POSSIBLE EFFECTS OF LANDFILL LEACHATE ON BROWN TROUT (*SALMO TRUTTA*) – A BIOMARKER INVESTIGATION AND CONTAMINANT ANALYSIS OF FISH TISSUE

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Abstract. Leachates from landfill sites contain a mixture of many chemicals, of which several are potentially harmful to nearby ecosystems. It is therefore important to monitor these adjacent systems and keep a lookout for signs of ecotoxicological effects so that action can be taken before the environment suffers severe harm. As much as 16 percent of the fish in Vallkärrabäcken, a stream in southern Sweden, situated in the close proximity of the closed down landfill St: Hans, have shown deformed caudal fins in recent studies. The aim of this study was to assess the environmental health status of a section of this stream. A part of Vallkärrabäcken further north which was considered unaffected by possible leachates was used as reference site. The present study consisted of sampling by means of electrofishing, an *in vitro* biomarker investigation and subsequent analysis of fish tissue for persistant organic pollutants and heavy metals. The malformed fish from Vallkärrabäcken South show higher HSI values than fish from the same site without malformations, but the fish of the different sites did not differ in condition factor. The organic compounds found in the fish, with an exception of HCB and PCB congeners 66 and 95 appear to be of other origin than the landfill, since concentrations were higher in fish from the reference site. Six of the analyzed heavy metals were found in significantly higher concentrations in fish from the reference site, which can be seen as an indication of leakage from the landfill.

INTRODUCTION

Since January 2005, it is prohibited to store organic waste in landfills in Sweden. Prior to this ban the majority of the waste generated in Sweden (organic and inorganic) was dumped, no notice taken of the potential extractable energy or recyclable material in it. As a consequence of this the number of landfills in Sweden has grown, and today there are approximately 6000 closed down sites. Leachate from these sites contains a mixture of many potentially toxic chemicals and an uncontrolled release can give rise to negative effects on adjacent ecosystems. One of these landfills is St: Hans in Lund, where household and industrial waste, ashes etc. was dumped between 1948 and 1967. In Vallkärrabäcken, a stream with its origin in the close proximity of the landfill, as much as 16 percent of the brown trout (*Salmo trutta*) exhibited deformed caudal fins or other skeletal malformations in 1999 (Eklöv, 2002). This, however, is only valid in the southern of the two parts of Vallkärrabäcken which further downstream become one stream. The cause of these malformations remains unknown but toxic leachate from the landfill has been suggested. Similar deformities have been observed in fingerlings of another species of freshwater fish (*Labeo rohita*) as a result of exposure to mercury chloride (Jagadeesan, 2005). Studies on other areas polluted by leachate from Swedish refuse dumps have shown higher EROD-activity, low percentage of fertile females and hepato-somatic indices different from those of fish from reference sites (Noaksson *et al*, *2005*).

The aim of this study is to further investigate if toxic leachate from St: Hans landfill appears to pollute the southern part of Vallkärrabäcken and if the fish at the site show any negative effects (apart from the observed caudal fin malformations) that could be due to pollution. This particular stream is of special interest because of its status as a watercourse worthy of regional protection (County administrative board of Skåne, 2005) and because of the high frequency of malformations in the fish there. The northern part of Vallkärrabäcken, which appears out of reach for a possible leakage from the landfill, is used as reference site. Fish are sampled by means of electrofishing and their condition factor and hepato-somatic index (HSI) is calculated. A HSI which differs from the reference can be a sign of pollution. In addition, samples of fish from both sites are analysed for concentrations of some heavy metals and organic pollutants, pollutants that are known to bioaccumulate (Folt *et al*, 2002 and Walker *et al*, 2001).

MATERIALS AND METHODS

Study area

The studied watercourse Vallkärrabäcken is situated just north of Lund. Its southern arm originates at St: Hans landfill and its northern arm further north. The two arms join and become one watercourse further downstream. The surrounding area is dominated by agricultural landscape.

