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Eel (*Anguilla anguilla*) and Brown Trout (*Salmo trutta*) Target Species to Assess the Biological Impact of Trace Metal Pollution in Freshwater Ecosystems

A. R. Linde[1], P. Arribas[2], S. Sanchez-Galan[1], E. Garcia-Vazquez[1]

[1] Departamento de Biología Funcional (Genética), Universidad de Oviedo, C/J. Claveria s/n, 33071 Oviedo, Spain
[2] Departamento de Ingeniería Química (Tecnología del Medio Ambiente), Universidad de Oviedo, 33204 Gijón, Spain

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Abstract. Copper, lead, and cadmium pollution were measured in water and sediments of two Northern Spanish rivers: Piles and Pigüeña. Liver contents of these heavy metals were analyzed in two fish species (eel, *Anguilla anguilla,* and brown trout, *Salmo trutta*) collected from the same locations. Significant levels of heavy metal pollution were found in a 38% of fish that are potential catches for sport fishermen. The results indicate that adult eel could be a good metal bioindicator if sampled at a homogenous age. In contrast, brown trout could be considered as a bioindicator only during the first year of life.

Heavy metals are a problem of magnitude and ecological significance due to their toxicity and their ability to accumulate in living beings (Purver 1985). They are not biodegradable, and are not eliminated from ecosystems. They undergo a global ecobiological cycle in which natural waters are the main pathway (Nurnberg 1985); they derive from a considerable number of sources and pose a severe threat to the aquatic environment (Forstner and Wittmann 1981).

Source assessments of trace metal pollutants entering local river systems has not been enough addressed to date. The significance of trace metal bioaccumulation in living beings inhabiting polluted ecosystems is even more important when those living beings are subjected to exploitation by human and enter the food chains. The most common example of animals directly exploited by human in both freshwater and seawater ecosystems are fishes. In freshwater ecosystems such as Spanish rivers, fish populations are exploited only by sport fisheries (usually angling) and catches are consumed fresh. Any possible metal pollution of these fishes will pass directly to consumers.

Fish uptake heavy metals from a polluted environment which bioaccumulate in their tissues. The level of metal uptake will vary depending on the way of uptake diet and/or waterborne exposure (Miller *et al.* 1993). Diet seems to be a significant route of uptake of copper and zinc by fish, whereas waterborne exposure plays a dominant role in the accumulation of cadmium and lead (Spry *et al.* 1988) which interact in the gill (Playle *et al.* 1993). Among the variety of fishes subjected to direct exploitation, their feeding and migrating behavior would also determine their degree of pollution. The incidence of pollution in human through food chains derived from freshwater ecosystems may depend on the species subjected to exploitation in the concrete ecosystems. Not all fish species are useful bioindicators for all the heavy metals: species able to regulate their body levels of any metal (at least partly) should be rejected as bioindicators for this metal (Bryan 1984). As an example, Amiard-Triquet *et al.* (1987) found that elvers (young eels) were useful to reveal cadmium but not copper and lead pollution in French estuarine ecosystems. This finding should be contrasted in other developmental stages; adults living in freshwater conditions may show greater tolerance to heavy metals or be able to bioaccumulate them, etc.

Among the species naturally distributed in Northern Spanish rivers, eel (*Anguilla anguilla* L.) and brown trout (*Salmo trutta* L.) are the most abundant. They inhabit very diverse freshwater ecosystems because of their adaptability to very different environments (from lakes and reservoirs to small mountain streams). Two characteristics common to these two species are very interesting from the point of view of their potential as bioindicators: their mobility and their ability to adapt to different salinity conditions. In Spain, both species are subjected to exploitation by sport fisheries, the minimum size required to be caught being 20 cm for eel and 18 cm for trout in Spanish regulations (Regional Government of Asturias, "Normas de Pesca en aguas continentales de Asturias" 1992–1995). Consumed fresh, they may cause heavy metal bioaccumulation in human, with subsequent serious risk to consumer health.

