Potential Effects of Rare Metals on Periphyton Communities: Platinum, Palladium, Indium & Silver

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Abstract

This paper is focused on potential effects of Pt, Pd, In and Ag on lotic periphyton communities that were collected in the relatively unpolluted river (Lärjeån) in Gothenburg, Sweden. The experiment was to combine analytical methods like ICP-MS and ICP-LA-MS with toxicity tests to get a wider view of bioavailability trends of platinum, indium, palladium and silver. By comparing two aquatic exposure environments: river water and Milli-Q water, it was possible to register differences in effects caused on periphyton communities.

Generally, to different extent, there is a linear relation between exposure and uptake of metals noticed. The highest uptake was associated with exposure to Pt and In and the lowest to Pd.

The exposure concentrations used (1, 10, 100, 1000nM) were not toxic to periphyton communities, however analysis of In and Pt a trend indicated that further increase of exposure concentration might cause a toxic effect.

Like cobalt, iron seems to be characteristic for biota only. Not only the signal during ablation of periphyton sample was higher than in case of clean disc but also a correlation between Fe and In was observed.

This pioneer study provides general information on exposure/uptake relation of Pd, Pt, Ag and In in the periphyton community and thus future research is advised to verify and to deepen current findings.

List of tables and figures

1.Introduction

There is a raising trend in application of rare metals in modern technology. As a side effect a new phenomenon in nature has been created. Some elements are being introduced at the bigger scale into environment that biota has not been in contact before due to either their scarcity in the Earth crust or due to their specific chemical properties that usually their concentration in water was negligibly low.

This study is focused on the potential toxic effects induced on water microorganisms by four rare metals: platinum, indium, silver and palladium. The aquatic toxicity of those four chosen metals has not been yet deeply studied but their industrial use increases.

Because different studies showed that toxicity tests with periphyton communities provide important information about the environmental toxicity of compounds like sulfonylurea herbicides, zinc or antrazine it was decided to perform similar tests.

The aim was to combine analytical methods like ICP-MS and ICP-LA with toxicity tests to get a wider view of bioavailability trends of platinum, indium, palladium and silver. By comparing two aquatic exposure environments: river water and Milli-Q water, possibly effects caused on periphyton community could be registered.

This paper is a contribution to pioneer ecology studies on aquatic toxicity of rare metals in the lotic system periphyton communities.

2. Metals

The table of elements is divided into groups in respect to their properties. Since this paper is focused on Pt, Pd, Ag and In three groups of elements must be mentioned that are called group A, group B and borderline group.

Palladium, platinum and silver belong to the B metal class that is known for strong affinity to ligands containing sulfur and nitrogen. These binding preferences would indicate that those metals interact principally with macromolecules such as proteins and nucleotides, in which sulfhydryl groups and purine/pyrimidine bases are rich in sulfur and nitrogen.

Indium belongs to metal group that is considered as borderline metals. This group shows ligand-binding characteristics that are intermediate to those of group B and group A. Since group B was described above, it must be said that group A has the strongest ligand-binding preferences for oxygen and phosphorous. The complexation trends of the borderline group are relatively complex and strongly dependent upon their immediate environment.(Mason & Jenkins 1995)

All four metals are soft, ductile and lustrous. Some additional information is presented in Table 1.

Table 1. Comparison of selected properties of Pt, In, Ag and In

Property	Platinum (Pt)	Indium (In)	Silver (Ag)	Palladium (Pd)
Atomic number	78	49	47	46
Atomic weight	195.08	114.8	107.7	106.42
Melting point	1,772 °C	156.6 °C	961.9 °C	1,554 °C
Boiling point	~3,827 °C	~2,080 °C	~2,212 °C	~2,970 °C
Valence	+2, +4	+1, +2, +3...	+1, +2	+2, +3,+4
Known since	1736	1863	~4000 BC	1803

2.1 Indium

Indium was named after the brilliant indigo line in its atomic spectrum. This element is found in very low concentrations in many ores and is produced mainly from residues generated during zinc ore processing. Canada produces the majority of the world's supply of indium.
The first large-scale application for indium was as coating for bearings in high-performance aircraft engines during World War II. Afterwards, production gradually increased, as new uses were found in fusible alloys, solders, and electronic. Indium phosphide, arsenide and antimonide are semiconductor materials used in photocells, thermistors and rectifiers.
In the middle and late 1980's the development of indium-tin-oxide thin films for Liquid Crystal Displays (LCD). Nowadays they are very popular in portable computers, digital clocks, microwave ovens, digital clock, CD players etc. (web ref. 1, web ref. 2)

The most common form of indium in fresh water is either $In(OH)_2$ or $In(OH)_3$. Because of high affinity to sulfur-containg ligands, indium might be a potential enzyme inhibitor. In the tests performed on bacteria cultures 57mg/L was enough concentration to inhibit the growth (Anderson & Apanna 1993)

2.2 Silver

It is not a chemically active metal, however attacked by nitric and hot concentrated sulfuric acid. Of all the metals, pure silver has the whitest colour, the highest optical reflectivity, and the highest thermal and electrical conductivity. Owing to these properties, silver has many industrial applications, such as in mirrors and electrical and electronic products. (web ref. 3) Silver chloride, bromide, and iodide are used in photography because of their sensitivity to light. (web ref.1) Silver's catalytic properties make it ideal for use as a catalyst in oxidation reactions. (web ref. 3) Another application of this metal is jewellery.

