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1	Linking diatom community structure to pesticide input as evaluated through a spatial
2	contamination potential (Phytopixal): a case study in the Neste river system (South-West
3	France)
4	
5	S. Morin ^{a,*} , M. Bottin ^a , N. Mazzella ^a , F. Macary ^b , F. Delmas ^a , P. Winterton ^c , M. Coste ^a
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7	^a Cemagref, UR REBX, 50 avenue de Verdun, F-33612 Cestas cedex, France
8	^b Cemagref, UR ADBX, 50 avenue de Verdun, F-33612 Cestas cedex, France
9	^c Université Paul Sabatier – Toulouse III, 118 route de Narbonne, F-31062 Toulouse cedex 4,
10	France
11	

^{*} Corresponding author: Tel.: +33557890800; Fax: +33557890801; e-mail address: soizic.morin@cemagref.fr

12 Abstract

13	A large-scale field survey was undertaken in the Neste system (South-West France) to
14	investigate the impact of pesticide inputs on the structure of riverine benthic diatom
15	communities. A total of 18 sites in the "Coteaux de Gascogne" study area were sampled
16	during periods of pesticide use over two consecutive years (2005-2006).
17	The typology of the diatoms collected was determined using artificial neural networks
18	generating patterns in diatom community composition that indicated the species influenced by
19	pesticide inputs, combined with organic pollution. Small, pioneer species of the Achnanthales
20	group were more frequent in the pesticide-contaminated assemblages, whereas slower
21	colonizers were more often found in the sites where disturbance was lower. The Phytopixal
22	approach, a means to assess the spatial contamination potential, provided valuable
23	information about pesticide exposure and was a successful means of discriminating diatom
24	assemblages, suggesting that a global estimate of pesticide pollution risk, although rough,
25	would be more appropriate and more representative than sporadic pesticide analyses.
26	
27	Keywords
28	Bacillariophyceae; pesticides; Phytopixal; self-organizing maps
29	
30	1. Introduction
31	Over the last decades, agricultural herbicide application has become the predominant weed
32	control method but the introduction of herbicides into aquatic environments, mostly through

34 particular freshwater algae that form the base of the aquatic food webs. Hence, the effects of

surface water run-off, is likely to have subsequent consequences for non-target organisms, in

35 pesticides on the structure and functions of these communities may alter other components of

36 the ecosystem.

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Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4G-4WD7B25-2&_user=5403746&_coverDate=08%2F13%2F2009&_rdoc=5&_fm 37 So far, investigations into the effects of agricultural pollution (fertilizers and pesticides) on periphytic algal communities are scarce (Guasch et al., 1997; Guasch et al., 1998; 38 39 Hatakeyama et al., 1994; Navarro et al., 2002) and generally disconnected from field complexity. Most work on pesticides to date has been performed in the laboratory on diatom 40 41 isolates, often using single compounds. Since these compounds are rarely found alone in aquatic environments which present a variety of other substances, commercial formulations 42 and breakdown products, exhaustive screening of all potential pesticides by physicochemical 43 44 analyses is almost impossible. 45 Herbicides can affect algal growth and physiology, as well as community structure but the responses of diatoms to pesticide contamination have been shown to vary greatly among 46 compounds (Eullaffroy and Vernet, 2003; Källqvist and Romstad, 1994; Nyström et al., 1999; 47 Pipe and Cullimore, 1984) and their combinations (Hatakeyama et al., 1994; Hoagland et al., 48 49 1996) and concentrations (Abdel-Hamid et al., 1996; Källqvist and Romstad, 1994), the

50 species involved (Kasai et al., 1993; Lockert et al., 2006; Nyström et al., 1999; Pérès et al.,

51 1996) or even the strains (Kasai et al., 1993; Millie and Hersh, 1987) and experimental

52 conditions (Tlili et al., 2008 and references therein). Many laboratory bioassays have revealed

53 that the toxic effects of some pesticides on freshwater diatoms may occur at environmentally

54 realistic concentrations (*i.e.* < 1µg/L)(Guanzon and Nakahara, 2002; Källqvist and Romstad,

55 1994; Nyström et al., 2002). However, the effects on natural multispecific populations,

56 described through biological indices, did not express significant relationships between these

57 indicators and total pesticide concentrations (Dorigo et al., 2004; Liess et al., 2005). The main

58 determinants of diatom community structure may thus not only be pesticides, especially in

59 agricultural contexts where heavy fertilizer pollution is also observed, and there is a need for

60 alternative methods for the assessment of pesticide impact on aquatic ecosystems.

61 Here, we propose to use typologies based on diatom community structure first (Grenier et al.,

62 2006; Park et al., 2006; Tison et al., 2005) to group homogeneous species distributions in the

63 "Coteaux de Gascogne" area, then to allow the determination of the most structuring

64 environmental parameters explaining the biotypology, *i.e.* describing the largest part of the

65 variance in the diatom dataset. In this study, herbicide exposure was characterized via the

66 number of compounds recorded and the total and maximum pesticide concentrations

67 determined from field samplings. At the same time, a "spatial contamination potential"

68 approach (Phytopixal) based on the vulnerability of the environment (slope, pedology,

69 distance from the stream) and the agricultural pressure (Macary et al., 2007) provided data

70 that were compared to the patterns observed in the diatom communities.

71

72 <u>2. Materials and methods</u>

73 2.1. Study design

Located in the "Coteaux de Gascogne" region (SW France), the Neste system is part of an exclusively agricultural river basin with upstream dams that resupply rivers from which farmers directly withdraw water for irrigation (Trouvat, 2001). Over 75% of the watershed area is cultivated, and is especially under cereals. The hydrological regime of the rivers is pluvial, with low water period in Summer, with water circulating mainly superficial, making this area vulnerable to surface run-off and erosion processes which may enrich river waters with pollutants.

A total of 18 sites belonging to the Neste system were selected along 4 watersheds (Save,
Touch, Sousson and Gèze catchments) and 2 complementary sites (the Jasse and Vermeil
rivers) to match the following requirements: all-year flow; agricultural pesticide use; expected
various pesticide loads. The streams were monitored for 2 years (2005-2006) and sampled
during herbicide spraying periods (*i.e.* in Spring: from mid-March to mid-June).

86

- 87 The location of the sites is displayed in Figure 1. The following catchments were
- 88 distinguished:
- the Sousson river (catchment surface area: 120 km²; sites Aujan, Dareous and Auch) 89 90 and its tributaries Hay and Cedon streams, with steep slopes on the right bank and 91 wide, gently sloping irrigated corn crops on the left bank (Colin et al., 2000). 92 the Gèze stream (sites Organ and Peyret), a tributary of the Sousson river draining a 93 heavily cultivated area. 94 the Save river (catchment surface area: 1150 km²; sites Gorges, Anan, Espaon and -95 Cazaux) and its tributary Montoussé, with mixed farming and livestock upstream and 96 irrigated corn, oil-seed crops and cereals downstream. the Touch river (catchment surface area: 515 km²; sites Lilhac, Fabas, Savères and 97 98 Lamasquère), has its source in the forest of Lannemezan plateau and runs through 99 corn, oil-seed and soya crops and large agricultural plains downstream (from Savères 100 site). 101 On the right bank of the Garonne river, the sites Jasse and Vermeil are located upstream and 102 at the outlet (respectively) of a small hilly watershed subjected to intensive agricultural land
- 104

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105

2.2. Calculation of the potential risk

use (irrigated crops and cereals).

