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Sediment quality of lake Seealpsee: presence of micro-plastics and tire associated chemicals, ecotoxicological and biological assessments

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Summary

The water protection service of the Canton of Appenzell Innerrhoden mandated the Ecotox Centre for measuring microplastics in sediment samples collected at two different sites of Lake Seealpsee and performing in parallel an ecotoxicological test (ostracod test) on these samples. The Ecotox Centre complemented the investigations of these sites with measurements of tire associated chemicals in the sediment samples and examination of in situ oligochaete communities. Sediment chemical analyses showed some microplastics pollution and the presence of some tire associated chemicals in significant concentrations. The sources of this pollution could be both the atmospheric dust and the recreational activities around the lake. The results of the biological and ecotoxicological investigations indicated that the quality of sediments was altered, due to the presence of contaminants and/or to a eutrophication.

Content

1 Introduction

The water protection service of the Canton of Appenzell Innerrhoden mandated the Ecotox Centre for measuring microplastics in sediment samples collected at two different sites of lake Seealpsee and performing in parallel an ecotoxicological test (ostracod test) on these samples. The Ecotox Centre complemented the investigations of these sites with measurements of tire associated chemicals in the sediment samples and examination of *in situ* oligochaete communities.

Seealpsee is a small alpine lake at an altitude of 1’143 meters with a natural watershed. The lake provides hydroelectric power and drinking water to the town of Appenzell, though the small stream Schwendibach. The region is a popular hiking spot and touristic destination. There are two restaurants directly at the lake shore. Recreational activities include hiking, barbecue, picnicking and aquatic activities (swimming, stand-up paddle, rowing boat).

It is noteworthy that a red algal bloom was observed in this lake in the summers 2009 and 2010[1]. It resulted from the proliferation of the dinoflagellate algae *Tovellia sanguinea*, present as cyst in the sediment, which was caused by excess of nutrients (nitrogen, phosphorus). The proliferation of this algae could indicate that the lake was eutrophic (or unable to recycle all received nutrients). The causes of this excess of nutrients in the lake could have been the agricultural activities in the catchment area and/or the presence of effluents from the guest houses, or natural.

Thanks to the introduction of treatments of the effluents from alpine guest houses and protection against manure runoff from stables, the water quality of the lake has increased lately (News archives, Kanton Appenzell Innerrhoden). It was demonstrated in a Master thesis that the tourism led to the significant presence of litter and macroplastics in the surroundings of this lake (Niebling, 2024). The eventual presence of significant concentrations of microplastics in our samples could be partly attributed to the pollution generated by the recreational activities around the lake (fragmentation of macroplastics).

[1] www.swissinfo.ch/eng/climate-change/science-solves-the-mystery-of-the-red-lake/1006602
www.20min.ch/fr/story/un-miracle-rouge-dans-un-lac-appenzellois-262517608093,
de.wikipedia.org/wiki/Seealpsee_(Appenzeller_Alpen)

2 Material and Methods

2.1 Sampling sites

The sampling was performed during a diver camp on August 13th, 2024. Two sites (A and B) were chosen by the divers (Fig. 1).

Fig. 1: Sampling sites in Lake Seealpsee (left) and satellite view (right) (map.geo.admin.ch).

The sediments were sampled at 8 m and 12.5 m depth for site A and site B, respectively. Oxygen content in the water was relatively low (slight hypoxia) (Tab. 1).

Tab. 1: Water parameters at the sampling day.

Site	Depth (m)	pH	Temp. (°C)	O_2 (mg/L)	O_2 (%)
A	12.5	7.2	6	6.4	51
B	8	7.3	8	5.2	44

For the analysis of microplastics and tire associated chemicals, and the bioassay, sediment samples (top 2-5 cm) were collected using plastic manual corers at 3 locations per site, regrouped in a composite sample. The sediment was then homogenized and sieved at 2 mm. Samples were stored in glass (microplastics and chemical analyses) or plastic (bioassay) containers. They were preserved at 4°C before being sent to the Ecotox Centre and the external partner performing the bioassay.

For the assessment of oligochaete communities, sediments were sampled at three different locations (spaced about 5-10 m apart) per site. In total, 36 cores were sampled for each site (12 cores per location pooled into one 5 L recipient). The sediment samples were preserved in neutral buffered formalin immediately after sampling (final formaldehyde concentration of 4%) and preserved at 4ºC before being sent to the Ecotox Centre.

Unfortunately, some glass containers were damaged during the transport. While the microplastics and physico-chemical analyses could only be performed on the sample of site B, the ecotoxicological test could be applied on the samples of both sites.

2.2 Physico-chemical analyses

2.2.1 Grain-size distribution

The analysis was done on fresh material (of site B only) by the Department F.-A. Forel for Environmental and Aquatic Sciences at University of Geneva by Laser Diffraction Particle Size Analyzer (COULTER®). This sediment property can help in the interpretation of the results from the ecotoxicological assessments.

2.2.2 Total organic carbon (TOC)

The analysis was done on dry sediment sample (of only site B) by the Central Environmental Laboratory at EPFL in Lausanne by high-temperature combustion using the elementary analyzer Vario TOC cube (Elementar, Lyon, France).

