Effect Thresholds of Metals in Stream Sediments Based on In Situ Oligochaete Communities

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Abstract: Aquatic oligochaetes, comprising a large number of species showing various degrees of resistance to chemical pollution, are recognized as valuable bioindicators of sediments’ quality. In the Geneva area (Switzerland), oligochaete tools were previously tested for assessing the biological quality of stream sediments, and effect thresholds of combined metals (quotients) in sediments were defined. The aims of the present study were to update this previous work with new data acquired in different cantons of Switzerland and to establish effect thresholds on oligochaete communities for individual metals and for combined metals. The oligochaete metrics “Oligochaete index of sediment bioindication (IOBS)”, “oligochaete density” and “percentage of tubificids without hair setae” proved pertinent for assessing the effects of metals and organic matter in sediments. We established a threshold effect level (TEL oligo) and probable effect level (PEL oligo) for eight metals in sediments (Cr, Ni, Zn, Cu, Pb, Cd, Hg and As) as well as a probable effect level for these metals combined (mPEL oligo-Q). These thresholds could be used directly to screen for alteration of in situ communities restricted to sediments and/or for establishing sediment quality standards based on a combination of different biological and ecotoxicological tools.

Keywords: bioindication; oligochaetes; streams; fine/sandy sediment; metals; effect thresholds

1. Introduction

Sediments are an essential component of river and lake ecosystems. Indeed, they play an important role for many species as habitat or nesting site, thus representing a significant environment of biological diversity [1]. Sediments also perform major ecological functions, most of which are essential for the good functioning of biogeochemical cycles. Sediments also act as natural receptors for certain contaminants. Metallic trace elements, polycyclic aromatic hydrocarbons (PAHs), chlorinated polybiphenyls (PCBs), or organochlorine (OC) pesticides that are only found in trace concentrations in water, are examples of toxic and hydrophobic substances that tend to accumulate in the sedimentary phase [2]. These substances can reach concentrations sufficient to induce adverse effects on benthic organisms and thus disrupt the proper functioning of the ecosystem [1,3]. These effects can occur not only locally, but also at the hydrosystem level via sediment transport and release of contaminants. For example, contaminated sediments may be remobilized during high-water events [4]. Therefore, the lack of consideration of the sedimentary compartment as part of monitoring programs can lead to too-optimistic conclusions about the quality of streams [5]. It is crucial to assess the quality of this compartment to identify whether it can contribute to the degradation of an ecosystem. A complete evaluation of this compartment requires combining ecotoxicological tests, in situ communities’ assessment and chemical analyses (Chapman’s triad, [6]). Ecotoxicological tests and biological indices inform on the effects of contaminants present in sediments. Chemical analyses
inform on the presence or absence of measured chemical stressors. For appropriate sediment contamination assessment, chemically based sediment quality guidelines (SQG) are used that inform on the potential effects associated with the presence of target chemicals [7,8].

The most widely used set of freshwater sediment quality guidelines are the threshold and probable effect concentrations (TEC and PEC, respectively) derived by MacDonald et al. [7]. Although the TEC and PEC were determined as the geometric mean of multiple SQG, they rely heavily on the results of toxicity tests performed with single benthic invertebrates exposed in the laboratory to field-collected sediments (e.g., [9]). de Deckere et al. [8] also derived consensus-based SQG using the results of laboratory toxicity tests with single benthic species (the amphipod *Hyalella azteca*) and in situ community composition of all macroinvertebrates (identified to the genus, family or group level). de Deckere et al. [8] emphasized that these SQG included only information on effects on a part of the whole macroinvertebrates (only a fraction of all macroinvertebrates live on or in fine sediments), excluding many sensitive taxa when deriving the thresholds. de Deckere et al. [8] pointed out that the study of nematode communities, specifically used to assess the biological quality of fine sediments [1], could be well suited for deriving effect thresholds in sediments. Oligochaetes are also adapted for in situ sediment quality assessment. Indeed, they are restricted to this compartment, display low mobility and their trophic mode is primarily collector, based on the ingestion of fine sediments. In addition, the group includes a large number of species presenting a wide range of pollution sensitivity [10], and oligochaetes are generally abundant in sediments [11]. Different biological indices based on the study of the structure of oligochaete communities have been proposed for the assessment of the biological quality of stream and lake sediments. The oligochaete index of sediment bioindication (IOBS), developed and standardized in France, allows to assess the quality of fine/sandy sediments in streams [12]. In addition, according to the IOBS guideline, the analysis of percentage of tubificids without hair setae and oligochaete density provides supplementary ecological information specific to the effects of micropollutants and organic matter.

Among the large panel of contaminants that can impact aquatic ecosystems, metals are considered important because they are still largely released by human activities (multiple sources) and are persistent [3,13]. Metals are often monitored in the sediment compartment, and it is therefore essential to define suitable thresholds of metal effects for helping in sediment quality assessment and management [11]. To this end, oligochaete metrics were specifically tested for defining effect thresholds for metals in Switzerland by Vivien et al. [11]. These authors analyzed, in many sediment samples collected in streams of the Geneva area, oligochaete community structure and concentrations of metals. They defined effect thresholds based on metal contamination indices calculated using the TEC and PEC values proposed by MacDonald et al. [7]. These thresholds of metal contamination agreed with those established using data of a similar study conducted in another ecoregion (Artois-Picardie in France) [11].

In the present work, we studied the relationships between the different oligochaete metrics and concentrations of metals and organic matter in sediments collected in different cantons of Switzerland. The aim is two-fold: (1) to continue testing the different oligochaete metrics for assessing sediment quality in relation to metals and organic matter, and (2) to establish in situ effect thresholds per metal (threshold effect level (TEL), probable effect level (PEL)) and for all metals combined (mPEL-Q) according to existing guidance for deriving environmental quality standards [14].