The spatial indicator Phytopixal was used to assess the agricultural pressure and impact on the surface water pollution potential (Macary et al., 2007). It is calculated from data available at different observation scales (*i.e.* plot, elementary drainage basin, or the entire drainage area) and information has to be averaged or "aggregated" from the pixels on remote-sensing images to the targeted Optimal Spatial Resolution (Marceau et al., 1994), *i.e.* final observation unit.

111 To enable between-scale data transfer, this indicator of the pollution risk due to pesticide 112 treatments was developed on a pixel scale, using very high resolution images (20 m x 20 m 113 for a SPOT XS image, 30 m x 30 m for a LANDSAT image). This enables the detection of 114 the smallest geographical objects related to agricultural landuse (plots). Other variables of 115 importance in the pesticide pollution process were included in the GIS within the ArcGIS® 116 system and are scaled from 1 to 5, corresponding to an increasing risk of pollution. By means 117 of this scale, we used information relative to: (i) topography, related to increasing slopes, (ii) 118 pedology (for the Save watershed only) depending on soil types and their influence on 119 pesticide transfer mechanisms, (iii) hydrography, with decreasing distance to the stream, (iv) 120 landuse patterns and cropping practices (number of pesticides treatments, determined after 121 Agreste, Recensement Agricole 2000).

Then, aggregation to the plot scale enabled a description to be given of the elementary drainage basin; aggregation to the elementary drainage basin enabled a description of the drainage area; and aggregation to the drainage area enabled a description of the entire watershed. Indeed, the method developed can characterize functional investigation units at different spatial scales, ranging from the elementary drainage basin to a large river basin, with respect to the surface water pollution potential. In this study, information acquired at the pixel level was not relevant so we used a large-scale spatial organization level (drainage area).

129

130 2.3. Periphyton sample collection and diatom identification

Periphytic diatoms were sampled from natural substrates by scraping pebbles (following the protocol of the European standard NF EN 13946) and artificial substrates (glass slides, 300 cm² area for both sides) installed at each site for a 21-day immersion (as described in Morin et al., 2008). At each time, glass slides were retrieved, scraped to give 3 replicate samples of

periphyton and fixed with a 37% solution of formaldehyde. Bare slides were placed in the

- 136 water column for a new 21-day colonization.
- 137 Taxonomic analysis of diatom assemblages was performed for all the periphyton samples,
- 138 which were prepared according to the procedure described in ANSP protocols (Charles et al.,
- 139 2002).
- 140 After digestion of the samples in boiling hydrogen peroxide (30% H₂O₂) and hydrochloric

141 acid (35 %), permanent slides were prepared by mounting the cleaned diatom frustules on a

142 glass microscope slide in Naphrax[©] (Brunel Microscopes Ltd, UK), a high refractive index

143 (1.74) medium. A minimum of 400 diatom valves were identified on each slide at $1,000 \times$

144 magnification, using taxonomic literature from central Europe (Krammer and Lange-Bertalot,

145 1986 - 1991) and recent nomenclature updates. Relative abundances of each species and

146 species richness were estimated, diatom diversity was calculated using the Shannon index,

147 and Biological Diatom Index (BDI-2006; Coste et al., 2009) and Specific Polluosensitivity

148 Index (IPS; Coste *in* Cemagref, 1982) values were determined.

- 149
- 150 2.4. Stream water physicochemical parameters

151 Water quality was evaluated every 21 days through the determination of *in situ*

152 physicochemical parameters (pH, temperature, conductivity, dissolved oxygen; WTW,

153 Weilheim, Germany) during the experimental periods. Two-litre stream water samples were

simultaneously collected and brought back to the laboratory for nutrient (phosphate, silica,

ammonium, nitrite and nitrate) measurements and pesticide analyses. Nutrient concentrations

156 were determined according to French and international standards (NF T90-023, NF T90-007,

157 NF EN ISO 11732 and NF EN ISO 13395).

158

159 2.5. Chemicals

160 Acetonitrile supragradient, methanol gradient and water gradient (HPLC grade) were purchased from ICS-Science Groupe (Gradignan, France). Oasis HLB cartridges (6 mL, 500 161 162 mg, 60 µm) were provided by Waters (Saint-Quentin-en-Yvelines, France). All analytical 163 standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany): acetochlor, aclonifen, 164 alachlor, ametryne, atrazine, chlorotoluron, deethylatrazine (DEA), deethylterbuthylazine 165 (DET), deisopropylatrazine (DIA), diuron, 1-(3,4-dichlorophenyl)-3-urea (DCPU), 1-(3,4dichlorophenyl)-3-methylurea (DCPMU), 1-(4-isopropylphenyl)-3-urea (IPPU), 1-(4-166 167 isopropylphenyl)-3-methylurea (IPPMU), isoproturon, linuron, metobromuron, metolachlor, 168 metoxuron, monuron, monolinuron, prometryn, propazine, simazine, terbuthylazine.

169 2.5.1. Sample preparation and solid phase extraction:

170 The water samples were collected in glass bottles and maintained at 4 °C until sample 171 preparation. Preconcentration of the analytes from water samples was accomplished by using 172 solid phase extraction (SPE) with Oasis HLB cartridges. Prior to SPE, 200-mL water samples (pH adjusted to 7) were filtered using GF/F glass microfibre filters (0.7 µm pore size). SPE 173 174 was conducted using a VisiPrep 12-port manifold (Supelco, Saint-Germain-en-Laye, France). 175 The conditioning, extraction and rinsing steps were carried out under a 53.33 kPa vacuum. 176 The SPE cartridges were successively washed with 10 mL of methanol, conditioned with 10 177 mL of HPLC grade water, loaded with 200-mL water samples, then rinsed with 20 mL of 178 HPLC grade water and dried with a stream of nitrogen for 30 minutes. Elutions were achieved 179 with 5 mL of methanol. The 5-mL extracts were blown under a gentle stream of nitrogen and 180 dissolved within 1 mL of an acetonitrile and water (20:80, v/v) mixture prior to the analysis 181 with an ODS 2 column. The recoveries are reported elsewhere (Mazzella et al., 2007).