2.2.3 Microplastics

The analysis of microplastics were performed by the Central Environmental Laboratory at EPFL in Lausanne using the Laser Direct Infrared Imaging method. Three replicates of fresh sediment (of site B) were drought at 70°C in an oven. Dry samples were mixed with 100 mL distilled water and 50 mL of 30% H_2O_2. The samples were stirred (250-450 rpm) and heated at 90°C for 24 hours. After digestion, the samples were sieved at 100 µm. The material was then mixed with 50 mL of $ZnCl_2$. After 30 min, the supernatant was recovered and filtered (12 µm). The filtered was rinsed with ethanol. After evaporation with nitrogen at 60°C to 150 µl, the material was transferred to a LDIR microscope slide.

The instrument Agilent LDIR 8700 (Fig. 2) scans an area using light of a single wave number to locate all the particles. It takes 4 minutes to scan an area 10 x 10 mm at 5 µm pixel size. The instrument then targets the identified particles (appearing as bright spots) and collects an IR spectrum of each one. Each spectrum is then compared against a spectral library to identify the chemical composition of each natural or synthetic particle (e.g. chitin, sand, polypropylene, polystyrene, …). The LDIR has a high-magnification visible camera to photograph particles. The identification of the plastic is associated with a quality score. The Central Environmental Laboratory recommends using a quality score of at least 85%.

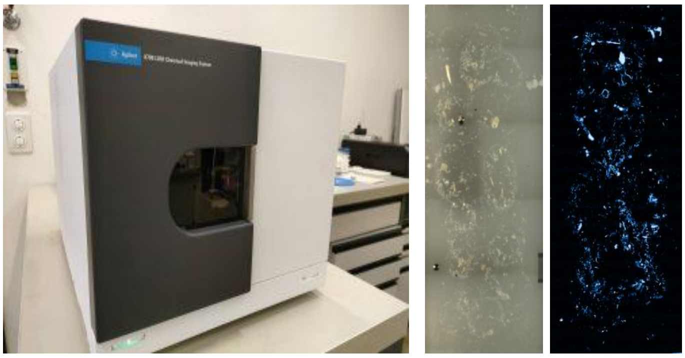

Fig. 2: LDIR at EPFL and one replicate slide for the sediment sample from site B (raw and scanned images).

2.2.4 Tire associated chemicals

The analysis of tire associated chemicals was performed by the Central Environmental Laboratory at EPFL in Lausanne. We had the opportunity to have them analysed in the context of a joint project between EPFL and Ecotox Centre[2] () and a citizen science project[3] (/). It was interesting to study if tire associated chemicals could be present in a lake where direct sources of these contaminants were absent or limited. It is acknowledged that tire associated chemicals can reach alpine lakes by dust deposition.

For this, the sediment sample was first freeze-dried. A subsample of sediment (500 mg) was spiked with a deuterated internal standard mixture and extracted with an Accelerated Solvent Extractor (ASE 350, Dionex®). A two-cycle extraction was performed at 100°C with a mixture of

[2] https://www.ecotoxcentre.ch/projects/sediment-ecotoxicology/ecotoxicity-of-tire-wear-particles
[3] https://actu.epfl.ch/news/a-citizen-science-initiative-evaluates-the-impac-2

Acetone/Dichloromethane (1:1, v:v). Extracts were concentrated under a gentle flow of nitrogen to 2 mL. The extract was then analysed by GC-MS/MS (TSQ Quantum XLS Ultra, Thermo Scientific®) for antioxidants and vulcanization accelerators: aniline, 2-hydroxybenzothiazole (2H-BT), 2-(methylthio)benzothiazole (MTBT), 1.3-diphenylguanidine (DPG), N-(1.3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and 6PPD quinone (6PPD-Q) and 4-hydroxydiphenylamine (HPDA).

2.3 Ecotoxicological test

The ecotoxicological test with the ostracod *Heterocypris incongruens* was applied on fresh sediments (from sites A and B) by Soluval Santiago. The test relies on the survival and the growth of synchronised organisms exposed during 6 days to the sediment.

The bioassay was performed according to the ISO 14371 standard experimental protocol (ISO 2012) using the Ostracodtoxkit F™ (MicroBioTests, Ghent, Belgium). Briefly, incubation of ostracod cysts began 52 h prior to testing. They were placed in a Petri dish containing 10 mL of standard fresh water (medium hardness) and incubated at 25°C under continuous illumination. After 48 h, the hatched cysts were fed a spirulina solution and incubated for a further 4 h. On the day of the test run, 6-well microplates (1 plate of 6 wells per sample/control) were filled with 2 mL of standard fresh water and 1 mL of sediment per well. Next, 2 mL of algae were added (*Scenedesmus spp*., also prepared in standard fresh water). As control sediment, the reference sediment supplied with the kit was used. Finally, 10 ostracods were transferred to each microplate well. The microplates were incubated in the dark at 25°C for 6 days. At the end of the exposure period, the surviving ostracods were recovered and immobilised with Lugol's solution. They were then counted for each well (calculation of the mortality rate) and transferred to a glass slide to measure their length using a binocular magnifier (CellD software, Olympus, Hamburg). The length was converted into growth (difference between the length at the end and the average length of 10 organisms at the start of the exposure).