In the present work, we analyzed the concentrations of heavy metals (copper, lead and cadmium) in eel (*Anguilla anguilla*) and brown trout (*Salmo trutta*) inhabiting a northern Spanish river. The aim of this work was to determine which species can constitute a vehicle to the accumulation of heavy metals by potential consumers. We analyzed metal content in liver,

Correspondence to: E. Garcia-Vazquez

considered to be the best indicator of chronic exposure to heavy metals because it represents the site of metal metabolism (Miller *et al.* 1992).

Materials and Methods

The Piles river, a small coastal stream which has at its mouth one of the most industrialized cities of Northern Spain (Gijón). Two different sites were sampled: upstream (Caldones), an agricultural area; and downstream (Viesques), an urban area near to the mouth. Two samples of water and sediments were taken at each of the two sites during July 1994. Twelve eels (*Anguilla anguilla*) and twelve trout (*Salmo trutta*) were caught at each site by electrofishing. Copper, lead and cadmium concentrations in all samples were determined by voltammetry (PSA).

Additional samples were collected on the same date in the Pigüeña river, a stream located more than 60 km apart in a wild area without apparent pollution. Samples of water, sediments and eels were taken from this river, in order to compare results for this species from a clean ecosystem to the results from the Piles river.

Water samples were taken 10 cm below the river surface. Temperature, pH and dissolved oxygen were determined in the field. Water samples were filtered under vacuum (Whatman nylon filters 0.45 μm pore), acidified with nitric acid to pH 2 and stored until analyzed. Sediment samples were collected by a core sampler 10 cm deep and kept frozen until analysis. Core samples were separated into two sections: top (0–3 cm) and bottom (7–10 cm) layer. 20 g of each fraction were dried at 105°C for 24 h, and disaggregated in a glass mortar, particles larger than 2 mm were eliminated and remaining sample was finely ground and taken to dryness. One gram was digested in Teflon reactors with 5 ml nitric/perchloric acid (3:1) for 7 min, using a microwave oven. Once cooled, the solution was transferred to a 20 ml flask to adjust the volume and settle the mineralized residue. Data were presented as μg metal/g dry weight.

Detection limits (in liver samples diluted 1:20) were estimated to be 0.49 ppb for copper, 0.38 ppb for Pb and 0.40 ppb for Cd. In aqueous solutions detection limits were 0.1 ppb for all the three studied metals.

Fish were weighed and fork lengths were recorded. Livers were removed, weighed fresh and dried at 24°C for 24 h. Dried livers were weighed and digested in Teflon reactors with 5 ml nitric/perchloric acid (5:1) for 7 min using a microwave oven. The contents of the reactor were transferred to a volumetric flask and volume was adjusted to 20 ml for subsequent analysis. Condition factor (CF) was estimated as $W \times 100/l^3$, where W = total weight, l = fork length. The liver weight relative to the total fish weight is also presented as an indicator of the degree of hepatic development in an attempt to detect hepatic hypertrophia or atrophia.

Statistical analyses were performed with a Macintosh Statview computer program. The chosen level of statistical significance was $P < 0.05$.

Results

Table 1 presents the environmental parameters measured. As the two water and sediment samples taken in each location were very similar, we present the average value of both samples for every site. Differences among sampling sites were evident. Upstream Piles river was the most polluted site with respect to heavy metals, showing higher content of Cu, Pb and Cd than the other two locations in sediments and water (except lead in water). Downstream Piles river showed similar levels of copper in sediments than Pigüeña river, sediment at downstream Piles river being less polluted with both Pb and Cd than upstream site. Water metal content was clearly lower in the Pigüeña. In every location, copper accumulated in the bottom whereas lead and cadmium remained in the top layer, near to the surface.