Silver is usually found in the combined state in nature and most silver used today is obtained from its ores. Mexico, USA (Idaho, Montana, Arizona, Colorado, Utah, Nevada, California, New Mexico, and Texas), the former USSR, Peru, Australia and Canada are leading producers.(web ref 1)

The most common forms of silver in freshwater are free ions Ag^+ and AgCl. Silver may be also bound to particles and organic matter or be accumulated in the sediment (Luoma et al. 1995) Under anoxic conditions Ag(HS) and Ag_2S are formed. The bioavailabilty of silver

depends on water chemistry very much. Binding to particles is clearly shown in the sewage system studies. (Shafer et al. 1998)
Silver accumulation in aquatic organisms is primary attributed to the bioavailability of the free silver ions but some reports suggest that AgCl (aq) is also available for biological uptake. (Reinfeld & Chang, 1999)

2.3 Platinum

Platinum is chemically inactive and unaffected by common acids, however, dissolves in aqua regia. It is attacked by halogens, sulfur and caustic alkalies. It does not combine with oxygen even at high temperatures. Like palladium it absorbs large quantities of hydrogen.(web ref. 1)

This metal is found in nature, alloyed with other metals of so-called platinum group. The other five metals in this group are iridium, osmium, palladium, rhodium and ruthenium.

Platinum group metals are found in alluvial deposits in the former Soviet Union South Africa, Colombia and Alaska. Platinum and the related metals are recovered commercially as a byproduct of the refining of nickel ores mined near Sudbury, Ont., Canada; from gold mines in South Africa; and from the alluvial deposits in the former Soviet Union.
Platinum and its alloys are used in surgical tools, laboratory utensils, electrical resistance wires, contact points, and standard masses. Because its thermal coefficient of expansion is nearly equal to that of glass, platinum is used to make electrodes sealed in glass. It is used extensively in jewerly and in dentistry. A platinum-cobalt alloy is used to make very powerful magnets.(web ref. 1) Platinum is also used in cancer therapy as a medicine. At present, the principal drugs used are cisplatin and carboplatin. (Rauch, 2001)
Platinum is specially prepared for use as a catalyst both in cars and industrial processes. (web ref 1)

Platinum , because of its inertness, was considered harmless for a long time. It has been included in the investigations into potential toxic substances to human health since the widespread introduction of catalytic converters for automobile exhaust gases. Platinum is regarded toxic to humans and an increase in concentration
Platinum mainly occurs in fresh water as $Pt(OH)_2$ and Pt(II) bound to organic matter. This element is relatively immobilised in soil and sediment. (Sternbeck & Oestlund 1999)

2.4 Palladium

Palladium is, directly after platinum, one element of the platinum metal group. It is strongly resistant to corrosion in air and to the action of acids (except nitric acid) at ordinary temperatures. However it is attacked by hot acids and dissolves in aqua regia. It forms different compounds like oxides, chlorides, fluorides, sulfides, phoshides and several complex salts. This metal, when finely divided, has a great ability to absorb hydrogen and one volume of palladium absorbs even 900 volumes of this gas.
Palladium is found in nature with platinum minerals and in association with nickel ores mined near Sudbury, Ont., Canada.
Because of its corrosion resistance, a major use of Pd is in alloys used in low voltage electrical contacts. It is used in watch bearings, springs and balance wheels and also for mirrors in scientific instrument. For use in dentistry it is alloyed with silver, gold and copper.
In chemistry it is a catalyst in sulfuric acid manufacture and in hydrogenation processes. (web ref1)

The use of palladium in three-way autocatalysts is now starting to increase, since palladium has similar catalytic properties as platinum and is much cheaper. (Jensen, 2000)

The most popular form in fresh water is $Pd(OH)_2$ and Pd(II) bound to organic material. Palladium is regarded as more mobile in ecosystem than platinum. Pd is found in ash coming from waste incineration and traffic.(Sternbeck & Östlund 1999)

3.Description of lotic periphyton community

The term *lotic systems* refers to running waters like rivers, streams and brooks. They are important elements of the hydrological cycle. Their role is to collect and convey water that is received from precipitation, runoff, subsurface flow and ground water flow (Paulsson 2000). If there is a risk that water has been contaminated by toxicants the lotic systems facility the migration of contaminants.

What influences the condition of running water? In general it is said that water chemistry and physics determine it. The climate and catchment characteristics determine the water flow that in turn affects the amount and rate of material entering the stream. The supply of material results in the properties of water like temperature, light, pH, salinity, hardness, concentration of particles, dissolved organic matter, oxygen and nutrients as well as land-delivered toxicants.

All those mentioned factors are essential but one cannot forget to mention living elements of lotic systems. Specific water conditions (chemistry and physics) determine the character of local biota. At the same time biota are able to change their habitat and thus influence the condition of water.