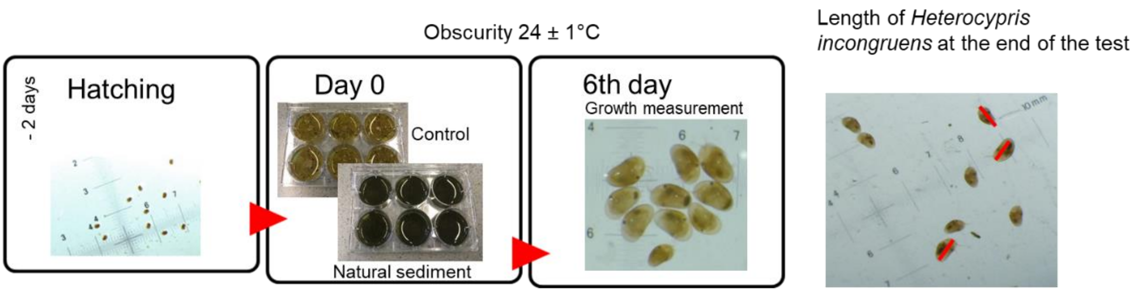

Fig. 3: Summary of the bioassay protocol with the ostracod H. incongruens.

To determine if the sample is toxic, the effects observed are compared to toxicity thresholds that have been defined taking into account the natural variability of organisms' responses to the intrinsic characteristics of sediments (granulometry, organic carbon content, etc.). These thresholds are 20% for mortality and 35% for growth inhibition compared with the control (Casado-Martinez et al. 2016).

2.4 Analysis of oligochaete communities

Aquatic oligochaetes include a large number of species ranging from sensitive to resistant to pollutions (Lafont, 1989; Rodriguez & Reynoldson, 2011) and play an important role in the mineralization of organic matter in sediments (Lafont et al., 2012). Analysis of oligochaete communities in a lake makes it possible to assess the biological quality of sediments and to estimate the capacity of sediments to assimilate and recycle nutrients (AFNOR, 2016; Lafont, 2007; Lafont et al., 2012).

Sediments were sieved through a column of sieves with 5 mm and 0.5 mm mesh sizes in the laboratory. For each site, the sieved sediment of each place was combined and preserved in absolute ethanol at -20°C. For the extraction of oligochaetes, the sieved sediment of each site was transferred into a square sub-sampling box (5 x 5 cells) and the content of randomly selected cells was examined under a stereomicroscope (in Petri dishes). Per site, 100 specimens were extracted, and the number of cells was used to determine the abundance of oligochaetes per 0.1 m^2 of sediments. Sorted oligochaetes were then mounted on slides in a coating solution composed of lactic acid, glycerol and polyvinylic alcohol (Reymond 1994) and identified to the lowest practical level (species if possible) using a compound microscope.

The oligochaete index of lake bioindication (IOBL), allowing to assess the potential of the sediments to assimilate and recycle nutrients from both water and sediments (AFNOR, 2016; LAFONT et al. 2012), was calculated. This index is computed according to the following formula:

$$IOBL = S + 3 \log_{10} (D+1)$$

The terms “S” is the number of taxa identified among 100 oligochaetes and “D” is the density of oligochaetes per 0.1 m^2. This index provides information on the functioning of the sediments and ranks the metabolic potential into 6 classes as described in Tab. 2.

Tab. 2: Classes of metabolic potential of lake sediments according to IOBL values.

IOBL value	Metabolic potential
>15	Strong (big lakes)
10–15	Strong
6.1–9.9	Moderate
3.1–6	Low
0.1–3	Very low
0	Null

In addition, oligochaete taxa were regrouped in four ecological groups according to the classification of Lafont (2007) and AFNOR (2016) (Tab. 3). Examination of the percentages of these ecological groups allows to complete the diagnosis by providing information, among others, on the trophic state of the environment (oligotrophic or non-oligotrophic) and on the effects of contaminants (in sediments) or of natural dystrophy.

Tab. 3: Definition of the different ecological groups of oligochaete taxa.

Ecological group	
1	Includes sensitive taxa to chemical pollution (toxic type and/or eutrophication)
2	Includes moderately resistant taxa to chemical pollution (toxic type and/or eutrophication)
3	Includes taxa which characterize a natural dystrophy (due to e.g., presence of peat, coarse vegetal detritus, abundance of Characeae)
4	Includes resistant taxa to chemical pollution (toxic type and/or eutrophication)

The biological quality of the sediments was assessed using the percentages of sensitive taxa to chemical pollution (group 1) according to the classification of Lafont (2007) and AFNOR (2016) (Tab. 4).

Tab. 4: Biological quality of sediments according to the percentages of sensitive oligochaete taxa (group 1).

% of sensitive taxa	Biological quality
>50	Very good
21–50	Good
11–20	Medium
6–10	Poor
≤5	Bad

Unlike those of group 1, the percentages of taxa of groups 2, 3 and 4 cannot be determined accurately because several tubificid species belonging to these groups cannot be identified when the individuals are in an immature state. The Tubificinae with hair setae not recognizable in an immature state can belong to groups 3 or 4 and the Tubificinae without hair setae not recognizable in an immature state to groups 2 or 4. All species of Tubificinae without hair setae belong to group 4, except *Limnodrilus profundicola* (group 2).

3 Results

3.1 Grain-size and total organic carbon

The average size of the particles from site B is 14 µm, which corresponds to silt (Fig. 4).