Table 1. Parameters measured in water and sediments in the three sampling sites. Metal content of water is expressed as μg/L, metal content of sediments, as μg/g dry weight Temp, temperature (°C); DO, dissolved oxygen; Cu, copper content of: water, top layer of sediments (Top sed), bottom layer of sediments (Bottom sed), mean of both layers (Mean sed). Idem for the other two metals

		Up Piles	Down Piles	Pigüeña
	Temp	17.4	18.3	14.0
	pH	8195	7895	8565
	DO (mg/L)	10.21	9.33	10.16
Cu	Water	3.205	3.097	0.088
	Top sed	9.10	7.20	7.81
	Bottom sed	10.85	8.91	8.51
	Mean sed	9.98	8.06	8.16
Pb	Water	3.207	4.327	0.125
	Top sed	25.44	13.97	9.23
	Bottom sed	22.93	11.40	6.80
	Mean sed	24.19	12.69	8.01
Cd	Water	0.607	0.298	0.040
	Top sed	0.30	0.17	0.22
	Bottom sed	0.19	0.05	0.12
	Mean sed	0.25	0.11	0.17

Table 2. Legal limits of metal content in water, sediments and fish, for Spanish rivers. Metal content of water is expressed as μg/L, metal content of sediments, as μg/g dry weight (values for sediments are considered to produce a negative effect on living beings in 10% of trials); metal content of fish is expressed as μg metal/g fresh tissue (wet weight)

Metal	Water	Sediments	Fish
Cu	5	70	20
Pb	7	35	3
Cd	5[a]	5	1

[a]Based on European regulation for effluents, 86/280/CEE

Table 2 shows the legal limits in Spanish legislation for heavy metals in water, sediments, and fish destined to human consumption (Regulation: Real Decreto 1521/1977). It seems that environmental heavy metal pollution did not reach illegal levels in any location.

Table 3 presents the data taken from Piles river eels. Asterisks indicate metal contents greater than legal limits. Eels were not significantly copper-polluted, following Spanish regulations; nevertheless, one eel caught upstream showed a lead content unsuitable for consumption and 6 eels (5 caught upstream and one downstream) were significantly cadmium-polluted. There was a clear relationship between the level of sediment pollution and the proportion of eels metal-polluted: A greater proportion of eels caught in the upstream location were unsuitable for consumption than eels caught downstream. The average metal content was also much higher upstream than downstream for all the three metals. Between-location differences in copper concentrations even reached statistical significance (ANOVA F value was 4.139, P = 0.054).

Contrary to metal contents, no clear between-location difference was found for the biological parameters considered (Table

Table 3. Parameters measured in the eels. Metal content, fork length (L), liver weight relative to total weight (LW/TW), and condition factor (CF). Asterisks indicate metal contents overcoming legal limits suitable for consumption. n.d., non detectable concentrations; S.E., standard error