2. Material and Methods

2.1. Study Area

The database used in this study includes 76 data entries from the Geneva area gathered from 2008 to 2012 [11], and 40 new data entries acquired in different cantons of Switzerland from 2013 to 2018. Of these 40 new data entries, 14 are from the canton of Geneva, 17 from the canton of Vaud, 7 from the canton of Lucerne, 1 from the canton of Bern and 1 from the canton of Valais (Supplementary Table S1). The 116 data entries correspond to 66 different stream sites. Twenty-nine sites were studied twice during the same year, in winter and summer (16 sites), in spring and summer (3 sites), in spring
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and autumn (6 sites) and in winter and autumn (4 sites). Of these 29 sites, most were studied in March and September/October. As both concentrations of pollutants in sediments and oligochaete community structure may greatly vary over time (during a year and between different years) (cf. § 3.1), it was pertinent to take into account the samples from the same sites for the comparison of chemical and biological data. The sites cover a significant gradient of anthropogenic pressures, including sources and sites in agricultural, industrial and urban areas.

2.2. Sampling and Examination of Oligochaete Communities

We followed the procedures described in the IOBS standard [12]. Sediments were collected using a Surber-type net (0.2 mm mesh size) at a water depth of 30–50 cm. At each site, 3 subsamples (one every 10–20 m) were collected, combined and fixed with 37% formalin (ThermoFisher Scientific, Ecublens, Switzerland) adjusted to a final formaldehyde concentration of 4%. Once in the laboratory, sediment samples were sieved through a column of sieves with 5 and 0.5 mm mesh size. The material retained on the 0.5 mm mesh size was transferred to a subsampling square box (5 × 5 cells). The content of randomly selected cells was transferred into a petri dish and examined under a binocular loupe. Oligochaetes were extracted until obtaining 100 identifiable specimens. Oligochaetes were mounted on slides in a coating solution and identified to the lowest practical level, species if possible, using a compound microscope.

The IOBS index was calculated for each sample according to the following formula [12]:

\[
IOBS = 10ST^{-1}
\]

where S is the total number of taxa identified among 100 oligochaete specimens examined and T is the percentage of dominant tubificids (including the subfamilies Tubificinae, Rhyacodrilinae and Phalldodrilinae) with or without hair setae, for all mature and immature worms combined. The index ranks the biological quality of sediments as follows: IOBS ≥ 6: very good, 5.9–3: good, 2.9–2: medium, 1.9–1: poor, <1: bad. We used additional interpretation criteria proposed by AFNOR [12] and Prygiel et al. [15]. Pollution by metals/PCBs is suspected when tubificids without hair setae dominate. Conversely, dominance of tubificids with hair setae indicate a pollution by organic matter. A high density of oligochaetes (>3000 individuals/0.1 m²) associated with a low IOBS value indicates excessive organic matter inputs. Some species of tubificids with hair setae, in particular Aulodrilus pluriseta and Tubifex tubifex, were described as highly resistant to metals [16–18]. Vivien et al. [11] showed that the sum of the percentages of tubificids without hair setae and Aulodrilus pluriseta (species identifiable in an immature state) were more highly correlated with an index of metal contamination than only the percentage of tubificids without hair setae.

The IOBS index is based on the general observation of the decrease of species richness and increase of abundance of resistant species when the pollution level increases. Pollution-intolerant and tolerant tubificid species with and without hair setae (mainly Tubificinae, less Rhyacodrilinae and Phalldodrilinae) are typical dwellers of fine and sandy organically enriched sediments [19]. In non-contaminated sediments, some species belonging to the families/subfamilies Enchytraeidae, Lumbriculidae, Naidinae, Pristininae, Propapidae and Haplotaxidae, including mainly sensitive species, are also significantly abundant. In very contaminated sediments, pollution-tolerant tubificid species represent more than 80% of the oligochaete communities. Conversely, in pristine sediments (i.e., in sources), resistant tubificid species are absent or in a very low abundance. The formula of IOBS takes into account all these observations. The study of more than 200 unpolluted and polluted sites [11,15,18,19] have shown that IOBS values ≥ 3 were systematically obtained at sites with no or low level of contamination, e.g., in sources and upstream of agricultural, industrial and urban areas. An IOBS value of 3 represents a limit below which adverse effects are observed. IOBS values less than 1 (low diversity of taxa and marked dominance of tubificids) describe severe adverse effects.

2.3. Chemical Analyses

Samples of sediment for chemical analyses were collected at the same time of the sampling for examination of oligochaete communities and on the same locations (same patches of sediment). The
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The top 10 cm of sediments were collected with a shovel and sieved in the field through a sieve of 2 mm. Once in the laboratory, sediment samples were directly pretreated or stored at −20 °C before pretreatment. The pretreatment consisted of drying the sediment samples in a heat chamber at 48 °C for at least 3 days. Once dry, they were ground for the subsequent analyses. Organic matter was estimated as the loss on ignition. Hg was measured by atomic absorption spectrophotometry with the advanced mercury analyser, AMA 254. For measurement of the metals Cr, Zn, Ni, Pb, Cu, Cd and As, about 1 g of each sediment sample was dissolved in 2M nitric acid overnight at 100 °C. It is assumed that this method allows for the extraction of the bioavailable fraction of metals. Metal concentrations were determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometer). Comparative samples of two reference sediments were processed and analysed simultaneously, and agreement was considered +/−20% of certified concentrations.