Sampling of fish

Brown trout (*Salmo trutta*) were collected from the two sites (Vallkärrabäcken North and South) by means of electrofishing in late April 2006. The sampling was continued until sufficient sample sizes were reached. 13 fish were collected from Vallkärrabäcken North and 15 fish from Vallkärrabäcken South. The collected fish were all in the same age class, 1+. Notes were made of malformed fish (8 of the 15 fish from the southern part but no fish from the reference site). The fish were weighed, measured for length and then frozen until further analysis.

Studied biomarkers

Condition factor, an index used as a measure of general fish health, was calculated according to the formula $K = 10^5 * (\text{body weight}/\text{body length}^3)$. The fish were weighed to the nearest 0,01 g and measured to the nearest 0,1 cm. The liver was excised and weighed to the nearest 0,001 g for HSI calculation ($HSI = \text{liver weight}/\text{body weight}*100$). A HSI which differs from the normal can be a sign of exposure to a variety of chemicals (Schmitt *et al*, 2000). Only fish in the 100-125 mm interval were included in this calculation.

Analysis for pollutants

The fish were homogenized and stored in separate plastic containers for heavy metal and persistant organic pollutant (POP) analysis.

Analysis for heavy metals. 7 ml of HNO_3 (Aristar quality from the supplier BDH) was added to each sample (0,5 g of homogenized fish). The mixture was digested by microwave oven technique (CEM, model mars 5) before analysis of elements in ICP-MS (Inductively coupled plasma with mass spectroscopy, Perkin-Elmer, ELAN-6000). Standards used were Atomic spectroscopy standards from Perkin-Elmer, SPEX, AccuStandard and Merck. Elements determined were Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, P, Sb, V and Zn.

Analysis for POPs. Samples of homogenized fish were weighed and oven dried for two days. The samples were weighed again and then Soxhlet extracted in hexane-acetone for two hours. The extracted fish samples were dried overnight and weighed to determine fat content. Water was added to the extract containing fat and pollutants. After phase separation, concentrated H_2SO_4 was added to oxidize the lipids. The hexane phase was separated and evaporated to dryness. After that it was redissolved in 400 µl of hexane and eluated with hexane-dichloromethane through a column containing two layers of silica gel to which NaOH and H_2SO_4 had been added, respectively (Bremle *et al* in Berglund *et al*, 1997). The eluate was evaporated to dryness and dissolved in isooctane and was then ready for gas chromatographic analysis.

Pollutants were analyzed by capillary gas chromatography electron capture detection with a 30-m db-5 quartz capillary column (Larsson *et al* in Berglund *et al*, 1997). The analyzed organic compounds were seven PCB congeners and the pesticides α-HCH, β-HCH, γ-HCH, HCB, p,p'- DDE, p,p'-DDT, p,p'-DDD, o,p-DDT and PCA.

Statistical analysis

One-tailed T-tests were performed to discover possible differences between the two sites in the investigated biomarkers and pollutant concentrations. T-tests were also performed between the fish from Vallkärrabäcken South with and without malformations. Concentrations of heavy metals in fish from the southern section and the reference site were compared, as well as concentrations of POPs.

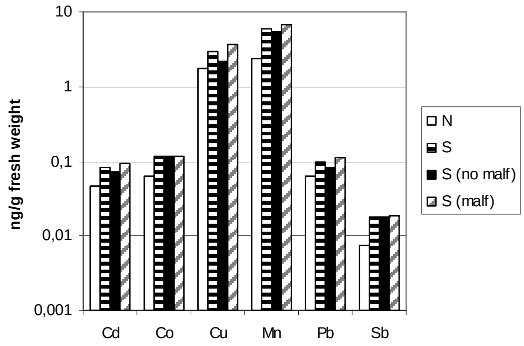

Fig. 2. Mean values of metal concentrations in fish (ng/g fresh weight).

RESULTS

All results are presented in the table in appendix 1. The malformed fish showed higher ($p < 0.1$) HSI values than the fish without malformations in Vallkärrabäcken South (fig. 1). None of the groups differed from the others in condition factor (appendix 1).

