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Accumulation of the precious metals platinum, palladium and rhodium from automobile catalytic converters in *Paratenuisentis ambiguus* as compared with its fish host, *Anguilla anguilla*

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Abstract

The platinum group metals (PGM) Pt, Pd and Rh are emitted into the environment mainly by catalytic exhaust gas converters of cars. As PGM accumulate in sediments of aquatic ecosystems, the study was focused on the uptake of the noble metals by European eels, *Anguilla anguilla* infected with the acanthocephalan *Paratenuisentis ambiguus*. Eels were exposed to ground catalytic converter material for six weeks. After exposure Pt and Pd were detected in the liver and kidney of the eels and in the parasites. Palladium was also found in fish muscle and intestine. No Rh uptake by the eel tissues and the parasites occurred. *Paratenuisentis ambiguus* contained the highest levels of both metals with 40 times higher Pt concentrations and four times higher Pd concentrations than the liver of its host. Due to its accumulation capacity for PGM, *P. ambiguus* can be applied as a sensitive accumulation indicator in field studies to assess the degree of environmental PGM contamination in aquatic ecosystems.

Introduction

Recent studies have revealed an exceptional heavy metal accumulation capacity of acanthocephalans parasitizing fish and consequently the use of intestinal fish parasites as sensitive indicators of environmental heavy metal contamination has much potential (Sures, 2003, 2004).

The use of catalytic converters for automobile exhaust gas purification related to the use of unleaded fuel has resulted in a considerable decrease in lead emissions by cars (Helmers, 2000). However, following the introduction of automobile catalysts in the mid-1980s in Europe and in the 1970s in the USA, the platinum group metals (PGM) platinum (Pt), palladium (Pd) and rhodium (Rh) are increasingly emitted with exhaust fumes into the environment (Zereini & Alt, 2000; Hoppstock & Sures, 2004). These elements operate as catalytic active metals in exhaust gas converters with the aim to reduce the emission of hydrocarbons, carbon monoxide and nitrogen oxides.

Although elevated PGM levels can be identified in road dusts and soils along heavily frequented roads (Schäfer *et al.*, 1999; Zereini *et al.*, 2000; Ely *et al.*, 2001; Gómez *et al.*, 2001; Jarvis *et al.*, 2001), there is a lack of information on PGM contamination of the biosphere and especially of the fauna (see Hoppstock & Sures, 2004). As the precious metals mainly occur in elemental form or as oxides in the exhaust fumes of cars and are attached to wash coat particles of aluminium oxide and other metal oxides (e.g. CeO_2, ZrO_2, La_2O_3) (Palacios *et al.*, 2000; Moldovan *et al.*, 2002), it was assumed that PGM are not biologically available. Recently, the biological availability of noble

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metals for animals has been verified in experimental studies using soluble metal salts, catalytic converter model substances, sediments of urban rivers, road dust or tunnel dust (reviewed in Hoppstock & Sures, 2004). Since PGM accumulate in sediments of aquatic ecosystems and exposure studies are more readily performed with aquatic than with terrestrial animal species, the majority of studies refer to aquatic organisms such as the freshwater isopod *Asellus aquaticus* (Rauch & Morrison, 1999; Moldovan *et al.*, 2001), the annelid *Lumbriculus variegatus* (Veltz *et al.*, 1994, 1996), the mussel *Dreissena polymorpha* (Sures *et al.*, 2002; Zimmermann *et al.*, 2002), and the fish species *Danio rerio* (Jouhaud *et al.*, 1999a,b) and *Anguilla anguilla* (Sures *et al.*, 2001; Zimmermann *et al.*, 2004).

Having demonstrated the biological availability of noble metals, it is now important to assess the distribution of PGM contamination in the biosphere thereby evaluating possible threats to the environment. To date, the accumulation of PGM in parasites has only been investigated using eels naturally infected with the eoacanthocephalan *Paratenuisentis ambiguus* (Sures *et al.*, 2003), in which higher uptake rates of Pt and Rh were described compared with different eel tissues. Unfortunately, no information about the bioaccumulation of Pd is available for this host–parasite system and this is surprising as Pd has a high biological availability (Zimmermann *et al.*, 2002).