2.4. Derivation of Thresholds

2.4.1. Effect Thresholds Per Metal

The threshold effect level (TEL) and probable effect level (PEL) were estimated for Cr, Zn, Ni, Pb, Cu, Cd, As and Hg according to the incidence of biological impact on the oligochaetes community, as measured by the IOBS index. The TEL_{oligo} was defined as the concentration below which the percentage of samples with a biological impact on the oligochaetes community (incidence of effect) was ≤10% but as close to 10% as possible. The PEL_{oligo} was defined as the concentration above which the incidence of effect was ≥90% but as close to 90% as possible. This is in agreement with the definition of TEL as the concentration below which biological effects are unlikely to occur and PEL as the concentration above which biological effects are likely to occur [14]. A site was considered affected (adverse biological effects) when the IOBS quality class was bad, poor or medium (IOBS < 3). Conversely, a good or very good IOBS quality (IOBS ≥ 3) was considered unaffected (absence of adverse biological effects, = no effect). Entries associated with adverse effects were considered relevant for a given metal only if the concentration of the metal was at least 2-fold above the background [14,20]. For calculation of the TEL_{oligo} the entries showing both an IOBS result < 3 and a concentration of metal < 2-fold the background concentration were therefore not considered. In the absence of background concentrations for metals in sediments (<2 mm) in Switzerland, they were estimated as the 10th percentile of the metal concentrations in our database [14]. If more than one entry was available per site, only the sample showing the lowest level of metal contamination globally was considered for the estimation of the natural background. Sites with elevated metal concentrations due to known point source discharges were also eliminated from the database for the estimation of background concentrations [14], which finally included 63 entries. Background concentrations could not be determined by calculating the average concentration (of a given metal) in unpolluted sites because only 4–6 sites of our dataset could be identified as completely unimpacted by human activities.

2.4.2. Metal Contamination Index and Effect Threshold for all Metals Combined

We calculated a metal contamination index based on the combination of concentrations of Cr, Zn, Ni, Pb, Cu, Cd, Hg and As. This index (mPEL_{oligo-Q}) was expressed as the sum of the ratios between the concentration of each metal (M^z) and its corresponding PEL_{oligo i} determined in the present study, divided by the number of metals considered (N):

\[
mPEL_{oligo} - Q = \frac{\sum_{i} [M_{i}^{z+}]_{i}}{\frac{PEL_{oligo i}}{N}}
\]

A threshold based on the mPEL_{oligo-Q}, above which effects of all metals combined on oligochaete communities were likely to occur, was established. This threshold was calculated according to the following criteria: above the threshold value of mPEL_{oligo-Q}, the percentage of samples with a
biological effect was ≥90%, but as close to 90% as possible. The derivation of a mTEL\textsubscript{oligo-Q} threshold, below which effects of all metals combined on oligochaete communities were unlikely to occur, could not be derived because of the exclusion of entries with both a biological effect (IOBS < 3) and a metal concentration < 2-fold the estimated background concentration of the metal (see above). Because of this exclusion of entries, the number of metals per site that could be used for the calculation of a mTEL-Q would be variable (from 0 to 8).

2.5. Study of Relationships between the mTEL\textsubscript{oligo-Q} Percentage of Organic Matter and Oligochaete Metrics

Linear regressions and Pearson’s test were applied to study the relationships between the chemical and biological variables. We assumed that all observations were independent. The coefficient of determination, R\textsuperscript{2}, of each relationship and the Pearson test were calculated using the Free Statistics and Forecasting Software [21]. Prior to the statistical analysis, a log-linearization was applied to all relationships as log(log(Yn)) = α + βlog(Xn) + ε for the relationships mTEL\textsubscript{oligo-Q} – IOBS, %organic matter – IOBS and metal-specific concentrations – IOBS; log(Yn) = α + βlog(Xn) + ε for the relationships mTEL\textsubscript{oligo-Q} – % of tubificids without hair setae, mTEL\textsubscript{oligo-Q} – % of tubificids without hair setae + A. pluriseta and %organic matter – oligochaete density. The total number of samples was 116 (n = 116) for the relationships mTEL\textsubscript{oligo-Q} – IOBS, metal-specific concentrations – IOBS, mTEL\textsubscript{oligo-Q} – % of tubificids without hair setae and mTEL\textsubscript{oligo-Q} – % of tubificids without hair setae + A. pluriseta, 115 for the relationship %organic matter – IOBS and 106 for the relationship %organic matter – oligochaete density.

3. Results

3.1. Description of the Database

Metal concentrations and organic matter content were very variable among sites, with metal concentrations ranging over several orders of magnitude (Table 1). Overall, metal concentrations were higher in sediments collected in summer/autumn than in winter/spring (Supplementary Table S2). For all metals, except As, and for organic matter, the mean and median values obtained based on all data were higher than those obtained based on the 63 data used for calculation of background concentrations. This is mainly explained by the exclusion in the restricted dataset of samples whose high level of contamination was due to known point source discharges. The background concentration of each metal is indicated in Table 1.