182 2.5.2. HPLC-DAD determination of herbicides:

183 HPLC system: Finnigan SpectraSYSTEM SCM1000 Solvent Degasser, Finnigan
184 SpectraSYSTEM P4000 Quaternary Pump, Finnigan SpectraSYSTEM AS3000 Autosampler

185 (column oven set at 30°C) and Finnigan UV6000LP photodiode array detector (Thermo

186 Electron Corporation, MA, USA). Detection wavelengths: λ =220 nm for acetochlor,

aclonifen, alachlor, ametryne, atrazine, deethylatrazine (DEA), deethylterbuthylazine (DET),

188 deisopropylatrazine (DIA), metolachlor, prometryn, propazine, simazine and terbuthylazine.

189 λ =240 nm for chlorotoluron, diuron, 1-(3,4-dichlorophenyl)-3-urea (DCPU), 1-(3,4-

190 dichlorophenyl)-3-methylurea (DCPMU), 1-(4-isopropylphenyl)-3-urea (IPPU), 1-(4-

isopropylphenyl)-3-methylurea (IPPMU), isoproturon, linuron, metobromuron, metoxuron,monuron and monolinuron.

193 The HPLC determination of herbicides was adapted from Dupas et al. (1996) and performed with a Prontosil Spheribond ODS 2 column (250 x 4 mm, 3 µm) with a C₁₈ (10 x 4 mm, 6 194 195 um) guard column (Bischoff Chromatography, Leonberg, Germany). The injection volume 196 and composition were 50 µL and acetonitrile/water (20:80, v/v), respectively. The limits of quantification after the SPE preconcentration (about 200:1) were between 20 and 50 ng L^{-1} for 197 198 the chemical of interest. The binary gradient was made up of acetonitrile and HPLC water. 199 The proportion of acetonitrile was maintained at 20 % for 5 minutes, then increased to 50 % 200 after 60 minutes, and further increased to 90 % after 5 minutes, and kept constant for 10 minutes. Afterwards, the proportion of acetonitrile was decreased to 20 % in 3 minutes and an 201 isocratic phase was applied for 7 minutes. The flow rate was maintained at 0.4 mL min⁻¹ for 5 202 minutes, then increased to 0.5 mL min⁻¹ after 60 minutes, and kept constant for 15 minutes. 203 Lastly, the flow rate was returned to initial 0.4 mL min⁻¹ after 10 minutes. The total running 204 205 time was 90 minutes.

206

207 2.6. Data analysis

208 Self-organizing maps (SOM; Kohonen, 1995) are known to be an efficient alternative to

209 traditional statistical methods to deal with datasets ruled by complex, non-linear relationships,

Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4G-4WD7B25-2&_user=5403746&_coverDate=08%2F13%2F2009&_rdoc=5&_fm 210 providing a topology-preserving projection of the data space onto a more comprehensible 211 two-dimensional space. These unsupervised neural networks were trained under a Matlab ® 212 environment (v7.0.4.365, The MathWorks, 2005), and the SOM toolbox (version 2.0 beta) developed by the Laboratory of Computer and Information Science, Helsinki University of 213 Technology (http://www.cis.hut.fi/projects/somtoolbox/documentation/somalg.shtml). We 214 215 used Bray-Curtis (Bray and Curtis, 1957) distances between log-transformed relative 216 abundances of diatom species (Park et al., 2006). The optimal size of the map (12 rows \times 8 217 columns) was determined in order to minimize the topographic and quantization errors. A

218 hierarchical cluster analysis on the SOM output using the Unweighted Pair Group Method

219 Analysis algorithm allowed the determination of homogeneous clusters. Neighbouring

220 clusters of sites are represented by neighbouring hexagons on the map according to species

assemblages, whereas sites very different from each other are expected to be distant in the

222 map. For clarity it is the number of the clusters obtained (i.e. of the 4 groups) that are noted

223 on the SOM map rather than the sample numbers.

224 Then the specific weight of each physicochemical parameter on the SOM data clustered

225 together was determined by using Linear Discriminant Analysis (LDA) in the ade4 package

226 (Thioulouse et al., 1997) implemented in the R environment (Ihaka and Gentleman, 1996;

227 <u>http://www.r-project.org/</u>). This statistical technique aims to classify the dataset into mutually

exclusive groups (i.e. the 4 clusters) based on the physicochemical parameters that can best

separate the samples. The predictability of the clusters from the physicochemical data was

tested using the Jackknife procedure with the package MASS (Venables and Ripley, 2002).

The IndVal method (Dufrêne and Legendre, 1997) was then used to identify the indicator

value of species, in order to determine the ones that structured the dataset most.

233

234 <u>3. Results</u>

235 3.1. Pesticide monitoring

236	Pesticide	concentrations	were below	detection	levels most	t of the ye	ar but during	spreading
						2		

- 237 periods many compounds were detected (Table 1 and Table 2), with total herbicide
- 238 concentrations frequently exceeding the general EU drinking water threshold of 0.5µg/L
- (about 1/3 of the samples analyzed) and occasionally above $5\mu g/L$ (10.3 $\mu g/L$ in the Jasse and
- 240 6.3μg/L at Auch, Sousson river, in April 2005; 6.3μg/L at Lilhac, Touch river, May 2006;
- 241 5.1µg/L at Auch, June 2005). The compounds recorded, displayed in Table 2, were triazines
- 242 (5 herbicides + 3 breakdown products), phenylureas (8 + 4), chloroacetanilides (3 herbicides)
- and diphenyl-ethers (1).
- 244 The spatial contamination potential (Phytopixal) gives an annual average pollution risk for
- 245 years 2005 and 2006 (Figure 2). For both years, the maps indicate a gradient of potential
- 246 pesticide pollution from up- to downstream, Phytopixal values ranging from 1 (site Gorges,
- not far from the source of the Save river) to 6 at sites Cazaux and Montoussé (downstream of
- the Save catchment). Between 2005 and 2006, pollution tended to decrease slightly in some of
- the drainage areas.
- 250
- 251 3.2. Biotypology of the streams

A total of 473 species (including 69 abnormal forms) were identified. The clusters based on

diatom communities (Figure 3) were compared to environmental data (Table 3, Figure 4), and

- allowed us to separate two main clusters and four sub-clusters corresponding to the following
- 255 stream characteristics:
- Clusters 1 & 3 (111 samples) with elevated pesticide inputs

- Clusters 2 & 4 (316 samples) with lower pesticide concentrations.