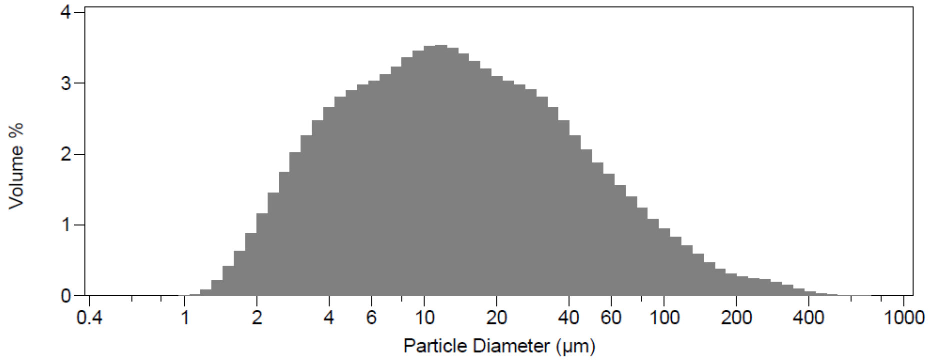

Fig. 4: Distribution of the size of sediment particles from site B.

The measured total organic carbon was 2.8% (average of 3 measurements). Assuming that organic carbon constitutes approximately 50% of the organic matter content of sediments, it is possible to convert this value to the organic matter content using a factor 2. This would give 5.6% of organic matter.

3.2 Microplastics

Ignoring natural particles like chitin, cellulose and polyamide, and applying the quality score threshold of 85%, we could estimate the presence of 23'000 particles / kg of dry sediment with an average size of 307 µm (size range 75 - 1855 µm). The three most detected plastics were acrylonitrile butadiene styrene (ABS, 27%), polypropylene (PP, 26%), polyurethane (PE, 26%), while rubber (11%), PET (9%), PVC (1%) and polytetrafluoroethylene (1%) were the less abundant. No polystyrene was detected.

3.3 Tire associated chemicals

Five out of the seven measured tire associated chemicals were detected (Tab. 5). The concentrations ranged from 0.05 ng/g dw for HDPA to 41 ng/g dw for aniline. We compared the obtained concentrations to those measured in sediments and suspended particulate matter (SPM) collected in Lake Geneva in 2023. While measured concentrations of 6PPD, DPG and HDPA in sediments of Lake Seealpsee were much lower than in sediments and SPM of Lake Geneva, the concentrations of 2-hydroxybenzothiazole (2H-BT) and aniline were higher in sediments of Lake Seealpsee. MTBT and the transformation product 6PPD-Q were not detected.

Tab. 5: Concentrations of tire associated chemicals measured in sediments of Seealpsee (present project) and in sediments (collected in 2023 at 100 m depth) and suspended particulate matter (SPM) of Lake Geneva. SPM was collected (in Lake Geneva) at 30 m depth from February to July (SPM 1) and from July to November (SPM 2). All mentioned concentrations are in ng/g dw; LOQ = limit of quantification.

Compound	Seealpsee	Lake Geneva sediments	Lake Geneva SPM 1	Lake Geneva SPM 2	LOQ
Aniline	41.0	9.3	9.1	22.8	0.06
2-hydroxybenzothiazole (2H-BT)	9.77	<LOQ	<LOQ	<LOQ	0.97
2-(methylthio)benzothiazole (MTBT)	<LOQ	5.54	<LOQ	2.44	1.46
1,3-diphenylguanidine (DPG)	2.83	23.1	25.1	56.9	0.05
N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)	1.33	28.6	22.5	54.9	0.58
6PPD quinone (6PPD-Q)	<LOQ	1.0	0.9	2.2	0.09
4-hydroxydiphenylamine (HDPA)	0.05	0.17	0.16	0.26	0.05

3.4 Ecotoxicological test

The results of the bioassay showed a strong mortality of the ostracods in the samples of both sites (Fig. 5).

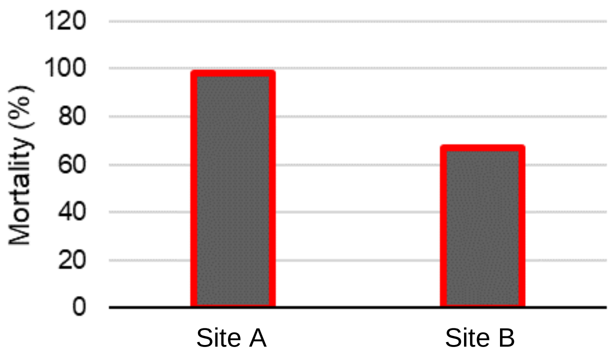

Fig. 5: Results of the bioassay with the ostracod H. incongruens. Red color indicates exceedance of the corresponding toxicity thresholds (20% mortality.)

The mortality rate was higher for sediment of site A (98%) than site B (67%). Both mortality rates largely exceeded the toxicity threshold of 20%, suggesting that the sediments are toxic to these benthic organisms. For site B, the surviving organisms showed a growth inhibition of 77% compared to the control (over the 35 % toxicity threshold). However, the test standard doesn't recommend using growth inhibition when mortality exceeds 30%. The raw data are available in Annexe 1.

3.5 Oligochaete communities

3.5.1 Oligochaete diversity

We observed a very poor diversity of oligochaetes at both sites. Only taxa belonging to the subfamily Tubificinae (Naididae) were found (Tab. 6). These taxa are very common in Swiss lakes.

