe | 14593 ± 185 | 14316 ± 200 | 12730 ± 425 | 10720 ± 245 | 20475 ± 512 | 23371 ± 198 | | |
| Naphthalene | 12.9 | 9.6 | 18.5 | 112.4 | 425.6 | 19.0 | 176 | 561 |
| Acenaphthylene | 3.6 | 2.9 | 2.7 | 32.7 | 22.3 | 23.3 | | |
| Acenaphthene | 4.4 | 3.7 | 5.6 | 59.0 | 238.2 | 2.0 | | |
| Fluorene | 1.3 | 0.6 | 39.7 | 104.8 | 2848.1 | 146.5 | 77.4 | 536 |
| Phenanthrene | 79.4 | 69.7 | 107.4 | 853.4 | 2159.0 | 70.8 | 204 | 1170 |
| Anthracene | 15.2 | 13.0 | 24.5 | 861.3 | 467.3 | 17.3 | 57.2 | 845 |
| Fluoranthene | 196.9 | 162.3 | 325.6 | 1908.8 | 3441.4 | 93.8 | 423 | 2230 |
| Pyrene | 164.0 | 132.7 | 296.2 | 1584.2 | 3795.4 | 131.2 | 195 | 1520 |
| Benzo(a)anthracene | 77.2 | 60.2 | 156.4 | 1253.9 | 1851.8 | 39.4 | 108 | 1050 |
| Chrysene | 132.1 | 114.6 | 234.7 | 1691.1 | 2585.3 | 58.5 | 166 | 1290 |
| Benzo(b)fluoranthene | 205.4 | 174.8 | 340.6 | 1456.9 | 2522.5 | 55.5 | | |
| Benzo(k)fluoranthene | 80.8 | 61.7 | 137.1 | 765.7 | 893.5 | 18.1 | | |
| Benzo(a)pyrene | 126.0 | 106.1 | 212.8 | 1391.8 | 1720.9 | 43.3 | 150 | 1450 |
| Dibenzo(a,h)anthracene | 28.5 | 23.0 | 41.8 | 241.8 | 274.4 | 4.4 | | |
| Indeno(1,2,3-cd)pyrene | 120.4 | 101.7 | 163.7 | 657.0 | 1079.6 | 23.4 | | |
| Benzo(ghi)perylene | 124.2 | 102.8 | 155.0 | 606.4 | 936.8 | 27.8 | | |
| ΣPAHs | 1372.4 | 1139.4 | 2262.5 | 13581.1 | 25261.9 | 774.3 | 1610 | 22800 |
| PCB 28 | 0.39 | 0.40 | 0.25 | 0.22 | 37.00 | 0.05 | | |
| PCB 52 | 0.54 | 0.52 | 0.49 | 0.26 | 32.33 | 0.06 | | |
| PCB 101 | 1.54 | 1.41 | 1.29 | 0.64 | 59.50 | 0.15 | | |
| PCB 118 | 1.31 | 1.18 | 0.95 | 0.63 | 35.98 | 0.11 | | |
| PCB 138 | 2.81 | 2.68 | 2.09 | 1.40 | 90.33 | 0.23 | | |
| PCB 153 | 3.48 | 3.14 | 2.41 | 1.75 | 112.87 | 0.28 | | |
| PCB 170 | 0.81 | 0.79 | 0.59 | 0.33 | 32.91 | 0.07 | | |
| PCB 180 | 1.77 | 1.65 | 1.16 | 0.92 | 54.52 | 0.12 | | |
| ΣPCBs | 12.66 | 11.77 | 9.23 | 6.15 | 455.43 | 1.06 | 59.8 | 676 |
| Mean PEC-$Q_{met}$ | 0.33 | 0.31 | 0.24 | 0.22 | 0.96 | 0.33 | | |
| Mean Consensus2-Q | 0.26 | 0.23 | 0.25 | 0.90 | 4.41 | 0.17 | | |

**Table 2. (left page)**

Median grain size (µm), percentages of organic matter, total organic carbon (TOC) and CaCO3 and contaminant concentrations (trace metal elements in mg/kg d.w., PAHs and PCBs in µg/kg d.w.) and values of the PEC-Qmet and Consensus2-Q at the six sampling sites of Lake Geneva. Toxic potential assessed using the threshold effect concentrations (TEC) and probable effect concentrations (PEC) proposed by MacDonald *et al.* (2000). In green: values below the TEC; in orange: values above the TEC but below the PEC; in red: values above the PEC.