258 The second level of discrimination was linked to trophic pollution:

- Clusters 1 & 2 exhibited high nutrient (nitrate, nitrite, phosphate) levels
- Clusters 3 & 4 had lower nutrient bioavailability.
- After cross-validation, clusters 2 and 4 were better predicted (respectively 63 and 80%) with
- LDA than clusters 1 and 3. However cluster 1 was predicted to 43% by the data of clusters 1
- and 2 (high nutrient availability), and cluster 3 to 56% by those of clusters 3 and 4 (lower
- 264 nutrient concentrations).
- Among the taxa used for the SOM analysis, 124 had significant IndVal values for the two
- 266 main clusters or four sub-clusters (Table 4).
- 267

268 <u>4. Discussion</u>

269 4.1. Land use

270 Neither wastewater treatment plants nor industrial facilities are found in the area studied;

271 hence we assumed that pesticide concentrations were due to non-point source pollution input

through runoff. The Phytopixal representation expresses the vulnerability of the soils and the

273 gradient of land use between up- (principally prairies) and downstream (short rotations spring

cereals / oil seed, and corn in the valley), with a theoretical increase in the use of pesticides

275 (in numbers of treatments per crop). Although the concentrations and numbers of compounds

- 276 recorded were highly variable between sites and dates, the herbicides detected are in
- accordance with those commonly sprayed on corn, cereals and oil seed crops in this area
- 278 (http://www.midipyrenees.chambagri.fr/). However, the use of some of the compounds

detected is forbidden in France (e.g. monuron since 1994; monolinuron since 2001; linuron,

atrazine, simazine, terbuthylazine, propazine and ametryn since 2003), suggesting soil storage

or illegal use.

282

283

4.2. Relationships between pesticide inputs and diatom community structure

As seen in the gradient up- to downstream of pesticide and nutrient concentrations (Table 1),

eutrophication and pesticides are major stressors affecting agricultural environments (Smith,

286 1998). The effects of organic contaminations on periphytic diatoms have been widely studied,

leading to the routine use of many diatom-based trophic indices in Europe (for a review, see

288 Prygiel et al., 1999). In the present study diatom indices BDI and IPS (Table 3) expressed the

trophic contamination levels better than they expressed pesticide exposure, as underlined by

290 Dorigo et al. (2004). This could indicate the unsuitability of these indices for the assessment

291 of pesticide contaminations, or imply a poor description of the water's physicochemical

292 characteristics (mainly pesticides) through point measurements. This was further observed in

the LDA performed on the physicochemical data where it was clear that nutrients were the

294 main discriminating factor.

295 It was expected that the abundances of valve abnormalities would indicate pesticide exposure,

following the laboratory results of Schmitt-Jansen and Altenburger (2005) and Debenest et al.

297 (2008). However, the percentages of deformities were very low (below 1%, see Table 3),

variable, and not clearly related to pesticide exposure. Experiments with triazines and

sulphonylureas showed a decrease in specific diversity (Abdel-Hamid et al., 1996; Hamala

and Kollig, 1985; Hamilton et al., 1987; Seguin et al., 2001), which was also observed in this

301 survey with multi-substance contamination (Table 3).

302 Herbicides exert selection pressure when the exposure reaches a certain level for a sufficient

303 period of time, which varies depending on the environmental conditions. It has been shown

that the toxicity of triazines is influenced by light and temperature (Guasch and Sabater, 1998;

305 Guasch et al., 2003; Krieger et al., 1988; Millie et al., 1992) and by their degradation stage

306 (Stratton, 1984). Agricultural streams being more likely to have little shading, periphyton

307 communities would consequently be more sensitive to the pesticides and diatom-dominated,

308 according to previous studies (Guasch et al., 1998; Hamala and Kollig, 1985; Tang et al.,

309 1997). Furthermore, since organisms vary in their resistance to toxicants, the selection

310 pressure would exclude the sensitive ones which would be replaced by resistant strains or

311 species. Very little is known about diatom species sensitivity to pesticides. Of the main

312 structuring species of clusters 1 and / or 3, inferred from their IndVal value (Table 4), some

313 have already been shown to tolerate triazines, for example Achnanthidium minutissimum

314 (Herman et al., 1986; Kasai et al., 1993; Munoz et al., 2001; Seguin et al., 2001) or Cocconeis

315 placentula (Goldsborough and Robinson, 1986), and phenylureas, like *P. frequentissimum*

316 (Pérès et al., 1996). Melosira varians (Pérès et al., 1996; Spawn et al., 1997) has been shown

to be sensitive to pesticides, but some of the other species preferentially found in clusters 2

318 and / or 4 are likely to tolerate a number of pesticides: Cyclotella meneghiniana (Kosinski,

319 1984; Millie and Hersh, 1987), *Eolimna minima* (Pérès et al., 1996), *Encyonema minutum*

320 (Seguin et al., 2001), Fragilaria capucina (Carder and Hoagland, 1998; Guasch et al., 1998),

321 Navicula lanceolata (Guasch et al., 1998), Nitzschia fonticola (Kasai, 1999; Munoz et al.,

322 2001), N. palea (Guasch et al., 1998; Hatakeyama et al., 1994; Kasai et al., 1993; Kasai,

323 1999), and Ulnaria ulna (Hatakeyama et al., 1994; Kasai et al., 1993).

324 Diatom community responses to pesticide contamination are also affected by changes in the

325 physicochemical conditions (conductivity, nutrient concentrations, etc.) and environmental

326 factors such as interspecific interactions. This implies competition for resources (light,

327 nutrients: Lozano and Pratt, 1994; Turbak et al., 1986) and allelopathic interactions between

328 diatoms throughout succession (Guasch et al., 1998), as well as indirect disturbances of the

329 grazing pressure (deNoyelles et al., 1982). The affinities of many species for varied trophic

levels are well known (van Dam et al., 1994) and most of the structuring species were meso-

to hyper-eutraphentic. However, in clusters 3 and 4 species were found with lower trophic

indicator values (oligotraphentic to mesotraphentic). This was also underlined by the BDI and

333 IPS values (Table 3). It has been suggested that some taxa may also avoid direct deleterious

334 effects of herbicides by their ability to grow heterotrophically (Goldsborough and Robinson,

1986; Pérès et al., 1996), although this was not demonstrated by our data (Table 3).

336 Other factors accompanying flood events, such as increased scour, may have exerted species-

337 specific pressure on diatom community structure when combined with pesticide exposure:

338 Wang et al. (1997) demonstrated inhibited adhesion in the diatom *Achnanthes longipes*

339 exposed to the herbicide DCB. The consecutive changes in periphyton physiognomy may

340 influence the overall community responses to toxicants by modifying the diffusion gradients

341 within the biofilm, and hence its sorption capacities (Guasch et al., 2003; Headley et al.,

342 1998).