| Table 1. Minimal and maximal value, mean and median of each metal (µg/g dry weight) and organic matter (OM) content (%) in the whole database and the database used for the estimation of background concentrations.背景浓度（第10百分位）金属。 |
|---------|---------|---------|---------|---------|---------|---------|---------|
| Cr      | Ni      | Zn      | Cu      | Pb      | Cd      | Hg      | As      |
| Min     | 7.7     | 7.0     | 13.9    | 1.4     | 3.1     | 0.04    | 0.004   | 1.2     | 0.4     |
| Max     | 117.1   | 111.1   | 1390.6  | 229.1   | 100.2   | 1.41    | 0.766   | 14.6    | 30.8    |
| Mean    | 34.2    | 28.4    | 108.9   | 35.1    | 20.8    | 0.23    | 0.054   | 3.5     | 5.1     |
| Median  | 27.7    | 22.3    | 56.1    | 16.2    | 13.8    | 0.17    | 0.025   | 2.7     | 2.6     |
| Whole database (N = 116) |

| Cr      | Ni      | Zn      | Cu      | Pb      | Cd      | Hg      | As      |
|---------|---------|---------|---------|---------|---------|---------|---------|
| Min     | 7.7     | 7.6     | 15.8    | 1.6     | 3.4     | 0.05    | 0.004   | 1.4     | 0.7     |
| Max     | 98.5    | 94.6    | 345.0   | 181.0   | 100.2   | 1.41    | 0.766   | 14.6    | 17.4    |
| Mean    | 27.6    | 23.7    | 66.5    | 24.6    | 18.3    | 0.21    | 0.045   | 3.8     | 3.8     |
| Median  | 23.1    | 19.8    | 50.0    | 13.9    | 10.4    | 0.15    | 0.022   | 2.9     | 2.5     |
| Background concentration | 15.1 | 11.4 | 20.2 | 5.12 | 5.2 | 0.09 | 0.009 | 1.6 |

All classes of biological quality were well represented (Supplementary Table S3). From the 116 samples, 36 had a good/very good quality (31%), 14 a medium quality (12%), 48 a poor quality (41%)
and 18 a bad quality (16%). The biological quality of sediments was very good or good at sites with no or very low impact from human activities (located upstream of agricultural, urban and industrial areas) and quasi exclusively medium, poor or bad at sites located in agricultural, urban and industrial areas. From the 29 sites studied in winter/spring and summer/autumn of the same year, the biological class obtained in summer/autumn was lower than in winter/spring at 18 sites, higher at 2 sites and unchanged at 9 sites. The degradation of the biological quality at these 18 sites could be related to the degree of intensity of agricultural activities (more intense in summer). The proportion of tubificids with and without hair setae varied from 0% to 91%–92%. The percentage of tubificids without hair setae and with hair setae clearly dominated (≥60%) in 33 and 24 samples, respectively. Oligochaete densities were very variable (range = 19–40,833 individuals per 0.1 m²) and high values (>3000) were observed in 34 out of 107 samples (density of 9 samples was not calculated).

3.2. Relationships between Metal Concentrations and IOBS

When we considered all samples, the correlations between the metal concentrations and IOBS index were highly significant for all metals except As (Figure 1, Table 2). The correlations were greatly improved (the correlation between As and IOBS became highly significant) when we excluded the entries showing both a biological effect (IOBS < 3) and a concentration of metal < 2-fold the estimated background concentration (Figure 1, Table 2).

Table 2. p and R² values of the relationships between the metal concentrations and Oligochaete index of sediment bioindication (IOBS) with all samples (n = 116) and without the samples showing both a biological effect (IOBS < 3) and a concentration of metal < 2-fold the background concentration (Selected samples; n = 68–100 depending on the metal).

|       | Cr | Ni | Zn | Cu | Pb  | Cd  | Hg  | As  |
|-------|----|----|----|----|-----|-----|-----|-----|
| All samples (n = 116) | R²  | 0.148 | 0.199 | 0.225 | 0.268 | 0.214 | 0.168 | 0.185 | 0.0144 |
| p     | 2 × 10⁻⁵ | 5.4 × 10⁻² | 7.7 × 10⁻⁴ | 2.6 × 10⁻⁸ | 1.7 × 10⁻² | 4.9 × 10⁻⁸ | 1.45 × 10⁻⁶ | NS |
| Selected samples | R²  | 0.476 | 0.493 | 0.455 | 0.499 | 0.517 | 0.483 | 0.434 | 0.366 |
| p     | 7.4 × 10⁻¹² | 2.7 × 10⁻¹³ | 1.7 × 10⁻¹³ | 2.2 × 10⁻¹⁶ | 1 × 10⁻¹⁵ | 3.2 × 10⁻¹³ | 6.9 × 10⁻¹⁵ | 4.7 × 10⁻⁸ |
| n     | 75 | 81 | 92 | 100 | 92 | 84 | 93 | 68 |

NS = not significant.
3.3. Derivation of the TEL<sub>oligo</sub> and PEL<sub>oligo</sub> Thresholds

The TEL<sub>oligo</sub> and PEL<sub>oligo</sub> obtained in the present study are presented in Table 3. The proportion of samples with a biological effect at concentrations < TEL<sub>oligo</sub> were 10% for Cr and Ni, 8% for Zn, 9% for Cu, Hg and As, 8% for Pb and 4% for Cd. The proportion of samples with a biological effect at concentrations > PEL<sub>oligo</sub> was 95% for Cr, 90% for Ni, 92% for Zn and Cu and 91% for Pb, Cd, Hg and As. For each metal, we observed a significant difference between the values corresponding to the TEL<sub>oligo</sub> and PEL<sub>oligo</sub>.