Biota act as a chemical modulators by transforming many inorganic chemicals into organic forms and stabilizing substrates. Periphyton communities in the running waters consist of populations of microscopic algae, bacteria, fungi and different grazers as well as silt particles. All populations are trapped in gelatinous polysaccharide matrix and interact with each other. Such communities living in a prescribed habitat are important primary producing biota in lotic systems.(Paulsson 2000)

Most often periphyton refers to microorganisms that live or are just attached to submerged substrates (both natural and artificial). The colonization of a clean substrate starts with the development of an organic matrix that enables bacteria to grow. Further small adnate diatoms settle followed by apically attached colonial diatoms and finally filamentous green algae and cyanobacteria (Biggs1996) When biofilm becomes thicker, even moving organisms can be found. (Round 1964)

The periphyton biomass is limited by carrying capacity that is reached when biomass accrual equals the biomass loss. (Biggs 1996)

Under unfavourable conditions like toxic stress or insufficient nutrient and light access the more sensitive species may move to a cleaner site. Mechanical stress such as grazing and floods will also affect the amount of loss from the periphyton biofilm. Current velocity is very important factor regulating the structure, biomass, export and colonization rate of periphyton.

The outer living organisms, living at the boundary layer of community, receive more of their nutrients from the surrounding water than organisms situated deeper into the biofilm. Due to the difficulty to gain nutrients from water, they have to depend more on internal cycling of nutrients or nutrients from substrate. With the increase of the biofilm thickness the internal cycling of nutrients increases too. At the same time, the increase of the biofilm thickness means the extension of the boundary layer and this may result in large gradients (e.g .pH) (Paulsson 2000)

3.1Bioavailability and toxicant uptake mechanism in algae

Bioavailaibility, according to U.S. EPA, is the degree of ability to be absorbed and ready to interact in organism metabolism. Bioavailability of given substance differs on different lotic systems due to individual properties, both chemical and physical, of water. In addition different species have different uptake capacities.
The direct way of studying bioavailability is to measure the amount of a substance that has been taken up by the organism and so it has been done in this thesis.

Given adequate time with constant exposure, the toxicant concentration in the organism will reach an equilibrium, where the internal concentration of the toxicant is generally considerably higher than the environmental concentration. This process is called bioconcentration. (web ref 5)
Risk assessment of chemicals in the environment is based on data for two important aspects: fate and effects. Evaluation of the risks of chemicals, however, is hampered by the fact that for most existing chemicals no data are available regarding their fate and effects in the environment. Effects assessment is usually based on relatively simple effect parameters such as survival, inhibition of growth or reproduction of species. (web ref. 6)

The short-term toxicity test can be performed to measure sensitivity to toxicants. These tests are based on different metabolic processes, such as photosynthesis, and help to estimate community tolerance.
A step forward in toxicity tests is to determine so called Pollution Induced Community Tolerance (PICT) that presents a long-term effect. The theory behind PICT includes the exclusion of sensitive species (individuals) and replacement by more tolerant ones, which results in an overall more tolerant community. This increase in tolerance can be quantified in a short-term metabolic toxicity tests. (Paulsson 2000)

As it was described in the previous chapter, the thicker the biofilm is, the more difficult is to take up nutrients from water by deeper situated microorganisms.
If there are toxicants in the water, they would be taken up mainly by the microorganisms living at the boundary layer if the biofilm is thick, in the same way as nutrients. Thus it is advisable to use relatively thin periphyton communities in toxicity studies.

Metal ions can enter a cell along via hydrophobic solution in the membrane, attachment to the membrane proteins and carbohydrates, attachment to the membrane lipids or endocytosis of membrane components. Another route is permeation that may occur though water channels, nonspecific and specific channels. Permeation maybe caused by general active processes like electrochemical potentials or by specific active processes like ATPases. (Tessier, A & Turner, D.R. 1995)

3.2 Co-tolerance mechanism

Co-tolerance in periphyton community may occur when pre-exposure to one toxicant induces tolerance not just to this particular toxicant but also to other toxicants. Multiple tolerance can be defined as when e.g. community possesses some tolerance mechanism that confers tolerance to several toxicants without pre-exposure to any of the some property related to chemical structure or mode of action. This phenomenon is common for metals in microbial communities and multiple tolerance has been reported too. Thus co-tolerance and multiple tolerance are obvious problems in field measurements. (Paulsson, 2000)

4. Method

4.1 Periphyton community

The sampling site has to be selected on the basis of specific requirements. The aquatic environment should not collect contaminated water. Any unnatural concentration of analysed metals in water is unwanted because algae cannot be in contact with them before the test exposition takes place. Chemistry and physics of running water is also required to be as stable as possible. Sampling site ought to be relatively easily accessible for sampling operations and thus an immediate transport of collected samples to laboratory could be provided.

In this study Larjeån was chosen as a sampling site. This river is not in contact with possible source of contamination. There are rather recreational areas that surround the river. It is possible to approach the sampling site by car but the last few hundreds meters to the riverbank must be passed on foot only.

The study performed in 1996 indicated diatom species composition in Larjeån (Table 2).