Therefore, in the present study exposure studies have been undertaken on eels naturally infected with *P. ambiguus* to test whether this acanthocephalan is also able to bioconcentrate Pd and wether this parasite could be recognized as a new promising accumulation indicator for all three automobile-emitted PGM.

Materials and methods

Experimental design

Eels naturally infected with *Paratenuisentis ambiguus* were caught from the river Weser near Petershagen, Germany, by professional fishermen, brought alive to the laboratory and divided into two groups with 9 and 15 individuals each (table 1). Eels were kept in 100 l glass tanks containing dechlorinated, aerated tap water and equipped with an aquarium power filter (Fluval 4, Hagen Deutschland GmbH, Holm, Germany). The water was replaced weekly and data on water parameters and eels are given in table 1. Eels were force fed weekly with commercial food pellets using a 2 ml syringe fitted with a 12 cm length of 2.5 mm diameter plastic tubing.

After six weeks of acclimatizing eels to laboratory conditions, PGM were added to the tank water of one group. The exposure was performed using 20 g ground catalytic converter material, which was added to 100 l water at the beginning of the experiment and after each water replacement. The material derived from unused automobile catalysts, which were ground and homogenized by the manufacturer (Engelhard Technologies GmbH, Nienburg, Germany) contained 0.36 mg g^{-1} Pt, 4.98 mg g^{-1} Pd and 0.36 mg g^{-1} Rh, which was equivalent to a theoretical exposure concentration of 72 μg l^{-1} Pt, 996 μg l^{-1} Pd and 72 μg l^{-1} Rh. Particle size was determined by raster electron microscopy to 0.6 ± 0.4 μm. The strong current generated by the aeration and the power filter guaranteed a rapid and homogenous distribution of the particles in the tank water.

After a six-week exposure, eels were transferred to clean water for 2 days to avoid contamination while sampling. Subsequently, eels were killed and immediately dissected. Samples of muscle, liver, intestine and kidney were taken with the aid of stainless steel scissors and forceps which had been previously cleaned with 1% ammonium-EDTA-solution and double-distilled water. The acanthocephalans were removed from the fish intestine using the same instruments. Tissue samples and parasites were frozen at -26°C in 20 ml polypropylene containers for further processing.

Analytical procedure

For PGM analyses samples of up to 1.4 g (wet weight) fish tissue and approximately 0.02 g (wet weight) parasites were digested with 4 ml HNO_3 (65%, subboiled) and 0.5 ml HCl (30%, suprapure, Merck, Darmstadt, Germany) in 70-ml quartz vessels using a high pressure asher (HPA®, Kürner, Rosenheim, Germany) according to Zimmermann *et al.* (2001). The resulting digestion solution was divided into two aliquots, one for the voltammetric determination of Pt and Rh (Alt *et al.*, 1994, 2001) and the other aliquot for Pd analysis by total reflection X-ray fluorescence spectrometry (TXRF) after co-precipitating the metal with mercury (Messerschmidt *et al.*, 2000; Sures *et al.*, 2001).

As only three eels of the exposed group contained sufficient acanthocephalans for analysis, the tissue samples of these infected eels were analysed for PGM. In the control group, the kidney, liver and the parasite

Table 1. Experimental design and parameters used in exposure of the eel *Anguilla anguilla* to platinum group metals (PGM) in tank water. Eel data were collected at the end of the experiment. Values for pH, water temperature and conductivity are means ± SD, for six observations made weekly during the six-week exposure period.

		A. anguilla				Water characteristics		
Group	PGM	Number	Size (cm)	Weight (g)	Prevalence of *P. ambiguus* (%)	pH	Temperature (°C)	Conductivity (μS cm^{-1})
Control	–	9	49 ± 5	178 ± 47	56	7.6 ± 0.08	10.1 ± 0.5	610 ± 20
CAT	200 mg l^{-1} converter material[a]	15	48 ± 7	156 ± 57	67	8.4 ± 0.04	18.3 ± 0.7	628 ± 15

[a] Corresponds to a theoretical exposure concentration in the tank water of 72 μg l^{-1} Pt, 996 μg l^{-1} Pd and 72 μg l^{-1} Rh.

samples of three eels were initially analysed as these organs usually demonstrate the highest metal contents (Zimmermann *et al.*, 2004), but as the samples showed PGM levels below the detection limits, no further analysis of the remaining control samples was conducted. For a sample weight of 0.01–1.4 g the detection limits varied between 0.001–0.2 ng g^{-1} for Pt, 0.025–3.5 ng g^{-1} for Pd and 0.01–2 ng g^{-1} for Rh.