343

Benthic communities are often exposed to a mixture, rather than to individual toxicants; few 344 345 studies have assessed whether such combinations act synergistically, additively or 346 antagonistically (Megharaj et al., 1990; Munkegaard et al., 2008; Stratton, 1984). Obviously, diatom species respond differently to pesticide exposure depending on concomitant organic 347 348 contamination. This confirms that conclusions based on standard single-species tests provide 349 limited assessment of possible environmental impacts. Toxicity endpoints that have been 350 established for numerous pesticides and many algal species (available in the PAN Pesticide 351 database: www.pesticideinfo.org; the EC_{50} of the widely studied green alga 352 Pseudokirchneriella subcapitata for the pesticides recorded in this survey are given in Table 2) do not provide reliable information about the effective toxicity of these compounds to 353 354 diatom communities. Indeed, it has been demonstrated for many herbicides that periphyton photosynthetic activity is affected at lower concentrations than the effective concentrations 355 356 published for standard single-species growth tests with phytoplankton species (Gustavson et 357 al., 2003). Furthermore effects of pesticides in the field are mostly related to multi-substance 358 contamination, but current risk assessment does not consider mixture toxicity. Hence, the data Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4G-4WD7B25-2&_user=5403746&_coverDate=08%2F13%2F2009&_rdoc=5&_fm
it provides may be useful in itself but unlikely to successfully evaluate the potential hazard of
chemicals at the ecosystem level. As the sensitivity / tolerance characteristics of species are
scarce and have generally been determined in simplified laboratory conditions, it is difficult to
extrapolate such results directly to stream ecosystems. From the present field study, the cooccurrence of the species reported in Table 4 may constitute a basis for the use of diatoms as
indicators of pesticide contamination.

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367 Considering the multitude of pesticides applied in this agricultural context (25 compounds 368 including pesticides and their metabolites were recorded here) and the moderate significance 369 of point measurements, it is difficult to ascribe direct toxicity to the concentration of a single 370 compound. The Phytopixal has been designed as an indicator of the potential run-off of 371 contaminants to the aquatic environment. The pesticides analyzed at each sampling date being 372 highly dependent on the local environmental conditions (rainfall and flooding events), some 373 of the measurements of elevated concentrations in the streams were almost synchronous with 374 changes in precipitation regimes (http://www.meteofrance.com/) and / or discharge 375 (http://www.hydro.eaufrance.fr/). The high level of prediction of clusters 2 and 4, when the 376 prediction of clusters 1 and 3 was grouped with clusters 2 and 4 respectively, indicates that 377 organic pollution was accurately predicted throughout the survey. The variations in pesticide measurements discarded the prediction of the clusters involved, whereas Phytopixal, while 378 379 not indicating the nature of the herbicides employed and possibly also other pollutants, or any 380 interactions occurring between them, nevertheless provides a rudimentary estimate for 381 understanding the stream community structure. It can be argued that, in chronically-382 contaminated conditions, a global indicator of pollution levels would give a better estimate of 383 pesticide stress than a single measurement, which would correspond either to a chronic stress

4.3. Use of a spatial contamination potential vs. point measurements

384 or a peak concentration. In this survey, the relationships between the Phytopixal and clusters

385 1-3 vs. 2-4 were more significant than those using pesticide concentrations or the number of

386 compounds detected. Experimental studies have previously shown that periphytic

387 communities grown in slightly contaminated conditions initially change in taxonomic

388 composition, but are less impaired when subjected to a secondary stress (Kasai, 1999;

389 Niederlehner and Cairns, 1992; Tlili et al., 2008). Moreover, field experiments of biofilm

390 translocation from polluted to unpolluted sites (and vice-versa) showed a high resilience of

diatom communities from polluted streams (Ivorra et al., 1999; Tolcach and Gómez, 2002).

392 As a consequence, a global estimate may still yield a good indication of potential pesticide

393 effects on the biota, despite variations in *in situ* measured concentrations.

394

395 <u>5. Conclusions</u>

396 Diatom classification based on community structure, and the relationship to environmental 397 descriptors, underlined that pesticide and nutrient inputs play an important role in structuring 398 the communities of the Neste system. The best estimate of pesticide exposure available in the 399 dataset was given by the Phytopixal approach, which does not necessarily express the 400 effective concentrations in the field. The use of passive integrative samplers to average out 401 water-soluble organic chemicals in aqueous environments has been proposed to provide time-402 weighted concentrations over long immersion periods, mimicking the exposure of aquatic organisms (Alvarez et al., 2004; Huckins et al., 1993; Mazzella et al., 2007). Further 403 404 investigations are needed to determine whether sampling strategies using passive samplers provide increased information and significance about the responses of periphytic 405 406 communities.

407

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624 Figure captions

- 625 Figure 1: Location of the study sites of the Neste system (SW France)
- 626 Figure 2: Spatial contamination potential (Phytopixal) in the Neste system in 2005 and 2006.
- 627 Figure 3: The patterned SOM map (left) and hierarchical cluster analysis (right) used to define
- 628 clusters on the units of the SOM map.
- 629 Figure 4: Linear discriminant analysis based on the physicochemical characteristics of the
- 630 water of each cluster (eigenvalues: Axis 1: 0.43, Axis 2: 0.16). (a) projection of the samples,
- 631 grouped by clusters (noted Cl); (b) projection of the environmental parameters.

Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste river system (South-West France). Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4G-4WD7B25-2&_user=5403746&_coverDate=08%2F13%2F2009&_rdoc=5&_fmt=high&_orig=browse&_srch=doc-info(%23toc%234974%232009%23



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Figure 3

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Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste river system (South-West France). Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4G-4WD7B25-2&_user=5403746&_coverDate=08%2F13%2F2009&_rdoc=5&_fmt=high&_orig=browse&_srch=doc-info(%23toc%234974%232009%23



Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste river system (South-West France). Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39. http://www.stifferedingerephysicology.accommentation potential (Phytopixal): a case study in the Neste river system (South-West France). http://www.stifferedingerephysicology.accommentation potential (Phytopixal): a case study in the Neste river system (South-West France). http://www.stifferedingerephysicology.accommentation potential (Phytopixal): a case study in the Neste river system (South-West France).