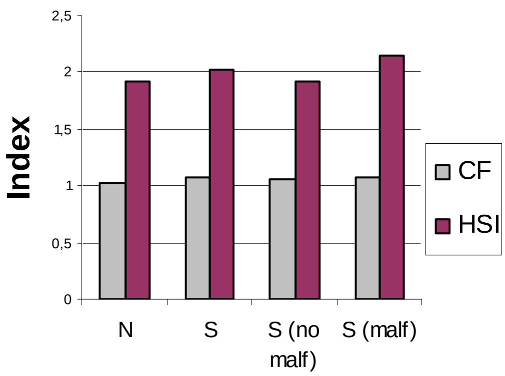

Fig. 1. Mean values of condition factor and HSI in Vallkärrabäcken North and South (with and without malformations).

Significantly higher concentrations of Cd, Co, Cu, Pb, Sb and Mn were found in the fish from Vallkärrabäcken South (fig. 2). When comparing the malformed fish from Vallkärrabäcken South with those from the same site showing no such symptoms, a higher concentration of Cu was found in the malformed fish (fig. 2).

Three of the organic compounds analyzed were not found. These were α-HCH, β-HCH and PCA. Analysis for POPs showed higher values in the reference streams for all other analyzed compounds except HCB and PCB congeners 66 and 95 (fig. 3). Comparison regarding lipid content between the two streams showed no significant difference (p = 0,81).

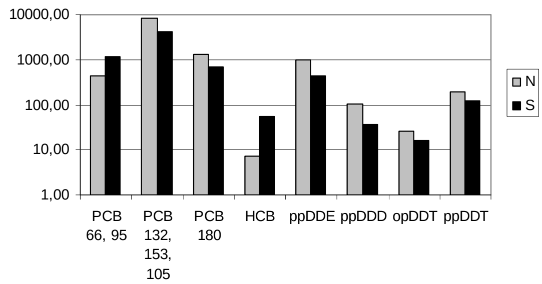

Fig. 3. Mean values of organic compound concenrations analyzed in fish from Vallkärrabäcken North and South

DISCUSSION AND CONCLUSIONS

Six of the metals were found in significantly higher concentrations in fish from Vallkärrabäcken South than in fish from the reference site. This can be seen as an indication of high metal concentrations in the leachate from the landfill. It is still uncertain whether metals can have contributed to the malformations in the fish from Vallkärrabäcken South, and more studies should be conducted in order to find out.

In the present study, HSI of the malformed fish in Vallkärrabäcken South was found to be higher than that of the other fish. However, no difference could be spotted in HSI between the fish without malformations from the two sites. In a study made by Noaksson *et al* in 2005 it is only the sexually mature fish that differ in HSI between polluted and reference sites. This could explain the small differences in our HSI results, as the sampled fish had not yet reached sexual maturity.

No differences were found when comparing condition factor between the different groups. This does not mean that condition factor is an insufficient biomarker, only that it showed no results in this particular situation. The higher liver weight in the malformed fish from Vallkärrabäcken South could have led to an increase in total weight, and thereby also in condition factor.

Analysis for POPs showed higher values in the reference streams for all analyzed compounds except two PCB congeners and the chlorinated aromatic compound HCB. The higher values in the reference site could be explained by environmental factors such as size of the catchment area, land use etc. The PCB values are close to those explained by natural deposition in other studies (Berglund *et al*, 1997). This indicates that the organic compounds do not come from the landfill, with a possible exception of HCB and PCB 66 and 95. This could imply that these two compounds leak from the landfill.

In a study made on aquatic ecosystems near landfills, considerable reproduction impairment (a very low percentage of fertile female fish) was found in streams and lakes recieving leachate from landfills (Noaksson *et al*, 2005). Bearing this in mind, it would be interesting to see if the same effects are present in Vallkärrabäcken.