Data analysis

To compare the accumulation capacity of the tissues for the different PGM, concentration factors (CF) were calculated as ratios of the mean metal concentration in the fish tissue or parasite and the applied exposure concentration in the tank water ((CF = $\bar{c}_{\text{animal tissue}}/c_{\text{water}}$).

For statistical analysis metal levels below the detection limit were set to zero. A Mann-Whitney U-test was applied to assess differences in the metal concentrations of the fish tissues or parasites between control and exposed eel groups.

Results

Metal concentration in eels and parasites

In all samples, the Rh levels were below the detection limit. In tissue and parasite samples taken from the control fish, the concentrations of Pt and Pd were also below detection limits. Furthermore, Pt was not found in any muscle or intestine samples of the exposed eels. In contrast, Pt and Pd were significantly ($P < 0.05$) taken up from the converter material by the kidney and liver of eels (fig. 1). A significant ($P < 0.05$) Pd uptake was also found for the muscle and intestine of exposed eels. The Pt levels in the kidney and liver ranged between 0.1 ng g^{-1} and 0.2 ng g^{-1}, whereas the mean Pd concentration of both organs was about 120 ng g^{-1}. Nevertheless, the highest metal levels were determined in the parasites with mean concentrations of 6 ng g^{-1} for Pt and 480 ng g^{-1} for Pd. Thus, the Pt concentration in the parasite was 30 and 60 times higher and the Pd concentration was four times higher as compared with fish kidney and liver, respectively.

Bioaccumulation

The CFs varied dependent on the metal and the tissue between 0.002 to 0.48 (table 2). For all tissues the CFs for Pd exceeded those for Pt. For example, the CF of fish kidney or liver was 60 times higher for Pd than for Pt. In accordance with the absolute metal level the highest bioaccumulation was found for *P. ambiguus* followed by the fish liver and kidney. The metal accumulation in the parasite was about 40 times higher for Pt and 4 times higher for Pd as compared with the fish liver or kidney.

Discussion

The present study demonstrates a considerable uptake of Pt and Pd from ground catalytic converter material by various eel tissues and by the intestinal acanthocephalan, *P. ambiguus*. The distribution of the noble metals within the eel resembled the distribution described previously for metals with kidney and liver as target organs, whereas eel muscle exhibited the lowest metal contents (Zimmermann *et al.*, 1999, 2004). Rhodium was not found in eel tissues nor in the acanthocephalans, most likely due to the low exposure concentrations in the water. A recent study has shown that *P. ambiguus* is able to bioconcentrate this metal following experimental exposure to higher Rh levels (Sures *et al.*, 2003). Interestingly, Pd was accumulated in the various fish organs and parasites to a very high degree, with CFs always surpassing those determined for Pt. Investigations on the PGM uptake by the freshwater isopod *Asellus aquaticus* with PGM standard solutions showed a similar trend (Moldovan *et al.*, 2001). Also in an experiment with *D. polymorpha* exposed to road dust, bioaccumulation decreased in the order Pd > Pt >