	Number of	pН	Conductivity	Ammonium	Nitrite	Nitrate	Orthophosphate	Oxygen	Suspended matter	Highest pesticide
	samples		(µS/cm)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L) *	concentration (µg/L)
										[number of compounds
										detected]
Sousson catch	iment		<u>. </u>		<u>. </u>	<u>.</u>			-	
Aujan	32	8.1 ± 0.1	518 ± 8	0.09 ± 0.02	0.25 ± 0.07	27.6 ± 2.3	0.23 ± 0.03	7.8 ± 0.6	29.6 ± 7.8	1.57 [18]
Dareous	31	8.3 ± 0.1	529 ± 15	0.14 ± 0.03	0.38 ± 0.07	23.2 ± 3.1	0.21 ± 0.03	8.7 ± 0.7	140.4 ± 51.3	1.88 [16]
Hay	10	8.2 ± 0.1	628 ± 12	0.05 ± 0.01	0.11 ± 0.01	22.8 ± 1.4	0.08 ± 0.01	<i>9.1</i> ± <i>0.7</i>	11.8 ± 3.7	1.84 [8]
Cedon	32	8.1 ± 0.1	440 ± 51	0.06 ± 0.01	0.10 ± 0.01	13.3 ± 1.7	0.12 ± 0.01	8.2 ± 0.6	32.4 ± 2.4	1.95 [16]
Auch	29	8.1 ± 0.1	596 ± 12	0.18 ± 0.06	0.29 ± 0.09	21.2 ± 1.7	0.13 ± 0.02	8.1 ± 0.6	42.5 ± 11.2	6.30 [21]
Gèze catchmo	ent									
Organ	10	7.9 ± 0.1	137 ± 5	0.03 ± 0.00	0.03 ± 0.00	8.6 ± 0.5	0.03 ± 0.00	8.3 ± 0.7	12.6 ± 0.3	0.10 [3]
Peyret	7	8.3 ± 0.1	323 ± 23	0.05 ± 0.01	0.09 ± 0.00	16.3 ± 1.2	0.17 ± 0.02	8.9 ± 0.8	10.8 ± 2.1	0.63 [8]
Save catchme	ent	-				·				
Gorges	29	8.3 ± 0.2	171 ± 4	0.03 ± 0.00	0.04 ± 0.01	4.6 ± 0.3	0.07 ± 0.01	8.7 ± 0.7	18.3 ± 4.4	0.88 [16]
Anan	32	8.4 ± 0.1	230 ± 11	0.05 ± 0.01	0.05 ± 0.01	6.7 ± 0.4	0.09 ± 0.01	9.0 ± 0.7	13.5 ± 1.2	0.35 [8]
Espaon	29	8.4 ± 0.1	236 ± 11	0.09 ± 0.01	0.10 ± 0.01	7.1 ± 0.5	0.12 ± 0.01	8.6 ± 0.7	40.2 ± 11.5	1.57 [16]
Cazaux	32	8.1 ± 0.1	344 ± 18	0.14 ± 0.01	0.19 ± 0.01	9.6 ± 0.6	0.15 ± 0.01	8.4 ± 0.7	20.7 ± 1.3	0.91 [13]
Montoussé	10	8.2 ± 0.1	842 ± 17	0.04 ± 0.01	0.09 ± 0.02	35.2 ± 0.5	0.08 ± 0.04	7.7 ± 0.6	6.1 ± 0.4	1.75 [5]
Touch catchn	nent	-	·		<u>.</u>	·		•		
Lilhac	31	8.3 ± 0.1	593 ± 8	0.02 ± 0.00	0.02 ± 0.00	4.9 ± 1.1	0.02 ± 0.00	9.0 ± 0.8	5.4 ± 1.4	6.29 [14]
Fabas	31	8.2 ± 0.1	598 ± 38	0.10 ± 0.04	0.08 ± 0.00	13.9 ± 1.5	0.06 ± 0.02	8.6 ± 0.8	30.4 ± 5.1	0.50 [9]
Savères	31	8.3 ± 0.1	629 ± 18	0.16 ± 0.05	0.10 ± 0.01	11.8 ± 1.0	0.05 ± 0.00	8.5 ± 0.6	31.8 ± 2.2	4.27 [13]
Lamasquère	32	8.2 ± 0.1	358 ± 32	0.06 ± 0.00	0.09 ± 0.01	6.3 ± 0.3	0.07 ± 0.00	8.5 ± 0.7	22.7 ± 1.8	1.38 [12]
Complement	ary sites		4		4	4				
Jasse	10	7.8 ± 0.1	794 ± 24	0.05 ± 0.01	0.09 ± 0.01	13.4 ± 1.2	0.06 ± 0.00	6.7 ± 0.4	6.3 ± 0.9	10.33 [5]
Vermeil	9	8.2 ± 0.1	772 ± 21	0.04 ± 0.01	0.18 ± 0.02	28.3 ± 1.3	0.15 ± 0.12	8.3 ± 0.7	10.9 ± 0.4	0.27 [2]

* intercorrelation with total phosphorus ($R^2 = 0.9034$; data not shown)

Data in italics represent sites from each catchment area that do not belong to the main tributary.

Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste river system (South-West France). Morin, S. etal. Aquatic Toxicology, 2009, vol. 94, n° 1, p. 28-39.

http://www.sciapcodineqt.sopm/sciensef2.sopfActicle/life/OrditBRTAPT4W20075,28Tysperit7A0820093_; vvrRestate98%2513%250999,84_59bert Fit _struttering 120059=br2ygeftesscopfactor/science/scie

Database (2007)) and concentrations of compounds recorded in the survey. DT50, water: hydrolysis half-life (or stability to hydrolysis) in aqueous neutral solutions at 20°C; K_{oc} : soil:water partitioning coefficient normalized by the organic carbon fraction of soil; Solubility: solubility in water at 20°C; K_{ow} : octanol:water partitioning coefficient at 20°C; EC₅₀: the concentration of a pesticide producing a 50% reduction in the abundance of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in one week.

Compound	Class	DT50, water ^a	$K_{oc}^{b,c,e}$	Solubility ^{a,b}	$\log K_{ow}^{a,b,e}$	$EC_{50}^{a,b,c,d,e}$	Highest
1		(days)	min - max	(mg/L)	0	$(\mu g/L)$	concentration
							(µg/L)
Linuron	Phenylurea	1460	341-620	63.8	3.00	67	9.10
Atrazine	Triazine	86	39-513	35	2.70	49	5.53
Deisopropyl atrazine	Triazine /	n/a	142	670	1.15	2	4.64
	Breakdown product						
Metolachlor	Chloroacetanilide	Stable	121-309	530	3.40	8	1.92
Prometryn	Triazine	30	277-400	33	3.34	12	1.90
Aclonifen	Diphenyl-ether	Stable	5318-12,164	1.4	4.37	16	1.65
Acetochlor	Chloroacetanilide	31	100-377	223	4.14	1.3	1.60
Alachlor		0.5	100-190	240	3.09	n/a	1.00
Isoproturon	Phenylurea	1560	36-241	70.2	2.50	35	0.93
Deethyl atrazine	Triazine /	n/a	72	3200	1.51	100	0.89
	Breakdown product					(12d-exposure)	
Metoxuron	Phenylurea	24	120	678	1.60	n/a	0.88
Chlorotoluron	Phenylurea	Stable	104-1000	74	2.50	n/a	0.81
Metobromuron	Phenylurea	170	95-646	330	2.41	n/a	0.80
Monuron	Phenylurea	n/a	150	230	1.79	n/a	0.49
Diuron	Phenylurea	Stable	161-1666	35.6	2.87	2.4	0.35
Simazine	Triazine	96	103-340	5	2.30	100	0.25
IPPU 3-(4-isopropylphenyl)-urea	Phenylurea /	n/a	n/a	n/a	n/a	n/a	0.21
DCPU 3-(3,4-dichlorophenyl)- urea	Breakdown product	n/a	527-861	940	2.35	n/a	0.21
Deethyl terbuthylazine	Triazine /	n/a	n/a	n/a	n/a	n/a	0.18
	Breakdown product						
IPPMU	Phenylurea /	n/a	n/a	n/a	n/a	n/a	0.16
3-(4-isopropylphenyl)-1-methylurea	Breakdown product						
Monolinuron	Phenylurea	Stable	67-500	735	2.20	1	0.11
Terbuthylazine	Triazine	Stable	162-306	8.5	3.21	3.20	0.08
DCPMU	Phenylurea /	n/a	498-1358	490	n/a	n/a	0.07
(3,4-dichlorophenyl)-1-methylurea	Breakdown product						
Propazine	Triazine	83	65-268	8.6	3.95	29	0.05
Ametryne		Stable	170-390	200	2.63	3.67	0.05