Tab. 6: Number of specimens per taxon at the two study sites; the ecological group of each taxon is indicated in brackets.

	Taxon	Site A	Site B
Tubificinae (Naididae)	Tubificinae with hair setae (that cannot be identified to the species level)	38	35
	Tubifex tubifex (3)		1
	Tubificinae without hair setae (that cannot be identified to the species level)	56	38
	Limnodrilus hoffmeisteri (4)	8	22

3.5.2 Metabolic potential and biological quality

Oligochaete communities indicated, at each site, a strong metabolic potential and therefore a good capacity of sediments to assimilate and recycle organic matter (Tab. 7). Oligochaete density was very high at both sites (4586 individuals per 0.1 m^2 for site A, 6173 individuals per 0.1 m^2 for site B) (Annexe 2).

Tab. 7: Oligochaete results (metrics) obtained at the two sites.

	Site A	Site B
IOBL index	14	15.4
Metabolic potential	Strong	Strong
% of taxa of group 1 (sensitive to chemical pollution)	0	0
% of taxa of group 2 (moderately resistant to chemical pollution)	0	0
% of taxa of group 3 (indicative of natural dystrophy)	0	1
% of taxa of group 4 (resistant to chemical pollution)	8	22
Biological quality of sediments	Bad	Bad

The biological quality indicated by the composition of oligochaete communities was classified as bad at each site. The percentages of groups 2 (moderately resistant to chemical pollution) and 3 (indicative of natural dystrophy) were null or very low at all sites and the percentages of group 4 (resistant to chemical pollution) were quite high at site A and high at site B. However, as mentioned previously, the percentages of groups 2, 3 and 4 cannot be determined precisely based on morphological identifications. The percentages of groups 3 and 4 are probably largely underestimated.

4 Discussion

4.1 Chemical results of sediments

The organic matter content of Lake Seealpsee sediment (5.6%) was in the median range of values usually found in sediments from Swiss water bodies (Beauvais et al., 2020). This percentage is comparable to findings from other Swiss lakes. For example, in a small alpine lake in the canton of Vaud (Lake Lioson, unpublished), similar in size and altitude to Lake Seealpsee, the measured organic matter content in sediments in June 2024 at a depth of 13 m was 5.73%. In Lake Geneva sediments, the percentage of organic matter in 2015 ranged between 3% and 11% (Loizeau et al., 2017).

Results showed some microplastics pollution. The sources of this pollution could be both the atmospheric dust and the recreational activities around the lake. Different types of plastics were found: acrylonitrile butadiene styrene (ABS), polypropylene (PP), polyurethane (PE), rubber, polyethylene terephthalate (PET), polyvinyl chloride (PVC) and polytetrafluoroethylene (PTFE). The results of the Master thesis (Niebling, 2024) mentioned in Introduction showed that food and drink packages, and cigarettes were the most abundant litter on the shores of this lake. The most abundant types of plastics were also PP, PE and PET but ABS was not found. In the same project, the analysis of microplastics (size range 300 - 5000 µm) in littoral sediments at two shore sites resulted in 9 and 3 particles per kg of dry sediment, which is about three orders of magnitude lower than what we obtained (23'000 particles / kg of dry sediment). This indicates that the microplastics concentrate in the deeper parts of the lake together with fine particles rather than at the shores. However, in the present project, we assessed smaller microplastics (size range 75 - 1855 µm), which may be more abundant in number and explain this difference.

We showed that tire associated chemicals could be found in this small mountain lake, only reachable by walk. The chemical 2H-BT, found at higher concentration than in sediments and SPM of Lake Geneva, is also known to be used in cosmetics and personal care products. Therefore, this chemical could have been introduced in the lake by swimmers. For this substance, a "predicted no effect concentration" for sediment ($PNEC_{sed}$) of 10 ng/g dw has been proposed for the protection of benthic organisms (Hu et al., 2021). The measured concentration in Lake Seealpsee sediments (9.77 ng/g, dw) is just below this threshold. Given that this $PNEC_{sed}$ value is considered provisional[4], it would be necessary to determine a PNEC value for this substance based on ecotoxicological tests on benthic organisms to exclude potential adverse effects due to the presence of 2H-BT on the sensitive species.

We also found a higher concentration of aniline in sediments of Lake Seealpsee than in sediments and SPM of Lake Geneva sediments. A concentration of 9.3 ng/g dw was measured in Lake Geneva sediments at 100 m depth, while in the present study a concentration of 41 ng/g dw was found. In anoxic conditions, aniline may be persistent in sediments. Such conditions could be prevalent in summer in Lake Seealpsee. The results in this project should however be interpreted with caution as the extraction of aniline from sediment samples may suffer from a matrix effect and the concentrations could have been overestimated (personal communication from the Central Environmental Laboratory). A $PNEC_{sed}$ for this substance of 153 ng/g dw, so much higher than the concentration that we measured, is proposed by ECHA[5]. This $PNEC_{sed}$ was derived using data on the effect on the chironomid *Chironomus riparius* and the oligochaete *Lumbriculus variegatus*. However *L. variegatus* is not considered particularly sensitive to chemical pollution by ecologists (Lafont, 1989). This may explain why effects could still be observed in more sensitive benthic species, despite the assessment factor of 100 applied in the PNEC derivation.