Table 2.Diatom species composition in Larje river.(based on Nyström 1997)

Very dominating species	**Dominating species**	**Other diatoms**
Achnanthes minutissima	Cocconeis placentula	Navicula lanceolata and N.sp.
Achnantes sp.	Cocconeis sp.	Nitzschia palea and Nitzschia. sp.3
	Gomphonema angustatum	Eunotia sp.
	Gomphonema sp.	Eunotia/Pinnularia like
		Synedra sp. and Synedra ovalis
		Cyclotella meneghiniana

The same study reported the algal biomass found. In case of Larje river it was described as 447865 algal cells/cm^2 and 1.65 µg/cm^2 of chlorophyll a.

4.1.1 Sampling and exposure

Periphyton communities were colonized for 2-6 weeks during summer 2001 on glass discs of area 1.5 cm^2 in the river called Lärjeån. The glass discs were mounted vertically on holders carrying 10 discs each and were inserted in a polyethylene racks. The racks were floating parallel to the main current at a depth of around 0.5 m.

Collected glass discs with periphyton were cleaned on all but the rough front surface and put into scintillation vials with the periphyton-covered side pointing upward. Vials were closed with lids. Samples were pre-incubated in 2 ml of filtered river water and 2 ml of solutions of each metal in concentrations from 10^{-9} to 10^{-6} M. Another set of samples was pre-incubated

with Mili Q water instead of river water. In both cases, control samples were incubated but not exposed to any metal solution. Five replicants were used per concentration. Pre-incubation was performed in a thermostat-controlled water bath under continuos shaking for 1 hour.
Two samples for determination of background activity were treated with formaldehyde and run together with the concentration series. The light and temperature were adjusted in such way to imitate natural conditions.

4.1.2 Toxicity tests

Periphyton photosynthetic activity was measured as incorporation of radioactive carbon added as ^{14}C-bicarbonate. $H^{14}CO_3^-$ solution was added to all vials after the 1 hour pre-incubation with metals. After 1 h of incorporation a formaldehyde was added to terminate the incorporation process. To drive off the remaining inorganic carbon 200 μl of concentrated acetic acid was added and the samples dried at 60^o under a stream of air. To enhance the release of incorporated ^{14}C- carbon from periphyton 1 ml of dimethylsulfoxide wasadded.
After adding 9 ml of scintillation cocktail, the amount of ^{14}C-carbon was measured in scitillation spectrometer. The activities, as disintegrations per minute (dpm) were calculated from the counts per minute (cpm) data, using external standard calibration and automatic quench compensation. Then the background activity was substracted from the obtained dpm values. (Nyström 1997)

There were used three replicates at every exposed concentration. The tests performed on control samples were in particular important. In each case the boarding lines were plotted to determine the minimum and maximum toxic effect registered for control samples (not exposed to metals). On this basis it was assumed the area between boarding lines indicates no toxic effect.

4.2 Metals analysis

The discs with periphyton, after performed toxicity tests, were kept in the refrigerator before the further analysis. The preparation of the samples for ICP-MS analysis started with the microwave digestion. Digestion was performed using microwave HP-500, Mars 5 (USA). Each disc was immersed in the separate vessel with 8 ml of aqua regia and put in the microwave. The samples were run under the program Assellus.

After the digestion was completed, the liquid content of the vessels, including biomass dissolved in aqua regia, was poured to Erlenmayer flasks and evaporated. The dry residue was redissolved in the 1% HCl. Thus the samples were prepared for ICP-MS analysis. At this stage of analysis Quadrupole ICP-MS ELAN 6000, Perkin Elmer was used.

ICP-MS results required mathematical correction due to interference that took place during analysis. Usually, argides, oxides and double charged ions are formed while determining Pt and Pd. To estimate the contribution of interfering species the analysis of standard solutions was performed simultaneously at concentration from 0 to 1000 ppt.

The corrections were calculated according to following equations:

$$I_{Pt} = I_{Pt,s} - \left(I_{Hf,s} \times R_{HfO,Hf}\right) \qquad 1.1$$

$$I_{Pd} = I_{Pd,s} - \left(I_{Cu,s} \times R_{ArCu,Cu} + I_{Y,s} \times R_{YO,Y} + I_{Sr,s} \times R_{SrO,Sr} + I_{Rb,s} \times R_{RbO,Rb}\right) \qquad 1.2$$

Where for Pt*:
I_{Pt} is Pt intensity after correction,
$I_{Pt,s}$ is apparent Pt intensity in the sample

$I_{Hf.s}$ is the Hf intensity in the samples and $R_{HfO,Hf}$ is the ratio of HfO^+/Hf^+, determined previously in Hf containing standards.

There were several steps in analysis of ICP-MS data. Firstly, the concentrations indicated by mass spectrometer, in the liquid extracts obtained through the microwave digestion of perifyton, were plotted against the exposure concentrations. This was performed to check whether there is any trend possibly seen.
Secondly, the uptake was calculated in relation to the dry weight of perifyton. Those results presented the approximated metal concentration in the algae cells.