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Number of samples	53	155	58	161
Water physicochemical parameters				
pH	8.0 ± 0.0	8.2 ± 0.0	8.2 ± 0.0	8.2 ± 0.0
Conductivity (µS/cm)	459.7 ± 19.4	518.7 ± 13.1	610.8 ± 28.1	323.0 ± 12.9
Nitrate (mg/L)	14.2 ± 1.7	17.7 ± 0.7	14.7 ± 1.7	9.2 ± 0.8
Nitrite (mg/L)	0.24 ± 0.06	0.13 ± 0.01	0.09 ± 0.03	0.13 ± 0.02
Ammonium (mg/L)	0.18 ± 0.04	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.01
Orthophosphate (mg/L)	0.14 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
Temperature (°C)	16.9 ± 0.3	13.2 ± 0.2	13.3 ± 0.3	16.4 ± 0.3
Oxygen (mg/L)	7.6 ± 0.1	9.2 ± 0.2	8.7 ± 0.3	8.3 ± 0.1
Suspended Particulate Matter (mg/L)	44.6 ± 6.5	18.7 ± 1.3	13.3 ± 3.2	49.7 ± 10.9
Total pesticide concentration (μ g/L)	0.96 ± 0.17	0.77 ± 0.10	1.65 ± 0.40	0.57 ± 0.06
- Triazines and their metabolites	0.22 ± 0.07	0.27 ± 0.07	0.55 ± 0.18	0.16 ± 0.04
- Phenylureas and their metabolites	0.30 ± 0.08	0.10 ± 0.02	0.95 ± 0.36	0.21 ± 0.03
- Chloroacetanilides	0.31 ± 0.10	0.26 ± 0.04	0.14 ± 0.04	0.12 ± 0.02
- Diphenyl ethers	0.04 ± 0.02	0.04 ± 0.01	0.01 ± 0.00	0.03 ± 0.01
Number of compounds detected	4.9 ± 0.4	3.3 ± 0.2	3.2 ± 0.3	3.5 ± 0.2
Phytopixal value	3.2 ± 0.2	3.1 ± 0.1	4.0 ± 0.2	2.7 ± 0.1
Diatom indices				
Shannon's diversity index	3.0 ± 0.1	4.3 ± 0.1	2.8 ± 0.1	4.4 ± 0.0
BDI-2006	15.4 ± 0.1	14.8 ± 0.1	18.2 ± 0.2	16.0 ± 0.1
IPS	14.6 ± 0.1	13.9 ± 0.1	16.7 ± 0.2	15.0 ± 0.1
Valve abnormalities (‰)	3.2 ± 0.5	8.7 ± 1.3	3.2 ± 1.0	5.4 ± 0.6
% N-heterotrophic	2.5	8.8	5.2	8.9

Table 3: Characteristics of strea	n waters and diatom	communities of eac	h cluster determined by
SOM. Data are mean \pm standard	deviation.		

Table 4: Main structuring species of the clusters.

Species	Cluster	IndVal	<i>p</i> -value
Achnanthidium minutissimum (Kützing) Czarnecki	1 - 3	0.734	0.001
Cocconeis placentula Ehrenberg var. lineata (Ehrenberg) Van Heurck	1 - 3	0.670	0.001
Cocconeis placentula Ehrenberg var. placentula	1 - 3	0.655	0.001
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	1 - 3	0.614	0.001
Achnanthidium exiguum (Grunow) Czarnecki	1 - 3	0.009	0.001
Cyclotella fottii Hustedt	1 - 3	0.009	0.001
Eunotia exigua (Brébisson) Rabenhorst var. tenella (Grunow) Nörpel & Alles	1 - 3	0.009	0.001
Gomphonema biceps Meister	1 - 3	0.009	0.001
Gomphonema clavatulum Reichardt	1 - 3	0.009	0.001
Gomphonema sarcophagus Gregory	1 - 3	0.009	0.001
Nitzschia nana Grunow	1 - 3	0.009	0.001
Psammothidium sacculum (Carter) Bukhtiyarova & Round	1 - 3	0.009	0.001
Sellaphora stroemii (Hustedt) Mann	1 - 3	0.009	0.001
Tabellaria fenestrata (Lyngbye) Kützing	1 - 3	0.009	0.001
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	1 - 3	0.514	0.006
Navicula cryptotenella Lange-Bertalot	2 - 4	0.854	0.001
Navicula gregaria Donkin	2 - 4	0.848	0.001
Navicula tripunctata (O.F. Müller) Bory	2 - 4	0.812	0.001
Nitzschia dissipata (Kützing) Grunow var. dissipata	2 - 4	0.811	0.001
Surirella brebissonii Krammer & Lange-Bertalot var. brebissonii	2 - 4	0.782	0.001
Encyonema minutum (Hilse in Rabenhorst) Mann	2 - 4	0.729	0.001
Melosira varians Agardh	2 - 4	0.693	0.001
Navicula reichardtiana Lange-Bertalot var. reichardtiana	2 - 4	0.664	0.001
Nitzschia sociabilis Hustedt	2 - 4	0.659	0.001
Ulnaria ulna (Nitzsch) Compère	2 - 4	0.658	0.001
Navicula capitatoradiata Germain	2 - 4	0.653	0.001
Nitzschia recta Hantzsch	2 - 4	0.647	0.001
Nitzschia palea (Kützing) W. Smith	2 - 4	0.629	0.001
Navicula antonii Lange-Bertalot	2 - 4	0.593	0.001
Nitzschia heufleriana Grunow	2 - 4	0.554	0.001
Diatoma vulgaris Bory	2 - 4	0.551	0.001
Eolimna minima (Grunow) Lange-Bertalot	2 - 4	0.534	0.001
Navicula lanceolata (Agardh) Ehrenberg	2 - 4	0.533	0.001
Fragilaria capucina Desmazières var. vaucheriae (Kützing) Lange-Bertalot	2 - 4	0.533	0.001
Caloneis bacillum (Grunow) Cleve	2 - 4	0.484	0.001