		Metal concentrations (μg/g)					
Site	Sample	Cu	Pb	Cd	L (cm)	LW/TW	CF
Up Piles	E1	15.19	n.d.	n.d.	30	0.0147	0.256
	E2	12.21	n.d.	n.d.	30	0.0106	0.219
	E3	11.72	0.43	n.d.	32	0.0141	0.180
	E4	14.72	n.d.	n.d.	33.5	0.0137	0.218
	E5	12.01	1.85	1.57*	25.5	0.0139	0.199
	E6	13.38	0.17	0.27	35	0.0151	0.194
	E7	7.62	n.d.	0.78	29.5	0.0150	0.203
	E8	0.18	n.d.	1.83*	30	0.0122	0.193
	E9	8.92	5.69*	1.48*	28.5	0.0128	0.203
	E10	2.85	n.d.	0.12	31	0.0149	0.215
	E11	10.44	n.d.	1.75*	27	0.0148	0.173
	E12	6.08	n.d.	1.09*	31	0.0197	0.205
	Average				30.25	0.0140	0.205
	S.E.				0.75	0.0010	0.003
Down Piles	E1	6.66	n.d.	0.36	31	0.0127	0.178
	E2	10.61	n.d.	1.36*	38	0.112	0.182
	E3	6.37	0.06	0.26	31	0.0156	0.164
	E4	4.37	0.15	0.40	31	0.0155	0.185
	E5	11.54	0.48	0.49	28.5	0.0098	0.168
	E6	4.44	n.d.	0.49	29	0.0120	0.213
	E7	2.39	n.d.	0.11	35	0.0158	0.210
	E8	6.42	n.d.	0.35	30	0.0127	0.226
	E9	1.63	n.d.	0.31	26.5	0.0184	0.172
	E10	9.72	0.04	0.67	32	0.0107	0.235
	E11	11.14	n.d.	0.43	30	0.0122	0.204
	E12	2.82	0.05	0.05	28	0.0092	0.187
	Average				30.8	0.0130	0.194
	S.E.				0.90	0.0010	0.004
Pigüeña	E1	7.01	0.13	0.68	36	0.0154	0.163
	E2	5.42	0.11	2.45	27.7	0.0158	0.188
	E3	0.73	0.49	0.30	28.5	0.0101	0.143
	E4	7.20	0.003	1.25	30	0.0091	0.156
	E5	n.d.	0.11	0.69	21.5	0.0121	0.141
	E6	1.35	2.00	0.84	23.7	0.0109	0.128
	E7	n.d.	0.82	0.59	21.2	0.0158	0.147
	E8	8.43	0.03	0.36	28	0.0110	0.137
	E9	3.64	0.64	1.10	22.5	0.0147	0.149
	E10	7.94	n.d.	0.67	32	0.0130	0.165
	E11	2.42	0.13	0.49	27.7	0.0136	0.122
	E12	1.05	0.18	0.25	30	0.0108	0.148
	Average				27.4	0.0127	0.149
	S.E.				1.29	0.0001	0.024

3). The most relevant feature is that all the eels analyzed were large enough to be legally subjected to sport fisheries.

Table 4 shows the data of Piles river brown trouts. Contrary to eels, a considerable proportion of trout were copper-polluted, specially in the sample caught upstream (58%). Eight of the 11 copper-polluted trouts were large enough to be legally subjected to sport fisheries; in this river, trouts of this length are two years of age (2+). Conversely, the only lead-polluted trout and the three cadmium-polluted ones were under the legal minimum size. Between-location differences in trout metal content were not statistically significant for any metal. In spite of this lack of statistical significance, average metal content of trout fits slightly the corresponding metal content of sediments for copper and lead (upstream higher content of both environmental and fish-pollutant metals), but not for cadmium (higher trout content downstream but higher environmental cadmium upstream). On closer examination of Table 4, we observe that downstream there were only two small trout (T5 and T7) accounting for most of the total content of cadmium, whereas upstream all the trout except one presented measurable levels of this trace metal.

Concerning the biological characters of trouts (Table 4), the only significant difference was for the relative size of liver ($F = 10.391$, $P = 0.004$), being significantly less developed in trouts caught downstream. This could be explained by the high metal content of upstream trouts, especially copper. Taken into account that liver is the site of metabolism and storage of this metal, a hypertrophia of this organ is likely to be expected.

Comparing the metal contents of both species (Table 5), several interesting facts can be observed. First, between-species differences were not found for the contents of lead and cadmium. Second, statistically significant between-species differ-

Table 4. Parameters measured in trouts. Metal content, fork length (L), liver weight relative to total weight (LW/TW), condition factor (CF). Asterisks indicate metal contents overcoming legal limits suitable for consumption