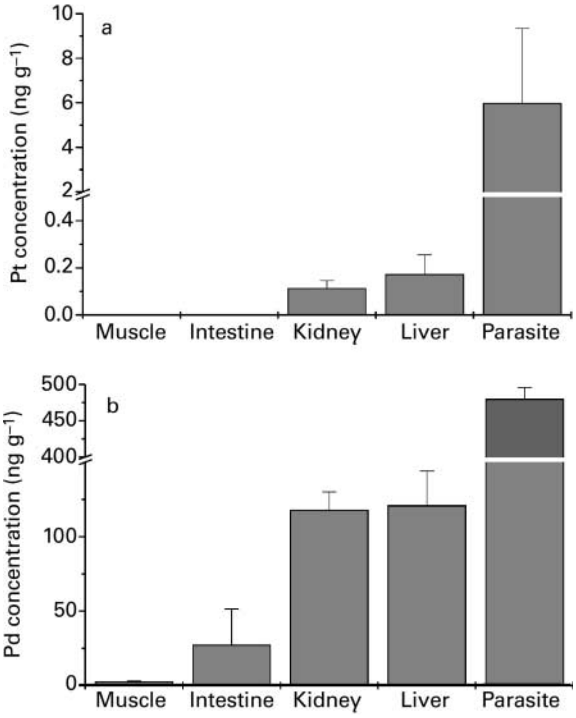

Fig. 1. Distribution of platinum (a) and palladium (b) in the tissues of the eel *Anguilla anguilla* and the acanthocephalan parasite *Paratenuisentis ambiguus* after six weeks of exposure to automobile catalytic converter material. The rhodium levels of all samples were below the detection limit. Values are mean element concentrations (n = 3), error bars represent standard deviation.

Table 2. Concentration factors (CF = $\bar{c}_{\text{animal tissue}}/c_{\text{water}}$) for various eel tissues and *Paratenuisentis ambiguus* after six-week exposure to automobile catalytic converter material.

	CF (Pt)	CF (Pd)
Muscle	–[a]	0.003
Intestine	–[a]	0.028
Kidney	0.002	0.12
Liver	0.002	0.12
P. ambiguus	0.083	0.48

[a] Pt levels in muscle and intestine were below the detection limit.

Rh (Zimmermann *et al.*, 2002). Although different metal sources and different test organisms were used in the experiments cited above, Pd appears to be the most biologically available element within the PGM and hence more emphasis should be made on this metal in future studies.

At a first glance, the concentration factors for the eel tissues and *P. ambiguus* seem to be very low with values ranging below 1. However, it should be noted that the CF was calculated with theoretical PGM concentrations, that were introduced into the tank, without taking into account the water soluble and thus biologically available fraction. Generally, the CF (synonym: bioaccumulation factor) is calculated by dividing the metal level in the test organism by the concentration of dissolved metal in the water (Streit, 1992). The solubility of the noble metals from catalyst material is very low as the PGM mainly occur in elemental forms. Experiments with ground catalytic converter material revealed a solubility of 0.01–0.025% for Pt and 0.05% for Rh at a pH of 5–8 (Zereini *et al.*, 1997; Sures *et al.*, 2003). Preliminary results on the solubility of Pd suggest a value of approximately 0.004% (Zimmermann, unpublished). Considering the solubility of Pt and Pd in the present study, the CF would be 4 orders of magnitude higher, yielding values for the parasites of several hundreds. In the exposure study of Sures *et al.* (2003) who also used ground catalytic converter material, the level of Pt was 50 times higher in *P. ambiguus* than in the tank water.

Although there is a cumulative increase of Pt, Pd and Rh in various environmental abiotic compartments (Zereini & Alt, 2000; Hoppstock & Sures, 2004), the environmental PGM concentrations, e.g. in road dust, are still about 2–3 orders of magnitude lower as compared with common heavy metals such as Pb. However, following proof of the biological availability of PGM, field studies on the anthropogenic PGM contamination of the biosphere especially the fauna are clearly necessary. A recent field study on the Pt levels in *Asellus aquaticus* from urban rivers and a stormwater detention pond in Sweden revealed values ranging from 0.04 to 12.4 μg g^{-1} (Rauch & Morrison, 1999). As crustaceans incorporate heavy metals in their exoskeleton their metal contents are dependent on the moulting status (Zauke, 1982) and are therefore less reliable than data obtained from the inner organs of fish or intestinal parasites.

The bioconcentration of metals in the parasites would be most useful in monitoring environmental PGM contamination as the PGM levels in the environmental compartments are usually very low and sophisticated (ultra) trace analyses are required. Bioaccumulation of the PGM in the parasites could facilitate analytical procedures with less sensitive detection methods (e.g. electrothermal atomic absorption spectrometry) as analytical pre-concentration steps prior to the metal detection may be unnecessary. On the other hand, the use of acanthocephalans as accumulation indicators in combination with extremely sensitive, analytical methods enables the detection of even lowest PGM contamination in the environment.

Apart from fish acanthocephalans, recent studies have shown that acanthocephalans and cestodes parasitizing mammals such as pigs or rats are able to bioconcentrate heavy metals (summarized in Sures, 2004). Therefore, studies on the PGM accumulation capacity of intestinal parasites from terrestrial hosts will be worthwhile as PGM concentrations in mammalian parasites may provide valuable information about the human exposure situation.

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