**Table 3.** Oligochaete results at the six studied sites: percentages of sensitive taxa (ecological group 1), biological quality, oligochaete densities per 0.1 m$^2$, IOBL index values, metabolic potential and percentages of taxa of ecological groups 2-4.

| | Site 6 | Site 21 | Site 32 | Site 36 | Site 53 | Site 78 |
|---|---|---|---|---|---|---|
| % of sensitive taxa to pollution (group 1) | 15 | 38 | 13 | 34 | 0 | 23 |
| Biological quality | Medium | Good | Medium | Good | Bad | Good |
| Oligochaete density | 381 | 107 | 147 | 347 | 45778 | 519 |
| IOBL index | 20.7 | 15.1 | 17.5 | 21.6 | 20.0 | 22.1 |
| Metabolic potential | Very strong | Very strong | Very strong | Very strong | Very strong | Very strong |
| % of moderately sensitive taxa to pollution (group 2) | 9 | 8 | 6 | 8 | 1 | 32 |
| % of taxa indicative of natural dystrophy (group 3) | 3 | 0 | 12 | 6 | 6 | 3 |
| % of tolerant taxa to pollution (group 4) | 11 | 11 | 5 | 9 | 23 | 6 |

**Table 4.** Nematode results at the six studied sites: densities of nematodes per 10 cm$^2$, numbers of species/taxa (S) found in all replicates per site (assessed individuals: n = 166 - 206), percentages of feeding types (BF = bacterial feeders; AF = algae feeders; SF = suction feeders; Pre & Om = predators and omnivores) and NemaSPEAR[%] index values (mean ± SD, n = 4). Colours represent the biological quality: blue = very good; green = good; orange = poor.

| | Site 6 | Site 21 | Site 32 | Site 36 | Site 53 | Site 78 |
|---|---|---|---|---|---|---|
| Density | 45 ± 19 | 269 ± 146 | 545 ± 86 | 353 ± 97 | 24 ± 14 | 1004 ± 197 |
| S | 19 | 19 | 23 | 21 | 25 | 22 |
| BF | 85 ± 12 | 66 ± 15 | 74 ± 9 | 55 ± 19 | 92 ± 4 | 71 ± 8 |
| AF | 1 ± 2 | 1 ± 2 | 9 ± 5 | 13 ± 3 | 1 ± 1 | 19 ± 8 |
| SF | 2 ± 1 | 8 ± 9 | 3 ± 2 | 9 ± 5 | 1 ± 1 | 6 ± 2 |
| Pre & Om | 13 ± 12 | 25 ± 8 | 15 ± 4 | 24 ± 12 | 7 ± 5 | 5 ± 2 |
| NemaSPEAR[%] | 43.9 ± 17.9 | 61.1 ± 2.2 | 78.1 ± 1.0 | 66.6 ± 7.1 | 14.0 ± 10.5 | 57.3 ± 4.7 |

# Appendix

**Table S1.** Number of specimens per oligochaete taxon at the six studied sites and classification as sensitive to pollution (ecological group 1) or not for each taxon.

| Taxon | | Sensitive | Sites | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 6 | 21 | 32 | 36 | 53 | 78 |
| **Tubificinae** | Tubificinae with hair setae (unidentifiable) | no | 36 | 6 | 18 | 18 | 50 | 17 |
| | *Tubifex tubifex* | no | 2 | | | 4 | 6 | 1 |
| | *Aulodrilus pluriseta* | no | 1 | | 12 | 2 | | 2 |
| | *Psammoryctides barbatus* | yes | 10 | | | 2 | | 16 |
| | *Embolocephalus velutinus* | yes | | 24 | | 18 | | |
| | *Spirosperma ferox* | no | 6 | 4 | | 6 | | |
| | *Potamothrix heuscheri* | no | 3 | | 1 | | | 1 |
| | *Potamothrix hammoniensis* | no | 3 | | | | | 1 |
| | *Potamothrix vejdovskyi* | no | | 1 | 5 | 2 | | 29 |
| | *Quistadrilus multisetosus* | no | | | | | 13 | |
| | Tubificinae without hair setae (unidentifiable) | no | 29 | 25 | 39 | 28 | 22 | 18 |
| | *Limnodrilus hoffmeisteri* | no | 4 | 3 | 2 | 1 | 11 | 2 |
| | *Limnodrilus claparedianus* | no | 1 | | | 2 | | 1 |
| | *Limnodrilus profundicola* | no | 3 | 1 | 1 | | 1 | 2 |
| | *Aulodrilus limnobius* | no | | | 6 | | | |
| | *Potamothrix moldaviensis* | no | | 5 | | 6 | | |
| **Lumbriculidae** | Lumbriculidae (unidentifiable) | yes | 4 | 3 | | 10 | | |
| | *Stylodrilus lemani* | yes | 1 | | | | | |
| | *Lumbriculus variegatus* | no | | | | | | 1 |
| **Naidinae** | *Piguetiella blanci* | yes | | | 3 | 2 | | 1 |
| | *Vejdovskiella intermedia* | yes | | | | | | 6 |
| | *Stylaria lacustris* | yes | | | 10 | 2 | | |
| | *Dero digitata* | no | | | 2 | | | |
| **Total analysed specimens** | | | **103** | **72** | **99** | **103** | **103** | **98** |

**Table S2. (right page)** Number of specimens per nematode taxon (mean of the 4 replicates) at the six studied sites, with indication of the feeding type (FT) and sensitivity to pollution (NemaSPEAR) of each taxon; BF = bacterial or deposit feeder, SF = suction feeder, AF = algae or epistrate feeder, Pre/Om = predators and omnivores or chewers.