Table 3. TEL<sub>oligo</sub> and PEL<sub>oligo</sub> thresholds determined for the metals Cr, Ni, Zn, Cu, Pb, Cd, Hg and As (µg/g dry weight).

|       | Cr | Ni | Zn | Cu | Pb | Cd | Hg | As |
|-------|----|----|----|----|----|----|----|----|
| TEL<sub>oligo</sub> | 35 | 24 | 46.9 | 10.5 | 12.3 | 0.18 | 0.0218 | 3.6 |
| PEL<sub>oligo</sub> | 45.9 | 34.1 | 88.1 | 31 | 23.9 | 0.23 | 0.054 | 5.7 |

3.4. Relationships between the mPEL<sub>oligo</sub>-Q and Percentage of Organic Matter and Oligochaete Metrics

The correlation between the mPEL<sub>oligo</sub>-Q and IOBS index was negative and highly significant ($R^2 = 0.274; p = 1.66 \times 10^{-5}; n = 116$) (Figure 2). The correlations between the mPEL<sub>oligo</sub>-Q = % of tubificids without hair setae was positive and highly significant ($R^2 = 0.126; p = 9.53 \times 10^{-5}; n = 116$). These results confirm that the tubificids without hair setae tend to proliferate in detriment to tubificids with hair setae and the other families/subfamilies in response to metal contamination. The correlations between the mPEL<sub>oligo</sub>-Q and the total of percentages of tubificids without hair setae and Aulodrilus pluriseta and between the mPEL<sub>oligo</sub>-Q and only the percentage of tubificids without hair setae were similar.
The correlation between the % of organic matter in sediment and the IOBS index was negative and highly significant ($R^2 = 0.181; p = 2.21 \times 10^{-6}; n = 115$). The correlation between the % of organic matter in sediment and the oligochaete density was positive and highly significant ($R^2 = 0.23; p = 1.95 \times 10^{-7}; n = 106$).

**Figure 2.** Relationships between the metal contamination mPEL_{oligo-Q} index and the Oligochaete index of sediment bioindication (IOBS). Dotted horizontal line is set at the threshold for good biological quality (IOBS = 3). The vertical line is set at the mPEL_{Q_{oligo}} = 0.92.

3.5. Determination of the mPEL_{oligo-Q} Threshold

The mPEL_{oligo-Q} threshold was fixed at a value of 0.92 (Figure 2). Above this value, 92% of the samples presented an altered biological quality. In addition, 92% of the samples presenting a good and very good biological quality had a mPEL_{oligo-Q} ≤ 0.92.

3.6. Comparison between the IOBS Results and Degrees of Metal Contamination of Sediments

In total, 33 samples showed values of IOBS ≥ 3 (no effect) and mPEL_{oligo-Q} ≤ 0.92 (case 1 in Table 4), while 33 samples showed values of IOBS < 3 (with adverse biological effect) for corresponding mPEL_{oligo-Q} > 0.92 (case 2 in Table 4). The IOBS and the metal contamination index mPEL_{oligo-Q} agree in the outcome of the evaluation in these samples. In case 1, most samples had one or no metal with concentration > TEL_{oligo} and less than four metals with concentrations ≥ TEL_{oligo}, but eight samples had four or more metals with concentrations ≥ TEL_{oligo} and/or two or more metals with concentrations > PEL_{oligo}. A reduced metal bioavailability could explain the good or very good biological quality in these eight samples.

Forty-seven samples showed an unacceptable biological quality (IOBS < 3) and mPEL_{oligo-Q} ≤ 0.92 (case 3 in Table 4). While we observed 20 samples at least with concentrations of four metals ≥ TEL_{oligo} and/or of two metals > PEL_{oligo}, 27 samples had less than four metals with concentrations ≥ TEL_{oligo} and two metals with concentrations > PEL_{oligo}. Some of these samples had no metal concentration ≥ TEL_{oligo}. In these 27 samples (identified with an asterisk in Supplementary Table S2), a high bioavailability and/or the presence of other pollutants could explain the degraded biological quality. A contribution of other pollutants to the adverse effects cannot be excluded and is even probable because these 27 samples are all located in areas impacted by human activities (agriculture in particular).

Three samples showed a good or very good biological quality and mPEL_{oligo-Q} > 0.92 (case 4 in Table 4). They all had at least concentrations of four metals ≥ TEL_{oligo} and/or two metals > PEL_{oligo}. While the mPEL_{oligo-Q} value of one sample (Hochdorf downstream (Ron) in March 2016) was close to the threshold of 0.92, the threshold was largely exceeded in the two other samples (amont busage (Lissole) in March 2010 and amont busage (Moulin-de-la-Ratte) in May 2012) (Supplementary Table S2).
S2). High concentrations of binding elements (Mn, Fe and organic matter) in these samples suggest reduced bioavailability of the metals to oligochaete communities.

### Table 4. Comparison between the Oligochaete index of sediment bioindication (IOBS) values and metal contamination expressed by the mPEL oligo-Q and numbers of metal concentrations exceeding the TEL oligo and PEL oligo thresholds.

| Case       | Incidence of Exceedance for Individual Metals                                                                 | Number of Samples | Comments                                                                                          |
|------------|---------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------------------------|
| **Case 1** | IOBS ≥ 3, mPEL oligo-Q ≤ 0.92, Less than 4 metals ≥ TEL oligo and 2 metals > PEL oligo                       | 25                |                                                                                                   |
| **Case 2** | IOBS < 3, mPEL oligo-Q > 0.92, Less than 4 metals ≥ TEL oligo and 2 metals > PEL oligo                       | 8                 | Reduced bioavailability of metals?                                                                 |
| **Case 3** | IOBS < 3, mPEL oligo-Q ≤ 0.92, Less than 4 metals ≥ TEL oligo and 2 metals > PEL oligo                       | 27                | Effects caused by other pollutants than metals and/or high bioavailability of metals.             |
| **Case 4** | IOBS ≥ 3, mPEL oligo-Q > 0.92, At least 4 metals ≥ TEL oligo and/or 2 metals > PEL oligo                   | 3                 | Reduced bioavailability of metals by Mn/Fe oxides and/or organic matter for two samples (high concentrations of Mn, Fe and OM)? For one sample, mPEL oligo-Q value (0.98) close to the threshold of 0.92. |