[4] This $PNEC_{sed}$ is derived using the Equilibrium Partitioning and the PNEC for pelagic organisms. $PNEC_{sed}$ derived using this approach are considered preliminary, and the results of the risk assessment can be refined using a PNECsediment derived using ecotoxicological data for benthic invertebrates.

[5] The registrations dossiers are available here: https://chem.echa.europa.eu/100.000.491/overview?searchText=aniline.

A possible cause of the presence tire associated chemicals is atmospheric dust deposition. Aniline and DPG being also used in other industrialized products (e.g. plastics), their presence in this lake could be also explained by the recreational activities around the lake.

4.2 Ecotoxicological and biological assessments

The results of the ecotoxicological test were quite striking, with 98 and 67 % mortality for site A and B respectively. Such results could be explained by the presence of chemical pollution (toxic and/or eutrophication) in sediments and/or by the properties of sediments.

The presence of fine sediment has shown to affect ostracods growth (Casado et al., 2016), and together with chemical pollution it is not known if also survival could be affected. Such high mortality was also observed in fine sediments of a river downstream of a wastewater treatment plant (not published) and to a lesser extent in suspended matter in Lake Geneva. In a study performed in 10 small lakes from Kanton Bern, such high mortality rates were only observed in a single lake that is anoxic most part of the year but also with intensive agricultural use of its catchment. However, sediments from lakes with high to medium amounts of organic matter showed some degree of toxicity in the ostracod test, which may be caused by oxygen depletion or ammonia and sulfide generation during organic matter decomposition (not published).

The causes of the bad biological quality indicated by oligochaete communities could be a toxic pollution and/or a eutrophication. Such results contrast with those obtained in 2024 in a mountain lake (Lake Lioson) at a similar depth (13 m), where 82% of sensitive oligochaete taxa were found (Vivien & Ferrari, 2024). Thus, as the oligochaete results confirmed the possible presence of chemical pollution at these sites, the results of the ostracod test could be at least partly explained by chemical pollution.

The eutrophication could be man-induced (agricultural activities, insufficient treatments of the effluents from alpine guest houses, etc.) or natural. Lakes represent an important sink for N, P and biogenic elements (Zhang et al., 2022). Eutrophication is not necessarily caused by excessive inputs of fertilizers (nitrates and phosphates) related to human activities (Zhang et al., 2022; Akinnawo, 2023). A lake can lose its self-purification capacity over certain decades, i.e. its ability to recycle all received nutrients (Akinnawo, 2023). Eutrophication in natural lakes can be explained by diverse factors such the age of the lake (old lakes tend to be more eutrophic), the duration of water retention (short duration favor self-purification) and the dynamics of hydrological exchanges between surface water and groundwater (exfiltration or alternance infiltration/exfiltration favor self-purification). Eutrophication causes a lack of oxygenation in water/sediments and can also result in excessive concentrations of toxic compounds for aquatic organisms, such as NH_3.

5 Conclusion

The results of the biological and ecotoxicological investigations indicated that the quality of sediments was altered at both sites, due to the presence of contaminants and/or to a eutrophication. In addition, some measured microplastics and tire associated chemicals, whose presence in the lake could be explained by atmospheric dust deposition and/or by the recreational activities around the lake, could be in sufficient concentration to possibly induce harmful effects to the benthic communities.

There is a need to extend the chemical analyses of the sediment, for instance by adding the measurement of metals and/or some micropollutants in sediments. The measurement of major elements (nitrates, nitrites, phosphorus, ammonium, etc.) in water at different periods could also provide information on the trophic state of the lake. Possibly, this information could be complemented by measurement in sediments of toxic compounds issued from the degradation of organic matter, such as NH_3. In addition, the following additional investigations would be possible to confirm our biological/ecotoxicological results: application of other bioassays (e.g. chironomid emergence test (AFNOR, 2010) and nematode growth and reproduction test (ISO, 2020) and examination of other benthic communities such as chironomids and/or nematodes.

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Appendix 1 Ostracod test report (Soluval Santiago)