The glass discs, to which biofilm was attached, contained beside perifyton also nonliving elements like detritus and particles. This creates a risk that metals might have been adsorbed on them. The microwave digestion, that was used to prepare samples for ICP-MS, was performed on the whole discs so if metals were somehow bound to any other part that algae, they were extracted into solution. In that case ICP-MS data may be a bit less reliable.

Due to the fact that perifyton samples were not homogenous **LA-ICP-MS** analysis was suggested. Laser ablation, using LSX-200 (CETAC), was performed in such way that laser beam passed mainly algae colonies. That was planned to eliminate the possible risk associated with ICP-MS analysis.

Due to time limitations only samples exposed to the highest concentrations of each metal were analyzed by laser ablation. It was decided to track also signals for Mo, Co, Fe, Si and C in addition to the elements of interest.
Only samples exposed in Milli-Q water were selected for analysis after the previous analysis of Ag in both waters. The test performed on Ag samples showed much more clear, noticeable results in case of Milli-Q water and thus it was decided to limit the analysis of the rest of metals just to Milli-Q water.
The parameters like spot size and ablation speed were adjusted during the analysis to obtain the best signal. A larger spot chosen enabled to ablate bigger area of the sample and thus increase the sensitivity. On the other hand, smaller spot size and lower energy provided more detailed spatial resolution leading to more detailed investigation of elemental distribution in the sample.

Analysis of samples by laser ablation gave just qualitative results, however to some extend it can be assumed that the strength of signal is proportional to the concentration of elements in the sample.
**The corresponding nomenclature refers to palladium too.*

4.3 Theory

4.3.1 Principle of operation of ICP-MS

The liquid sample is pumped at 1 mL/min (usually with a peristaltic pump) into a nebulizer, where it is converted into a fine aerosol with argon gas at about 1 L/min. The fine droplets of the aerosol are separated from larger droplets using a spray chamber. The fine aerosol then emerges from the exit tube of the spray chamber and is transported into the plasma torch via a sample injector.

The plasma is produced by the interaction of an intense magnetic field on a tangential flow of argon at about 15 L/min flowing through a concentric quartz tube (torch). This ionizes the gas and, when seeded with a source of electrons from a high-voltage spark, forms a very high temperature plasma discharge (~10,000 K) at the open end of the tube.

Once the ions are produced in the plasma, they are directed into the mass spectrometer via the interface region.. This interface region consists of two metallic cones (usually made of nickel), called the sampler and a skimmer cone. Each cone features a small (0.6 - 1.2 mm) orifice to allow the ions through to the ion optics, where they are guided into the mass separation device.

Once the ions have been successfully extracted from the interface region, they are directed into the main vacuum chamber by a series of electrostatic lenses called ion optics. A turbomolecular pump maintains the operating vacuum in this region at about 10^{-2} torr. There are many different designs of the ion optic region, but they serve exactly the same function - to electrostatically focus the ion beam towards the mass separation device and to stop photons, particulates, and neutral species from reaching the detector.

The ion beam containing all the analyte and matrix ions exit the ion optics and now pass into the heart of the mass spectrometer - the mass separation device, where a second turbomolecular pump maintains an operating vacuum of approximately 10^{-6} torr.

Separation device allows analyte ions of a particular mass-to-charge ratio (m/z) through to the detector and to filter out all the non-analyte, interfering, and matrix ions. In the final process, an ion detector converts the ions into an electrical signal. This electronic signal is then processed by the data handling system in the conventional way and converted into analyte concentration using ICP-MS calibration standards.(web ref4)

4.3.2 LA-ICP-MS

Laser ablation means the applications of a laser to ablate material from a surface. The energy of laser is focused and concentrated onto material surface. This energy can be absorbed by the material according to the absorption characteristics and the wavelength of the laser. When sufficient amount of energy is absorbed, vaporisation at the sample surface starts. Vaporisation is relatively slow so energy is absorbed under the surface before vaporisation and this leads to pressure rise and explosions. (Jensen, 2000)

If laser ablation is combined with other techniques like LA-ICP-MS a laser beam is used to directly sample solid material for ICP-MS analysis. The sample is placed in a cell in which a flow of argon flushes the ablated material into the ICP-MS (Rauch 2001)

Laser ablation has many advantages in comparison to other introduction methods because the sample does not have to be pre-prepared so solid samples can be analyzed directly. It is possible to analyse relatively small samples and perform spatially-resolved analysis including depth profiling. Furthermore, the production of dry aerosol leads to decreased interference from oxides effects that usually appear due to the solvent.

On the other hand, there are also disadvantages. Quantitative analysis from laser ablation requires a very close matrix-matched calibration standard. (Lu, 2000)

5.Results

5.1 Results of ICP-MS

The analysis of samples of ICP-MS showed the relation between exposure and uptake of metals by algae. Then those results were plotted against the toxicity data and the uptake/ toxicity relation was presented.

Generally, to different extent, there is a linear relation between exposure and uptake noticed for analyzed metals (Fig 1, Fig 2).