3.7. Comparison of TEL oligo and PEL oligo with Existing SQG

The effect thresholds derived for the individual metals in this work were globally lower than those developed previously based on ecotoxicological tests performed in the laboratory [8,9,22] (Table 5). Only the TEL/PELHAD10 (threshold effect level and probable effect level based on 10-day toxicity tests with *Hyalella azteca*) and the TELHAD28 (threshold effect level based on 28-day toxicity tests with *Hyalella azteca*) for Cr and Ni, the PELHAD28 (probable effect level based on 28-day toxicity tests with *Hyalella azteca*) for Ni and the PELHAD10 for Cd, Cu, Pb and Zn, TEL/PELHAD28 for Cd, Cu, Pb and Zn and TELHAD28 for As and PELHAD28 for Cr were more than twice as high than the TEL/PEL oligo of these metals, with the TEL/PELHAD10 for Hg and Cd and PELHAD28 for As, Cd and Zn being more than 5 times higher.

Our effect thresholds were also globally lower than those developed by de Deckere et al. [8] based on the study of macroinvertebrate community structure (Table 5). Except for the LELECO (lowest effect level based on macrozoobenthic community assemblages) for Cr and Ni that were lower than our TEL oligo, all thresholds based on macroinvertebrate community structure were higher than those obtained in the present study. The LELECO was >2-fold higher than the TEL oligo for Zn, Cd and As and >10-fold for Hg. The SEL eco (severe effect levels based on macrozoobenthic community assemblages) was >2-fold higher than the PEL oligo for Cu, >5-fold for Pb and As and >10-fold for Cd, Hg and Zn.

Finally, our effect thresholds were globally lower than those based on ecotoxicological and ecological studies [7,8] (Table 5). Only the TEC (threshold effect concentration)/consensus 1 (mean of the LELECO and TELHAD10) for Ni and Cr, consensus 1 for Cu and consensus 2 (mean of the SEL eco and PELHAD10) for Ni were close or lower than the TEL oligo or PEL oligo. The TEC/consensus 1 for Cd, Hg, Pb,
As and Zn and TEC for Cu were more than two times higher than the corresponding TEL_{oligo}, with the TEC/consensus 1 of Cd and Hg being >5-fold higher. While the PEC (probable effect concentration) for Ni and consensus 2 for Cr and Cu were moderately higher (1.4–1.9-fold) than the corresponding PEL_{oligo}, all the other PEC and consensus 2 were largely higher than the corresponding PEL_{oligo}, from 2.4-fold (PEC of Cr) to 35-fold (Consensus 2 of Cd).

Table 5. Comparison between the effect thresholds derived in the present study (TEL_{oligo}, PEL_{oligo}) and those proposed in previous studies based on ecological data and ecotoxicological tests with field-collected sediments.

| Type of Threshold | Cd  | Cr  | Cu  | Hg  | Ni  | Pb  | Zn  | As  |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| The present study | TEL_{oligo} | 0.18 | 35  | 10.5| 0.0218 | 24  | 12.3| 46.9| 3.6 |
|                   | PEL_{oligo} | 0.23 | 45.9| 31  | 0.054 | 34.1| 23.9| 88.1| 5.7 |
| Ecotoxicological tests | | | | | | | | |
| de Deckere et al. [8] | TEL_{HA10} | 1.2  | 26  | 16  | 0.18 | 7.5 | 31  | 163 | N.D.|
|                     | PEL_{HA10} | 2.6  | 45  | 34  | 0.47 | 19  | 68  | 305 | N.D.|
| US EPA [22]; Ingersoll et al. [9] | TEL_{HA28} | 0.58 | 36  | 28  | N.D. | 20  | 37  | 98  | 11  |
|                     | PEL_{HA28} | 3.2  | 120 | 100 | N.D. | 33  | 82  | 540 | 48  |
| Ecological studies | | | | | | | | |
| de Deckere et al. [8] | LEL_{eco} | 0.71 | 25  | 13  | 0.28 | 15  | 19  | 129 | 7.9 |
|                     | SEL_{eco} | 13   | 90  | 85  | 1.8  | 44  | 167 | 1300| 50  |
| Mix of ecotoxicological and ecological studies | | | | | | | | |
| MacDonald et al. [7] | TEC | 0.99 | 43.4| 31.6| 0.18 | 22.7| 35.8| 121 | 9.79 |
|                     | PEC  | 4.98 | 111 | 149 | 1.06 | 48.6| 128 | 459 | 33  |
| de Deckere et al. [8] | Consensus 1 | 0.93 | 26  | 14  | 0.23 | 11  | 25  | 146 | 7.9 |
|                     | Consensus 2 | 7.8  | 68  | 60  | 1.2  | 32  | 118 | 800 | 50  |

LEL_{eco} and SEL_{eco}: lowest effect and severe effect levels based on macrozoobenthic community assemblages; TEL_{HA10} and PEL_{HA10} threshold effect level and probable effect level based on 10-day toxicity tests with Hyalella azteca; TEL_{HA28} and PEL_{HA28}: threshold and probable effect level based on 28-day toxicity tests with Hyalella azteca; TEC and PEC: Threshold and probable effect concentrations based on consensus values from different ecotoxicological and ecological studies; Consensus 1: mean of the LEL_{eco} and TEL_{HA10}; Consensus 2: mean of the SEL_{eco} and PEL_{HA10}; N.D. = not determined.