Soluval Santiago

Analyses environnementales
Ecotoxicologie

Couvet, le 19 septembre 2024

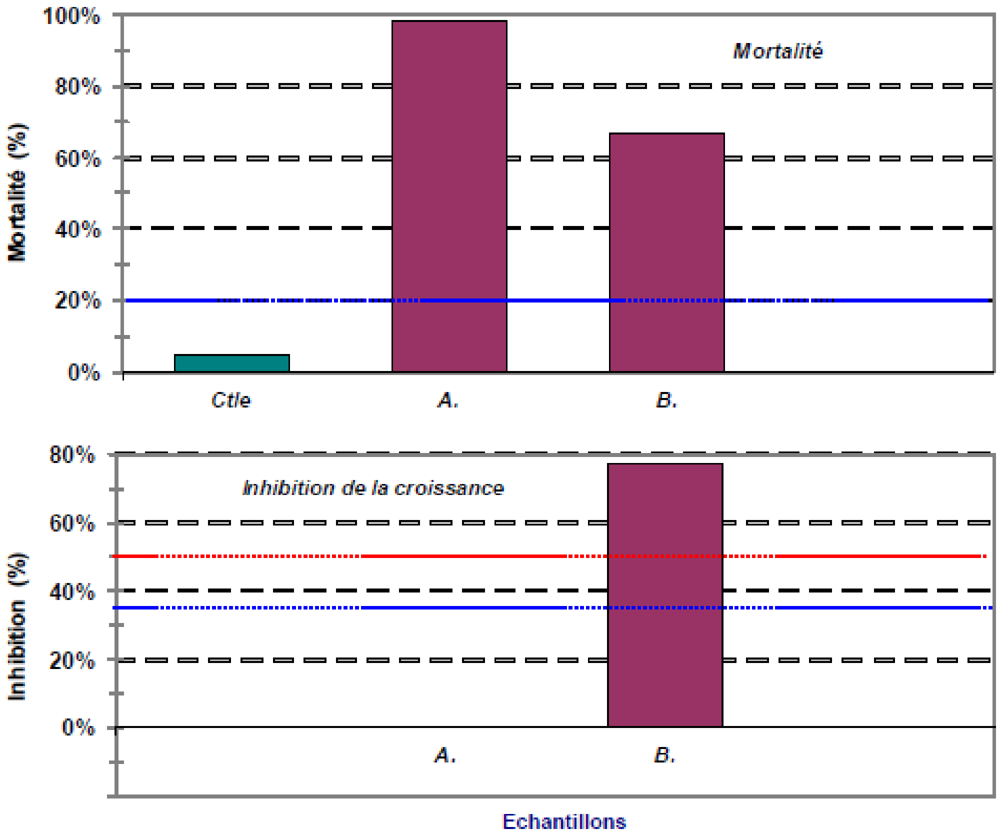
8872 – Appenzell Seealpsee Ostracodes_2024

Ecotoxicité associée à des sédiments du lac Seealpsee (Appenzell, AI) d'après le test avec Ostracodes

Résultats
des bioessais
en Août 2024

Pour : AMT FÜR UMWELT
APPENZELL INNERRHODEN
CH - 9050 APPENZELL

OEKOTOXZENTRUM
CH - 8600 DÜBENDORF



Sergio Santiago

Récapitulation des résultats

Soluval Santiago
Analyses environnementales
Rue Edouard-Dubied 2 Tél: 032 863 43 60
CH - 2108 COUVET e-mail: ssantiago@bluewin.ch

Bioessais de toxicité
Récapitulation des résultats

Identification

Origine :	*Seealpsee - Appenzell Rhodes intérieures (AI)*	Destinataires :	*Mme R. Beauvais*
Type d'échantillon :	*Sédiments bruts (< 2 mm)*	Société :	*Oekotoxzentrum*
		Adresse :	*8600 Dübendorf*
Prélèvements :	☑ *instantané* ❑ *intégré*		
Date :	*13 Août 2024*	Plan d'analyse(s) :	*Ostracodes (OstracodToxkit F)*
Effectués par :	*Plongeurs / Amt für Umwelt Appenzell AI*		
Echantillons n°:	*A.* *B.*	Date de réception :	*14 - 08 - 2024*
		Enregistrement n°:	*8872*
		Responsable :	*S. Santiago*

Remarque : gobelets en plastique [A : Flacon verre brisé ; B : flacon verre fissuré]

Test Ostracodes
Heterocypris incongruens
(selon ISO 14371)

Organisme : *Heterocypris incongruens (Microbiotests inc.)*
microplaque (PS; 6 puits); 25 ±1°C; obscurité
Dilution : milieu *SMHW*; nour. *Algues (Scenedesmus spp., R.s.)*

Date : 21-08-2024
Effectué par : SS
Contrôlé par :

Echantillon	Sédiment	Mortalité à 6 jours	Toxicité subléthale : Inhibition de la croissance des ostracodes — Longueur moyenne par puits à 6 jours (µm) — Puits (moyenne des survivants)			Moyenne	Ecart-type	Croissance (%)	Inhibition (%)
Contrôles témoins (= réf. Microbiotests) Long. initiale moy. J_0 = 205.6 µm		3 / 60 = 5,0 %	634 / 602	640 / 688	710 / 653	654.3	39.2 = 6.0 %	100%	0%
A.	*brut*	59 / 60 = 98,3 %	594 / (10 † /10)	(10 † /10) / (10 † /10)	(10 † /10) / (10 † /10)				
B.	*brut*	40 / 60 = 66,7 %	280 / 279	342 / 288	312 / 342	307.4	29.6 = 9.6 %	22.7%	77.3%

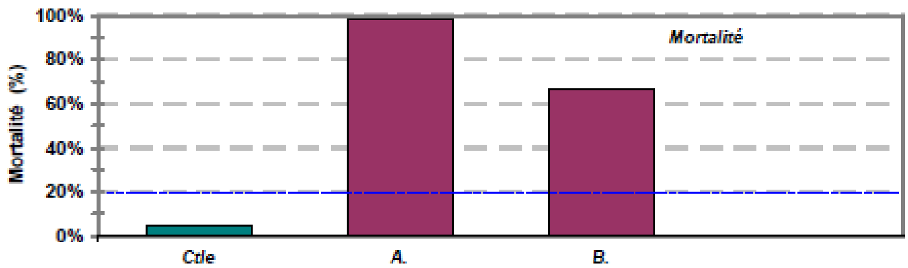
Remarques :