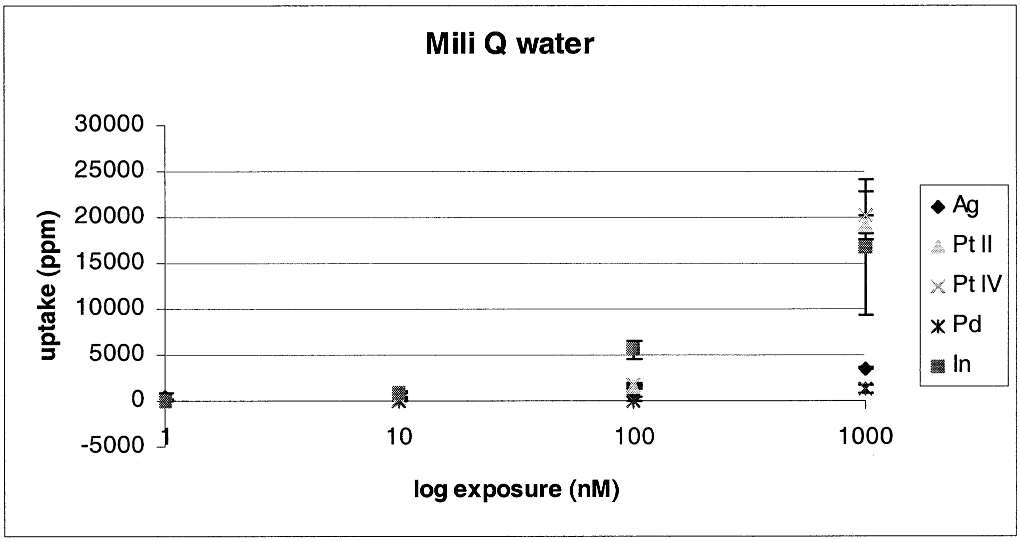


Figure 1. The uptake/exposure relation in Milli -Q water for all analyzed metals

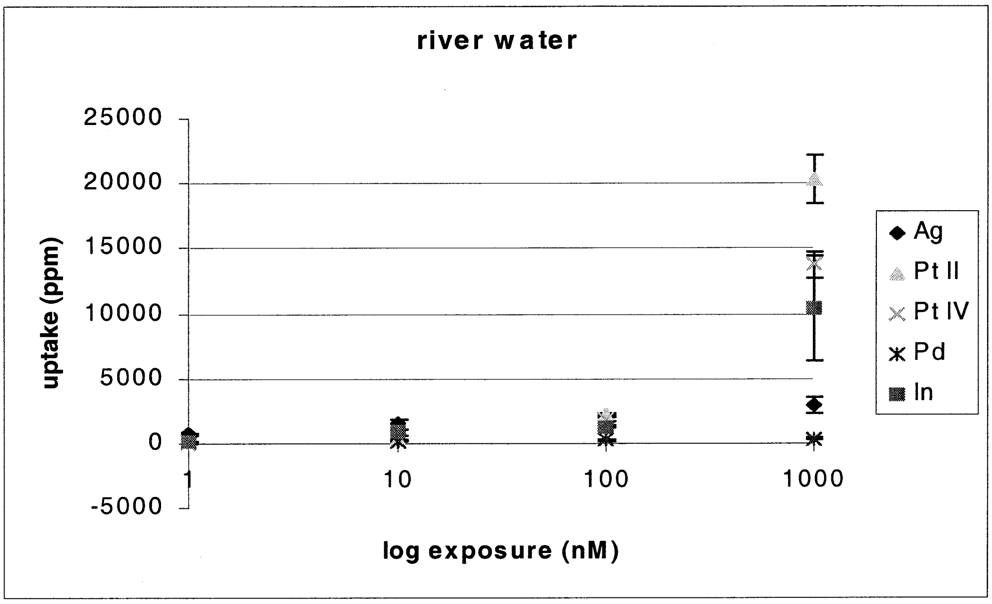


Figure 2. The uptake/exposure relation in the river water for all analyzed metals.

Uptake of the majority of metals, independently on the type of water used, was determined at the similar level within the exposure range from 0 to 100 nM. No significant differences in uptakes were noticed except for In at 10nM exposure in Milli-Q water.

Only exposure at the highest concentration (1000 nM) resulted in the wide range of uptakes individual for each metal (Fig. 1, Fig. 2). There is almost defined sequence of uptake of metals at this concentration and the bioconcentration of metals found is just a bit different in relation to type of water. The bioconcentration sequence for Milli-Q water can be defined as Pt>In>Ag>Pd and for river water PtII>PtIV, In>Ag>Pd.
As it can be seen above, the only difference concerns platinum. The uptake of both forms of platinum is similar for Milli-Q water but it is not so in case of river water. It can clearly be noticed that there was reasonably more Pt II taken up from river water than Pt IV.
Although there is no difference in uptake of PtII in both waters, uptake of Pt IV is much lower in the river than in Milli-Q water.
Like Pt IV, Pd and Ag seem to be a bit easier taken up in Milli-Q water rather than in river at 1000nM.

5.1.1 Platinum

During analysis it turned out that results for PtII were more precise than those for PtIV. In addition the best precision was reached for the exposure of Pt II in river water. In this case SD has not exceeded 15% of mean value at any exposure concentration.

It is characteristic that the biggest SD, ranging from 15 to 87%, accompany the control samples in both waters for PtII and Pt IV.