4. Discussion

In this work, the metrics IOBS index, percentage of tubificids without hair setae and oligochaete density were highly correlated with the level of sediment contamination by metals and/or organic matter. Effect thresholds per metal and for all metals combined could be derived. These results confirm that the study of oligochaete communities was valuable to describe the level of metals and organic matter contamination in sediments. The new results on the relationships between oligochaete communities and the concentrations of metals acquired since 2013 in different cantons were globally in agreement with those obtained until 2012 in a restricted geographic area [11]. These results suggest that our effect thresholds could be appropriate for a larger scale in Switzerland. We could not confirm the assertion that including the species A. pluriseta in addition to the tubificids without hair setae improved the metric indicating metal contamination in sediments. This result suggests, therefore, that it is not necessary to modify the calculation of this metric.

The establishment of thresholds concentrations for metals based on field data or natural contaminated sediments presents some limitations. First, the biological effects observed cannot be attributed unequivocally to a particular chemical stressor (e.g., one metal or metal contamination). In our study, we observed, in many samples (27), an altered biological quality, although the concentrations of metals were low. At these sites, other pollutants besides metals may have contributed to the observed adverse effects. Indeed, a recent study measured high concentrations of several pesticides in sediments of some streams located in agricultural areas in Switzerland leading to toxic effects on benthic organisms [23]. Secondly, complex mechanisms such as metal speciation
(binding of metals to Mn/Fe oxides, organic matter, etc.) and synergism or antagonism between metals and between metals and other pollutants (e.g., PCBs, PAHs, pesticides) can occur in field-collected sediments [24]. In two samples, the very good biological quality despite quite high concentrations of metals seemed to be explained by a reduced bioavailability of metals due to Mn/Fe oxides and/or organic matter that were at high concentrations in these samples.

Despite the discrepancies between biological and chemical data due to the confounding factors described above, we were able to define two different thresholds for each metal, a threshold referring to the concentration below which effects in the oligochaete community are unlikely to occur (TEL_{oligo}) and a second threshold above which effects in the oligochaete community are likely to occur (PEL_{oligo}). The TEL_{oligo} were determined by excluding the entries corresponding to metal concentrations that were below 2-fold the background concentration, thus susceptible to be too low to cause effects. This supports the hypothesis that metals contribute to some extent to the observed effects in the oligochaete communities.

Because oligochaete communities could respond more to the effect of a mixture of metals present in sediments than of a single metal, a threshold integrating all metals (mPEL_{oligo-Q}) above which effects of metals on oligochaete communities are likely to occur was also established. It is possible to calculate the probabilities that the IOBS value is $\geq 3$ or $<3$ using the mPEL_{oligo-Q} threshold of 0.92 and the number of true negatives (TN) (mPEL_{oligo-Q} $\leq 0.92$ and IOBS $\geq 3$, TN $= 33$), false negatives (FN) (mPEL_{oligo-Q} $\leq 0.92$ and IOBS $< 3$, FN $= 47$), true positives (TP) (mPEL_{oligo-Q} $> 0.92$ and IOBS $< 3$, TP $= 33$) and false positives (FP) (mPEL_{oligo-Q} $> 0.92$ and IOBS $\geq 3$, FP $= 3$). These predictive values help with taking into account the uncertainty around the proposed threshold when assessing metal concentration in sediments, e.g., in a first-tier screening approach. The probability that an IOBS result is $<3$ when mPEL_{oligo-Q} is $>0.92$ is calculated as the ratio between TP and the sum TP + FP, and corresponds in our study to 0.92. From a management perspective, this means that there is a high probability that the IOBS result will be $<3$ if the mPEL_{oligo-Q} threshold of 0.92 is exceeded. In that case, it could be concluded that metals are likely to affect the oligochaete communities. Conversely, the probability that an IOBS result is $\geq 3$ when mPEL_{oligo-Q} is $\leq 0.92$ is calculated as the ratio between TN and the sum TN + FN and corresponds in our study to 0.40. From a management perspective, this means that a site complying with the mPEL_{oligo-Q} threshold of 0.92 has less than one chance out of two of having an IOBS result $\geq 3$. In that case, a further step in a tiered approach should be triggered, i.e., implementing the oligochaete IOBS index. If the observed IOBS result is $<3$, it could be concluded that metals may be present in a highly bioavailable form or that contaminants other than metals or site-specific environmental factors may affect the oligochaete communities.

The effect thresholds derived for the individual metals in this work were globally lower than those derived previously based on ecotoxicological tests performed in the laboratory and on the study of macroinvertebrate community structure. They were also lower than the consensus thresholds based on ecotoxicological and ecological data, which is logical as the consensus values are derived as the mean of ecological and ecotoxicological thresholds (e.g., LEL_{eco} and TEL_{HAAD} for consensus 1 and SEL_{eco} and PEL_{HAAD} for consensus 2 [8]). The method of metal extraction used in the present study (nitric acid) could partly explain these differences, with aqua regia having been used in all or most of the previous studies for extracting metals, for example in de Deckere et al. [8]. According to a previous work performed in our laboratory, the traditional method of extraction with boiling nitric acid on a hot plate results in approximately 90% of the metal concentrations obtained with aqua regia extraction, a percentage that is lower for metals with a high geogenic contribution such as Cr [25]. Furthermore, there is good agreement between the TEL_{oligo} and TEL_{HAAD28} for Ni and Cr. Therefore, we think that the differences in the extraction capacity of the analytical methods used contribute rather little to the differences in the derived thresholds. Based on an analysis of the effects of metals, PAHs and PCBs on the composition of in situ nematode communities, Höss et al. [26] showed lower toxicity thresholds than those proposed by Ingersoll et al. [5] based on ecotoxicological tests. Indeed, Höss et al. [26] observed an unacceptable ecological status (medium to bad) of nematode communities in 80% of the samples with values of the index of contamination mPEC-Q > 0.17, while Ingersoll et al. [9] observed that 50% incidence of toxicity in the *Hyalella azteca* 28-day survival or
growth toxicity test corresponded to a PEC-Q > 0.63. The discordances of thresholds derived using ecotoxicological tests and oligochaete and nematode communities could be explained by the fact that the ecotoxicological tests are performed on species (e.g., *H. azteca*) that may not be representative or protective for sensitive oligochaete and nematode species. The conditions of the tests in the laboratory are, in addition, very different from environmental conditions [27]. In particular, the exposure of organisms to pollutants is in general shorter in laboratory toxicity tests than in situ and the physicochemical parameters that influence the bioavailability of contaminants (% of organic matter, pH, redox potential, etc.), constant in the laboratory, are variable in environmental conditions [28,29]. On the other side, even if oligochaetes and nematodes are used as indicators of the chemical quality of sediments, the composition of their communities in situ could also be influenced by physical factors, for example, extreme temperatures or instability of fine/sandy sediments.