0.001 Navicula catalanogermanica Lange-Bertalot & Hofmann 2 - 4 0.449 Gyrosigma sciotense (Sullivan & Wormley) Cleve 2 - 4 0.409 0.001 Navicula cryptotenelloides Lange-Bertalot 2 - 4 0.001 0.368 Surirella angusta Kützing 2 - 4 0.363 0.001 Fallacia subhamulata (Grunow in Van Heurck) Mann 2 - 4 0.360 0.001 2 - 4 0.240 0.001 Fragilaria capucina Desmazières var. capucina Fragilaria capucina Desmazières var. capitellata (Grunow) Lange-Bertalot 2 - 4 0.234 0.001 Nitzschia acula Hantzsch 2 - 4 0.209 0.001 2 - 4 0.004 Encyonema prostratum (Berkeley) Kützing 0.140 Fallacia pygmaea (Kützing) Stickle & Mann 2 - 4 0.114 0.004 2 - 4 0.235 0.005 Gyrosigma attenuatum (Kützing) Rabenhorst 2 - 4 0.124 0.005 Nitzschia angustatula Lange-Bertalot Cymatopleura solea (Brébisson) W. Smith var. solea 2 - 4 0.258 0.007 0.007 Diploneis oblongella (Naegeli) Cleve-Euler 2 - 4 0.190 Navicula(dicta) seminulum (Grunow) Lange Bertalot 2 - 4 0.178 0.007 Gyrosigma acuminatum (Kützing) Rabenhorst 2 - 4 0.276 0.009 Cocconeis placentula Ehrenberg var. euglypta (Ehrenberg) Grunow 1 0.607 0.001 0.019 0.001 Frustulia crassinervia (Brébisson) Lange-Bertalot & Krammer 1 Frustulia saxonica Rabenhorst 0.019 0.001 1 0.019 0.001 Surirella capronii Brébisson 1 1 0.002 Cocconeis pediculus Ehrenberg 0.338 Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova 1 0.305 0.005 Nitzschia linearis (Agardh) W.M. Smith var. linearis 2 0.570 0.001 Nitzschia vermicularis (Kützing) Hantzsch 2 0.479 0.001 0.001 Nitzschia linearis (Agardh) W.M. Smith var. subtilis (Grunow) Hustedt 2 0.423 2 0.001 Navicula trivialis Lange-Bertalot var. trivialis 0.419 Nitzschia linearis (Agardh) W.M. Smith var. tenuis (W. Smith) Grunow 0.313 0.001 2 2 Frustulia vulgaris (Thwaites) De Toni 0.234 0.001 Nitzschia pusilla (Kützing) Grunow 2 0.233 0.001 Nitzschia sigmoidea (Nitzsch) W. Smith 2 0.220 0.001 Navicula radiosa Kützing 2 0.211 0.001 Cyclotella ocellata Pantocsek 2 0.198 0.001 2 0.195 0.001 Nitzschia capitellata Hustedt Nitzschia dubia W.M. Smith 2 0.159 0.001 Gyrosigma spencerii (Quekett) Griffith & Henfrey 2 0.130 0.001 Mayamaea agrestis (Hustedt) Lange-Bertalot 2 0.122 0.001 0.002 Nitzschia acicularis (Kützing) W.M.Smith 2 0.177 0.259 0.003 Navicula cryptocephala Kützing 2 0.003 Tryblionella hungarica (Grunow) Mann 2 0.148 Nitzschia archibaldii Lange-Bertalot 2 0.091 0.003 Nitzschia gracilis Hantzsch 2 0.103 0.004

Navicula veneta Kützing 2 0.224 0.005 Tryblionella apiculata Gregory 2 0.183 0.005 Nitzschia dissipata (Kützing) Grunow var. media (Hantzsch) Grunow 2 0.118 0.005 Gomphonema micropus Kützing var. micropus 3 0.568 0.001 3 0.405 0.001 Gomphonema parvulum (Kützing) Kützing var. parvulum f. parvulum Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt 3 0.399 0.001 Gomphonema olivaceum (Hornemann) Brébisson var. olivaceum 3 0.374 0.001 3 0.291 0.001 Meridion circulare (Greville) Agardh var. circulare Gomphonema pumilum var. elegans Reichardt & Lange-Bertalot 3 0.001 0.275 3 0.223 0.001 *Gomphonema gracile* Ehrenberg Gomphonema tergestinum Fricke 3 0.001 0.145 0.001 Gomphonema parvulius Lange-Bertalot & Reichardt 3 0.110 Gomphonema angustum Agardh 3 0.001 0.103 0.001 Ulnaria biceps (Kützing) Compère 3 0.102 Navicula exilis Kützing 3 0.091 0.001 Eunotia minor (Kützing) Grunow in Van Heurck 3 0.085 0.001 3 0.062 0.004 Achnanthes linearioides Lange-Bertalot Gomphonema acuminatum Ehrenberg var. coronata (Ehrenberg) W. Smith 3 0.088 0.005 Encyonema silesiacum (Bleisch) Mann 4 0.583 0.001 4 0.400 0.001 Achnanthidium pyrenaicum (Hustedt) Kobayasi 4 0.001 Encyonema ventricosum (Agardh) Grunow 0.379 Amphora ovalis (Kützing) Kützing 4 0.348 0.001 Fragilaria arcus (Ehrenberg) Cleve var. arcus 4 0.339 0.001 4 0.310 0.001 Gomphoneis minuta (Stone) Kociolek & Stoermer var. minuta 4 0.001 Cyclotella meneghiniana Kützing 0.309 Reimeria sinuata (Gregory) Kociolek & Stoermer 4 0.306 0.001 4 Sellaphora pupula (Kützing) Mereschkowksy 0.290 0.001 Diatoma ehrenbergii Kützing 4 0.282 0.001 Nitzschia fonticola Grunow 4 0.245 0.001 4 *Surirella suecica* Grunow 0.244 0.001 4 0.243 0.001 Fallacia subhamulata (Grunow) Mann Navicula rostellata Kützing 4 0.199 0.001 Achnanthidium subatomus (Hustedt) Lange-Bertalot 4 0.184 0.001 Navicula submuralis Hustedt 4 0.180 0.001 Achnanthidium latecephalum Kobayasi 4 0.152 0.001 Navicula germainii Wallace 4 0.130 0.001 4 0.001 Navicula cryptofallax Lange-Bertalot & Hofmann 0.119 4 0.001 Encyonema mesianum (Cholnoky) Mann 0.095 Sellaphora bacillum (Ehrenberg) Mann 4 0.093 0.001 Amphora copulata (Kützing) Schoeman & Archibald 4 0.242 0.002 Surirella angusta Kützing 4 0.217 0.002 Hippodonta neglecta Lange-Bertalot Metzeltin & Witkowski 4 0.121 0.002 Navicula novaesiberica Lange-Bertalot 4 0.003 0.211

Caloneis silicula (Ehrenberg) Cleve	4	0.138	0.003
Surirella minuta Brébisson	4	0.122	0.003
Cyclotella cyclopuncta Håkansson & Carter	4	0.108	0.003
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin	4	0.133	0.004
Navicula viridula (Kützing) Ehrenberg	4	0.116	0.004