		Metal concentration (μg/g)					
Site	Sample	Cu	Pb	Cd	L (cm)	LW/TW	CF
Up Piles	T1	14.45	0.64	n.d.	18.1	0.0100	1.38
	T2	32.21*	n.d.	0.23	22	0.0096	1.37
	T3	11.67	n.d.	0.34	17.5	0.0118	1.23
	T4	9.06	0.40	0.23	16.7	0.0104	1.31
	T5	49.37*	n.d.	0.13	20.3	0.0105	1.29
	T6	9.27	n.d.	0.28	17.6	0.0133	1.41
	T7	11.39	n.d.	0.10	18.9	0.0121	1.34
	T8	36.55*	1.46	0.53	19	0.0119	1.18
	T9	93.47*	1.21	0.51	19.5	0.0108	1.13
	T10	46.52*	5.07*	1.27*	10.8	0.0106	1.25
	T11	33.27*	0.27	0.10	11.5	0.0132	1.42
	T12	105.12*	n.d.	0.07	18	0.0134	1.18
	Average				17.5	0.0110	1.29
	S.E.				1.47	0.0004	0.03
Down Piles	T1	62.07*	n.d.	0.40	17.5	0.0100	1.38
	T2	11.50	n.d.	n.d.	19	0.0141	1.41
	T3	19.53	n.d.	0.004	17.5	0.0103	1.41
	T4	63.00*	0.95	0.13	20	0.0106	1.43
	T5	11.00	0.95	10.91*	9	0.0058	1.21
	T6	13.91	0.43	0.43	10	0.0098	1.13
	T7	15.95	n.d.	2.05*	9.4	0.0064	1.07
	T8	11.33	n.d.	n.d.	21.3	0.0120	1.20
	T9	47.86*	0.21	n.d.	20.5	0.0070	1.07
	T10	30.42*	n.d.	0.14	19.7	0.0075	1.05
	T11	14.16	n.d.	n.d.	11.7	0.0084	1.22
	T12	10.80	n.d.	n.d.	11.2	0.0087	1.16
	Average				15.7	0.0090	1.21
	S.E.				1.35	0.0010	0.04

ences of copper content were found, trout accumulating more copper than eels did in the same river. This fact was evident in the proportion of fish of each species containing levels of copper unsuitable for human consumption (Tables 3 and 4). One more between-species difference could be observed from Tables 3 and 4: the different responses of fish to cadmium content of sediments. From Piles river data, eels seem to fit better sediment cadmium level than do trouts.

The results of the additional sample of eels caught in the Pigüeña river are in Table 3 (below). Eels in this sample were smaller and had lower CF than eels from Piles river. Data obtained for this location clarify some points indicated in the Piles river. First, there is a statistically significant positive relationship between the copper content of sediments and the copper content of eels ($r = 0.49$, 34 d.f. $P < 0.05$), confirming the utility of eels to indicate copper pollution. For lead content, concentrations in sediment (Pigüeña < downstream Piles < upstream Piles) were not correlated with the increasing order of eel pollution (downstream Piles < Pigüeña < upstream Piles). Moreover, Pigüeña river had the highest proportion of eels with measurable lead content (11 of 12, contrasting to 5 and 4 upstream and downstream Piles, respectively). For cadmium content, a similar fact can be observed: Pigüeña eels had the highest average, although the Pigüeña river site was not the most polluted. Data from Pigüeña river showed that cadmium concentration in eels did not fit as well as the sediment content of this metal. A possible explanation for the different response of eels to different metals pollution could lie on the ability of eels to bioaccumulate each of the studied heavy metals. As it is difficult to calculate the exact age of eels, we estimated it simply by the length. Considering all the analyzed eels (36) as a whole population, there was a statistically significant positive correlation between eel length and eel copper content ($r = 0.424$, 34 d.f. $P < 0.05$), indicating copper bioaccumulation with age or size. Statistical significance was not found for lead and cadmium but correlations eel length/metal content were negative ($r = -0.293$ and -0.242, respectively). Similar results have been found for trouts (Table 4), although statistical significance has not been reached in any case: trout length and trout copper content were positively correlated ($r = 0.338$, 22 d.f.) whereas trout length and lead and cadmium contents were negatively correlated ($r = -0.273$ and -0.404, respectively).