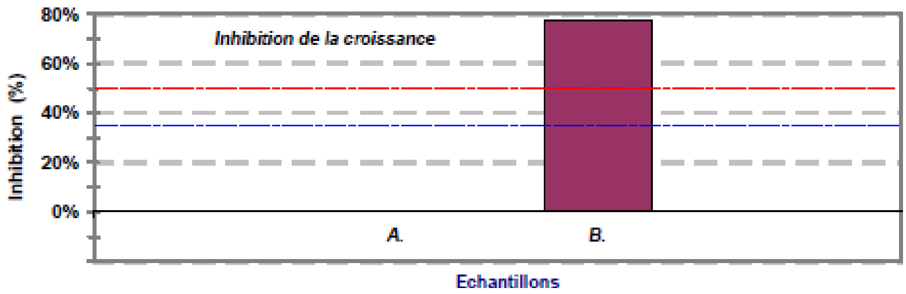
Conclusions - Commentaires

Mortalité	*Sédiments*	*Inhibition de la croissance*	
≤ 10% :	-	≤ 20% :	-
11% - 20% :	-	21% - 35% :	-
21% - 30% :	-	36% - 50% :	-
> 30% :	A ; B	> 50% :	B (A : non pertinent)

Essai valide ☑ oui - ❑ non
Contrôles (sédiment de référence) :
Mortalité < 20% ☑
Coefficient de croissance ≥ 1,5 ☑

Couvet, 19-09-2024 p. 1 / 2





Test Ostracodes - *OstracodToxkit F*
Heterocypris incongruens
Longueurs à 6 jours [en µm]

Origine : Appenzell
Type d'échantillon : Sédiment lac Seealpsee

Echantillons : A - B
Date : 13.08.2024

Enregistrement n° : 8872
Début de l'essai : 21.08.2024

Effectué par : *SS*
Remarques : x = mort
oo = vivant, non mesurable

Contrôle initial		
193.8	194.5	208.7
213.5	204.6	204.3
210.3	214.4	
200.4	206.7	
211.7	212.0	
201.9	201.8	
208.0	203.0	
Moyenne		**205.6**
écart-type		*6.2*
		3.0%

	Contrôle (sédiment de référence)						A. (séd. brut)						B. (séd. brut)					
Répl.	*1*	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*
1	652.0	635.9	770.9	749.6	657.4	657.3	594.5	*Aucun survivant [10 morts / 10]*	*Aucun survivant [10 morts / 10]*	*Aucun survivant [10 morts / 10]*	*Aucun survivant [10 morts / 10]*	*Aucun survivant [10 morts / 10]*	279.9	328.9	304.7	276.0	289.8	370.9
2	642.4	639.2	650.0	505.7	761.4	788.1	x						x	349.4	317.4	295.5	286.6	356.1
3	642.7	627.6	777.1	618.0	772.5	630.6	x						x	348.3	280.8	262.6	x	342.8
4	736.4	759.9	755.6	604.5	641.6	639.3	x						x	x	304.1	283.9	x	300.1
5	615.2	647.1	837.0	624.0	647.1	631.4	x						x	x	330.1	x	x	x
6	559.0	655.5	809.8	623.5	627.2	631.7	x						x	x	336.0	x	x	x
7	627.9	628.0	595.5	480.5	749.7	619.2	x						x	x	x	x	x	x
8	537.6	542.1	645.4	631.8	784.7	626.5	x						x	x	x	x	x	x
9	689.3	621.9	639.6	578.3	617.2	648.5	x						x	x	x	x	x	x
10	oo	x	621.6	x	623.7	x	x						x	x	x	x	x	x
Mortalité (%)	3 / 60 =	**5.0%**					59 / 60 =	**98.3%**					40 / 60 =	**66.7%**				
Longueur moyenne, par puits	633.6	639.7	710.2	601.8	688.2	652.5							279.9	342.2	312.0	279.5	288.2	342.5
Longueur moyenne (tous puits)			***654.3***	±	*39.2*	*6.0%*									***307.4***	±	*29.6*	*9.6%*
Croissance moyenne, par puits	428.0	434.1	504.6	396.1	482.6	446.9							74.3	136.6	108.4	73.9	82.6	136.9
Croisance moyenne (tous puits)			***448.7***	±	*39.2*										***101.8***	±	*29.6*	
Croissance / Inhibition moy. (%)	**100%**	(Facteur croissance = 2.2)													**22.7%**	/	**77.3%**	

Répl.																		
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
Mortalité (%)																		
Longueur moyenne, par puits																		
Longueur moyenne (tous puits)																		
Croissance moyenne, par puits																		
Croisance moyenne (tous puits)																		
Croissance / Inhibition moy. (%)																		

Contrôles : facteur croissance = 2.18

Critères de validité pour sédiment de référence :
Mortalité ≤ 20% ☑
Facteur croissance > 1,5 ☑

Couvet, 30-08-2024
S. Santiago

Draft

Inhibition de la croissance (%)
Inhibition ≤ 20%
21% ≤ Inhibition ≤ 35%
36% ≤ Inhibition ≤ 50%
Inhibition ≥ 50%

Appendix 2 Detail for calculation of oligochaete densities

	Site A	Site B
Surface in m^2	0.0572	0.0572
Number of specimens sorted per x examined cells (sub-sampling box)	105	106
Number of examined cells	1	0.75
Density (number of specimens per 0.1 m^2	4586	6173