Also, the use of the whole macroinvertebrates to establish effect thresholds in sediments tend to show higher values than those obtained in the present work. de Deckere et al. [8] considered the presence/absence of all taxa, sensitive and resistant taxa combined. They even removed many sensitive taxa (occurring at less than 5 sites) from their dataset for determining the thresholds. This absence of consideration of the effects of metals specifically on the presence/absence and abundance of sensitive taxa can explain the globally higher values of the thresholds observed in the study by de Deckere et al. [8] compared to those derived in our study. Additionally, in de Deckere et al. [8], macroinvertebrates were identified to the genus, family and group level. Because genera (and higher levels) include species with different degrees of resistance to pollutants [11,30–35], the loss of ecological information that results from the consideration of higher levels than species can lead to an imprecise or incorrect assessment of the effects of pollutants. Besides, to establish their effect thresholds, de Deckere et al. [8] collected macroinvertebrates in fine sediments using a grab sampler according to a methodology specifically dedicated to the study of the biological quality of sediments [33]. We emphasize that sampling macroinvertebrates in various habitats using a net (classical macroinvertebrate methodology) is appropriate for assessing the quality of aquatic ecosystems at large, but may not be appropriate for establishing effect thresholds in sediments, given that many insect taxa (e.g., stoneflies, mayflies and Coleoptera) have no or a limited contact with the fine fraction of sediments [33].

The IOBS index has proven largely sensitive enough to discriminate between “pristine” sites from sites under anthropogenic influence. The proposed TEL_{oligo}, PEL_{oligo} and threshold integrating all metals (mPEL_{oligo}-Q = 0.92) can be used for screening for alteration of in situ communities restricted to fine sediments based on the results of sediment metal analyses. According to the existing guidance for the derivation of environmental quality standards in Europe [14], field data should be considered, where available, in the derivation of quality standards for sediments. Threshold effect levels derived from field data (TEL) are preferred over probable effect levels derived from field data (PEL) because TEL have the same intended definition as quality standards, i.e., they refer to concentrations below which biological effects are unlikely to occur. According to Reference [14], if the TEL derived from field data for a given metal is lower than the quality standard derived from spiked-sediment toxicity tests, there might be the case of increasing the level of protection of the quality standard derived from spiked-sediment toxicity tests, given that field data are reliable. For example, the TEL_{oligo} is the lowest among the effect data available for Hg and lower than the quality standard derived from spiked-sediment toxicity tests for this metal [36], meaning that the quality standard of Hg may not be protective for oligochaete communities.

Although the TEL_{oligo}, PEL_{oligo} and mPEL_{oligo}-Q threshold are suitable for triggering further steps within a tiered approach, it would be important to validate these effect thresholds with more data and by testing different adaptations of the oligochaete index for optimally determining effect–no effect thresholds. An adverse effect on oligochaete communities, as described by the IOBS index, is mainly indicated by the proliferation of tubificids, which include highly resistant species, in detriment to all other species that are mostly sensitive or moderately sensitive to pollution. A potential improvement of the IOBS index would imply classifying all oligochaete species into different degrees of sensitivity to pollution and calculating the oligochaete index using the
percentages of the resulting sensitivity classes. Such classification, however, would be difficult based on the morphological identification of oligochaetes because only a fraction of the specimens present in a sample can be identified to the species level [37]. Only the identification of oligochaete specimens using DNA barcodes [37] would be suitable to establish such classification. A genetic oligochaete index based on the approach “high-throughput DNA barcoding”, that allows to identify each specimen sorted from a sample to the species level on a routine basis, was recently developed to assess the biological quality in stream sediments [38]. In the future, we will progressively implement this new genetic index, which will allow us to assign each oligochaete species to a specific sensitivity class and contribute to validate or refine the metal effect thresholds proposed here.

**Supplementary Materials:** The following are available online at www.mdpi.com/2076-3298/7/4/31/s1, Table S1: Details of the sampling (studied sites, sampling dates, geographical coordinates); Table S2: Details of the chemical data. Values of the metals Cr, Ni, Zn, Cu, Pb, Cd, Hg, As, Co, Mn and Fe (µg/g), of the percentage of organic matter (% OM) and of the mPEL_{olig}-Q obtained per site; Table S3: Details of the faunistic data. Values of the IOBS index, % of tubificids without hair setae (TUSP), % of tubificids with hair setae (TUCP), density of oligochaetes per 0.1m$^2$ (n ind), number of taxa (n taxa) and % of the species Aulodrilus pluriseta (% A. pluriseta) obtained per sample.

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