Discussion

Significant metal pollution has been found in two fish species subjected to sport fishing in Northern Spain. Trout of fishable size showed copper concentrations greater than regulations, while several eels were significantly polluted with cadmium in all the sampling sites. This finding is significant, because environmental measures of metal pollution (in water and sediments) were not over the level considered to be a risk.

Concerning copper bioaccumulation, our data showed that brown trout are able to increase their copper content with age. A possible reason for the divergence of our results with the ones

Table 5. ANOVAs (two-factor with replication) comparing species (eel and brown trout) and Piles river locations (upstream and downstream) by their metal content

	Cu content				
Source of variation	DF	SS	MS	F-test	P value
Location	1	15491.22	15491.22	1.655	0.205
Species	1	160037.96	160037.96	17.099	0.0002
Interaction	1	3815.44	3815.44	0.407	0.526
Within	44	411798.37	9359.05		
Total	47	591142.99			
	Pb content				
Source of variation	DF	SS	MS	F-test	P value
Location	1	115.23	115.23	3.329	0.075
Species	1	1.99	1.99	0.058	0.811
Interaction	1	0.56	0.56	0.016	0.899
Within	44	1523.14	34.61		
Total	47	1640.93			
	Cd content				
Source of variation	DF	SS	MS	F-test	P value
Location	1	4.06	4.06	0.091	0.764
Species	1	0.73	0.73	0.016	0.899
Interaction	1	76.05	76.05	1.703	0.199
Within	44	1964.29	44.64		
Total	47	2045.14			

of Amiard-Triquet *et al.* (1987), who did not find correlation between environmental and eel copper content, could be the fact that they analyzed copper content of the whole body of elvers, whereas we analyzed here the copper content of the liver, the organ actually metabolizing and storing metals.

The failure to detect bioaccumulation of lead and cadmium with age could have two alternative explanations. First, after a former bioaccumulation of both metals bioelimination could follow to protect fishes against deleterious effects of heavy metals. Second, a selective mortality of fishes accumulating high concentrations of lead and/or cadmium could occur, with metal-free fishes surviving and growing up preferentially. In addition, growth rate could be slower in fishes at highly polluted sites. Waterborne cadmium interacts in the gill (Playle and Dixon 1993), affecting severely ion uptake, ion regulation and oxygen-carrying capacity (Giles 1984). In this sense, the lower CF of Pigüeña eels could be a consequence of cadmium pollution (among other factors such as a lower water temperature). The action of cadmium interfering with vital metabolic processes could reduce the normal homeostasis of polluted individuals, decreasing their growth rate and consequently their CF.

Apparently, metal concentrations in the livers of eels were useful as biomonitors of metal concentrations in sediments, but not of concentrations in water. Significant between-location differences of copper content suggest that eel should enable a reasonable estimation of copper pollution gradients. Contrarily, trout bioaccumulate high levels of copper, which are not correlated to the environmental content. Trouts are probably more sensitive to heavy metals than eels, being disturbed by lead and cadmium much earlier. This sensitivity may provide a fine tool to detect heavy metal pollution, but may not be suitable for evaluating pollution gradients. Eels may be better for this latter purpose. The behavior of eel, living and feeding into sediments and being in general less mobile than trout (Neveu 1981; Mann and Blackburn 1991) is another reason for its better ability to act as pollution bioindicator.

The results suggest that fish of similar age should be used to compare populations, given possible variation of metal content with age. Eels of homogeneous age (and consequently length) will probably be good bioindicators of heavy metal pollution of sediments. Eels 30 cm large could be about 6–8 years old (Mann and Blackburn 1991) and probably less in temperate Spanish rivers. The use of smaller eels should be evaluated (3–4 years, near to 20 cm) and the degree of sensitivity of this species to detect small variations of metal pollution should be tested in following works.

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