Taking into account relatively high SD it might be concluded that, regardless of the type of water, the uptake was almost equal for both forms of platinum and thus a clear increasing trend in uptake can be noticed for PtII and PtIV (Fig.3).

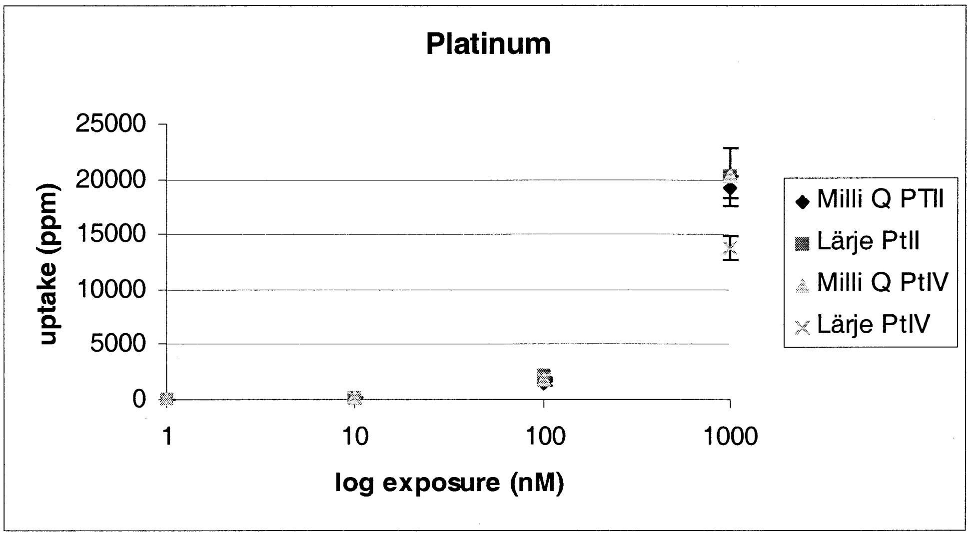


Figure. 3 An uptake/exposure relation in both types of water obtained for platinum.

For both platinum forms, the uptake at exposure 1 and 10 nM is at the same level as for other metals however the increase in exposure concentration to 100 nM resulted in significant increase in uptake in both type of water. Similar scale of that increase has not been noticed for other metals at 100 nM.

At higher exposure concentration (1000 nM) uptake was indicated at around 20 000ppm. Only, Pt IV in river water was taken up at the lower range (around 15 000ppm). Although the exception for Pt IV in river water, in general, platinum uptake was the highest among all metals analysed. Moreover, the uptake of Pt II and IV at 1000nM was significantly (at least 4 times) higher than any other metal at corresponding concentration in Milli-Q water.

5.1.2 Indium

The analysis of the uptake of In either in Milli-Q or river water shows the increasing trend along the exposure curve. (Fig.4)

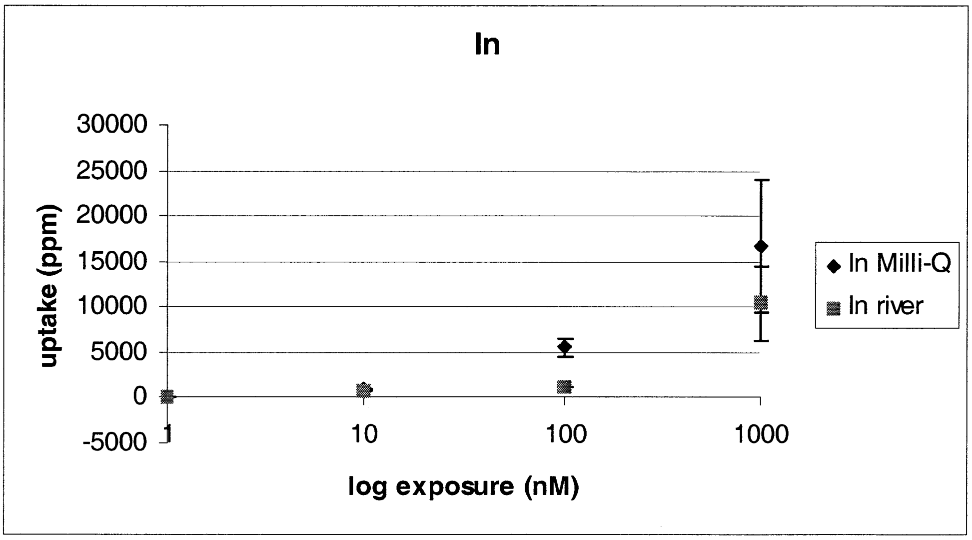


Figure 4. The uptake/exposure relation in both types of water obtained for indium.

The uptake is higher for Milli-Q water than in river water. Uptake in Milli-Q water is just a bit lower than uptake of Pt. Values representing the uptake in Milli-Q water are associated with bigger SD than the ones in case of river water.