| Taxon | FT | Nema SPEAR | Sites | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 6 | 21 | 32 | 36 | 53 | 78 |
| *Aphanolaimus aquaticus* | BF | yes | 3.0 | 1.0 | 1.0 | 2.0 | 0.0 | 0.5 |
| *Aporcelaimus superbus* | SF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Brevitobrilus stefanskii* | Pre/Om | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.5 |
| *Bursilla monhystera* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Cephalobus persegnis* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Chromadorina berczki* | AF | no | 0.0 | 0.0 | 2.5 | 5.9 | 0.6 | 18.3 |
| *Chromadorina bioculata* | AF | no | 0.5 | 0.5 | 0.5 | 0.0 | 0.0 | 0.5 |
| *Chromadorita leuckarti* | AF | no | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Daptonema dubium* | BF | no | 52.7 | 10.7 | 14.9 | 15.3 | 0.0 | 3.0 |
| *Daptonema sp.* | BF | no | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Diplogaster rivalis* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 4.8 | 0.5 |
| *Diplogasteritus nudicapitatus* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 51.2 | 0.0 |
| *Diploscapter coronatus* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 7.2 | 0.0 |
| *Dorylaimus stagnalis* | SF | no | 0.5 | 7.8 | 2.0 | 8.9 | 0.0 | 4.5 |
| *Epitobrilus medius* | Pre/Om | yes | 6.0 | 17.5 | 5.0 | 9.9 | 0.6 | 1.0 |
| *Ethmolaimus pratensis* | AF | yes | 0.0 | 0.5 | 2.0 | 1.0 | 0.0 | 0.0 |
| *Eumonhystera andrassyi* | BF | yes | 3.0 | 14.1 | 4.5 | 2.0 | 2.4 | 20.3 |
| *Eumonhystera barbata* | BF | yes | 2.5 | 3.4 | 4.0 | 4.4 | 0.0 | 1.0 |
| *Eumonhystera filiformis* | BF | no | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Eumonhystera longicaudatula* | BF | yes | 0.0 | 7.3 | 2.0 | 3.0 | 0.0 | 4.0 |
| *Eumonhystera pseudobulbosa* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 | 2.5 |
| *Eumonhystera simplex* | BF | yes | 0.0 | 1.5 | 0.0 | 0.5 | 0.0 | 1.5 |
| *Eumonhystera vulgaris* | BF | yes | 2.5 | 1.9 | 13.4 | 6.4 | 0.6 | 5.0 |
| *Helicotylenchus spec.* | SF | no | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 |
| *Hemicycliophora typica* | SF | yes | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Hofmaenneria brachystoma* | BF | yes | 5.0 | 4.9 | 3.0 | 2.5 | 0.0 | 7.4 |
| *Ironus tenuicaudatus* | Pre/Om | yes | 4.0 | 7.3 | 8.5 | 11.3 | 0.0 | 1.5 |
| *Mermithidae gen. sp.* | Pre/Om | no | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 |
| *Mesodorylaimus sp. 1* | SF | no | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Mesodorylaimus sp. 2* | SF | no | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Monhystera paludicola* | BF | no | 8.5 | 4.4 | 0.5 | 1.5 | 1.2 | 7.9 |
| *Monhystera stagnalis* | BF | no | 4.5 | 2.4 | 0.5 | 0.5 | 0.0 | 0.0 |
| *Monhystrella macrura* | BF | no | 0.0 | 13.1 | 0.0 | 2.5 | 0.0 | 0.0 |
| *Monhystrella paramacrura* | BF | yes | 0.0 | 1.0 | 9.5 | 8.9 | 0.0 | 16.8 |
| *Mononchus aquaticus* | Pre/Om | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Neotobrilus longus* | Pre/Om | yes | 0.0 | 0.0 | 0.0 | 0.0 | 1.8 | 0.0 |
| *Panagrolaimus rigidus* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 3.6 | 0.0 |
| *Paraphanolaimus anisitsi* | BF | yes | 0.0 | 0.0 | 5.0 | 0.5 | 0.0 | 0.0 |
| *Paraplectonema pedunculatum* | BF | yes | 0.0 | 0.0 | 15.9 | 4.9 | 0.6 | 1.0 |
| *Pelodera punctata* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 6.6 | 0.0 |
| *Pelodera strongyloides* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 6.6 | 0.0 |
| *Pelodera teres* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Plectus aquatilis* | BF | yes | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | 0.0 |
| *Plectus opisthocirculus* | BF | yes | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Prismatolaimus intermedius* | AF | yes | 0.0 | 0.5 | 3.0 | 5.9 | 0.0 | 0.0 |
| *Prodesmodora circulata* | AF | yes | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 |
| *Rhabditis sp.* | BF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| *Semitobrilus pellucidus* | Pre/Om | yes | 2.5 | 0.5 | 1.0 | 0.0 | 0.0 | 0.0 |
| *Tobrilus gracilis* | Pre/Om | no | 0.0 | 0.0 | 0.0 | 2.5 | 3.0 | 1.5 |
| *Tripyla glomerans* | Pre/Om | no | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Tylencholaimellus spec.* | SF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 |
| *Tylenchus sp.* | SF | no | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 |
| **Total analysed specimens** | | | **201** | **206** | **201** | **203** | **166** | **202** |