To conclude, the malformed fins can not be explained by exposure to POPs, with the possible exception of HCB and PCB congeners 66 and 95. Six heavy metals, on the other hand, showed significantly higher values in Vallkärrabäcken South, which could indicate leakage from the landfill. To further investigate the cause of the malformations in fish from Vallkärrabäcken South, more studies should be made. These could include laboratory studies of effects of the six heavy metals found in elevated concentrations and analysis of contaminants in sediment.

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Appendix 1.

	Sample mean with std. deviation				p-values	
	Vallkärra North (ref)	Vallkärra South (all fish)	Vallkärra South (no malf)	Vallkärra South (malf)	North - South(all)	South(malf)-South(no malf)
Biomarkers						
condition factor	1.03 ± 0.05	1.07 ± 0.05	1.06 ± 0.09	1.08 ± 0.12		
HSI	1.92 ± 0.11	2.02 ± 0.28	**1,91 ± 0.14**	**2,15 ± 0.11**		0.1
Metal conc. (µg/g fresh fish)						
Al	71.177 ± 35.258	74.79 ± 29.93	49.334 ± 15.103	74.791 ± 39.056		
As	0.129 ± 0.036	0.19 ± 0.79	0.79 ± 1.138	0.192 ± 0.064		
Cd	**0.047 ± 0.037**	**0.09 ± 0.03**	0.072 ± 0.031	0.092 ± 0.031	0.07	
Co	**0.062 ± 0.025**	**0.12 ± 0.02**	0.116 ± 0.02	0.118 ± 0.026	0.0005	
Cr	0.328 ± 0.087	0.38 ± 0.07	0.342 ± 0.018	0.382 ± 0.095		
Cu	**1.727 ± 0.430**	**3.71 ± 0.96**	**2.174 ± 0.218**	**3.714 ± 0.696**	0.01	0.01
Hg	0.033 ± 0.008	0.03± 0.01	0.034 ± 0.016	0.033 ± 0.012		
Mn	**2.402 ± 0.865**	**6.72 ± 1.53**	5.387 ± 0.660	6.718 ± 2.016	0.00009	
Ni	0.187 ± 0.091	0.23 ± 0.08	0.157 ± 0.025	0.231 ± 0.106		
Pb	**0.062 ± 0.04**	**0.11 ± 0.04**	0.081 ± 0.021	0.111 ± 0.057	0.1	
Sb	**0.008 ± 0.002**	**0.02 ± 0.01**	0.018 ± 0.011	0.018 ± 0.003	0.005	
V	0.181 ± 0.103	0.28 ± 0.11	0.173 ± 0.037	0.278 ± 0.140		
Zn	36.233 ± 6.435	34.13 ± 4.48	38.466 ± 4.794	34.13 ± 3.625		
POPs conc.						
PCB 66, 95	**434.46 ± 87.26**	**1156.73 ± 193,88**	1186.58 ± 277.32	1126.88 ± 119.99	0.000004	
PCB 132, 153, 105	**8321.17 ± 1358.61**	**4217.7 ± 1159.4**	4117.94 ± 353.01	4317.47 ± 1216.95	0,0001	
PCB 180	**1300 ± 271.52**	**698.31 ± 232.02**	676.47 ± 258.71	753.08 ± 220.14	0,0001	
HCB	**7.19 ± 0.53**	**53.36 ± 28.78**	54.11 ± 41.37	52.61 ± 18.92	0.007	
g-HCH		1.85 ± 0.14		1.85 ± 0.14		
ppDDE	**990.66 ± 223.73**	**446.53 ± 105.72**	438.96 ± 139.79	454.21 ± 90.67	0,0002	
ppDDD	**102.58 ± 74.26**	**35.28 ± 20.37**	24.98 ± 28.47	42.28 ± 15.25	0,04	
opDDT	**26.34 ± 4.84**	**16.51 ± 4.33**	13.76 ± 2.26	19.32 ± 4.35	0,003	
ppDDT	**195.97 ± 96.94**	**124.25 ± 45.20**	112.26 ± 55.28	136.24 ± 40.25	0,07	