5.1.3 Silver

Although the uptake of silver is not high in comparison to platinum and indium there is still a trend visible and uptake changes along exposure range. Apart from the lowest exposure concentration (0 and 1nM) and the highest (1000nM) uptake there are separate uptakes for Mill-Q and river water. Usually the uptake of silver is higher from river than from Milli-Q water. (Fig.5) The exposure at the highest concentration gave unclear results. The standard deviation for uptake in river water is reasonably big thus it is questionable whether silver is taken up at the same level in both type of water at concentration equal to 1000 nM.

The results for Milli-Q water are quite precise and carry small SD. The uptake trend in this type of water shows a smoothly increasing (except for 1 nM) curve along exposure range until it reaches 100 nM. At that exposure point, a curve increases dramatically reaching the highest value at 1000nM. The uptake at 1000nM differs approximately by 3000ppm from the previous one.

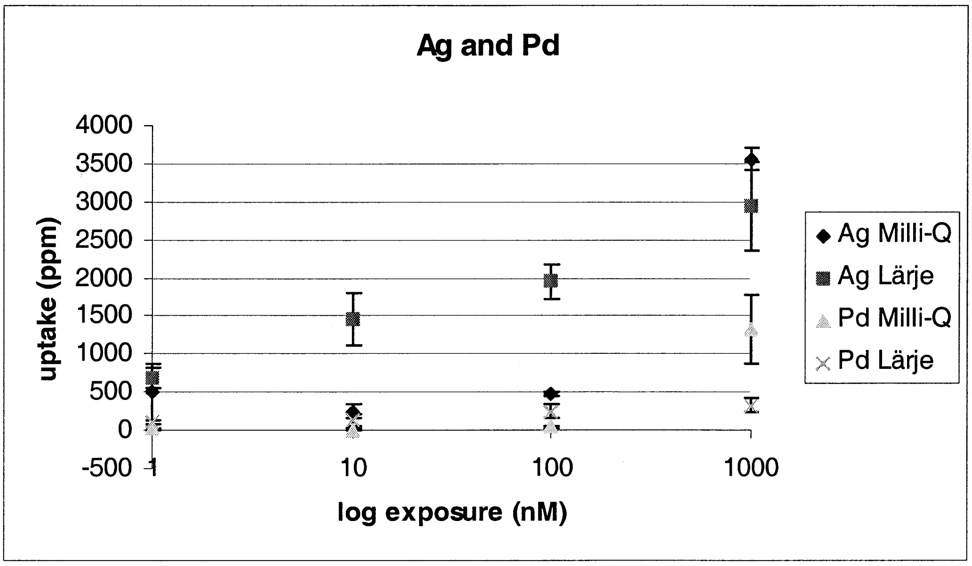


Figure 5. An uptake/exposure relation in both types of water obtained for silver and palladium.

The fact that Ag uptake is higher in river water than in Milli-Q water is controversial. Usually, the presence of organic matter, that is natural for river environment, inhibits the uptake of metals by binding them. Theoretically, bioavailability of Ag on river water should be lower than in Milli-Q water.

Moreover, the uptake of silver in this study is lower than expected. A reasonably strong affinity to this metal was in other algae studies (Ratte 1999) and bioaccumulation was to occur mainly by adsorption on cells. (Fisher & Reinfelder 1995).

5.1.4 Palladium

It seems that the uptake of Pd is lower than uptake of other metals that were simultaneously analyzed (Fig.1). Moreover, Pd uptake data are not precise as the rest of metals. Standards deviations, in general, are very high (in percent) in comparison to the results of other analyzed metals.

The uptake of Pd in river water did not change even if the perifyton was exposed to increasing concentration of that metal (Fig. 5). Almost similar level of uptake was kept within the exposure range and was always lower than 500 ppm.

A bit clearer trend is visible for uptake of Pd in Milli-Q water. The uptake remains stable and low (< 50 ppm) until the perifyton was exposed to 100 nM. The change of exposure concentration from 100 to 1000 nM caused the rapid increase of uptake that oscillated around 1200 ppm. It must be added that this result carries reasonable SD that can be compared to the SD of Ag uptake result obtained at the same concentration but in the river water.

The results obtained for palladium were quite surprising. It was expected that uptake of this metal would be higher due to reports from the previous study on uptake Platinum Group Elements by aquatic macroinvertebrate Asellus aquaticus. Palladium concentration found in Asellus aquaticus after exposure was higher than concentration of platinum. A comparison of Pd/Pt ratio in river sediment and Asellus body indicated the occurrence of Pd in more bioavailable form than Pt. (Rauch & Morrison, 1999)

5.2 Results concerning toxicity

The next step in analysis was to plot uptake data versus toxicity results. The toxicity tests were performed independently to ICP-MS on the same samples.

None of the metals studied showed a clear increase in toxicity. All values oscillated between the boarding lines that were created for control samples. No toxic effect was determined.

In some cases like In and Pt in Milli-Q water a trend is visible. Although no toxic effect was registered a gradual decrease in percentage of control can be seen along with increasing uptake. (Fig 6, 7, 8). This gradual increase was limited within the 'boarding region'. That might mean that metals are biaccumulated in algae but the concentration is too low to cause toxic effect or metals are just adsorbed on the alga membranes and thus uptake is visible but toxic effect can't take place because there is no transfer into the cell.

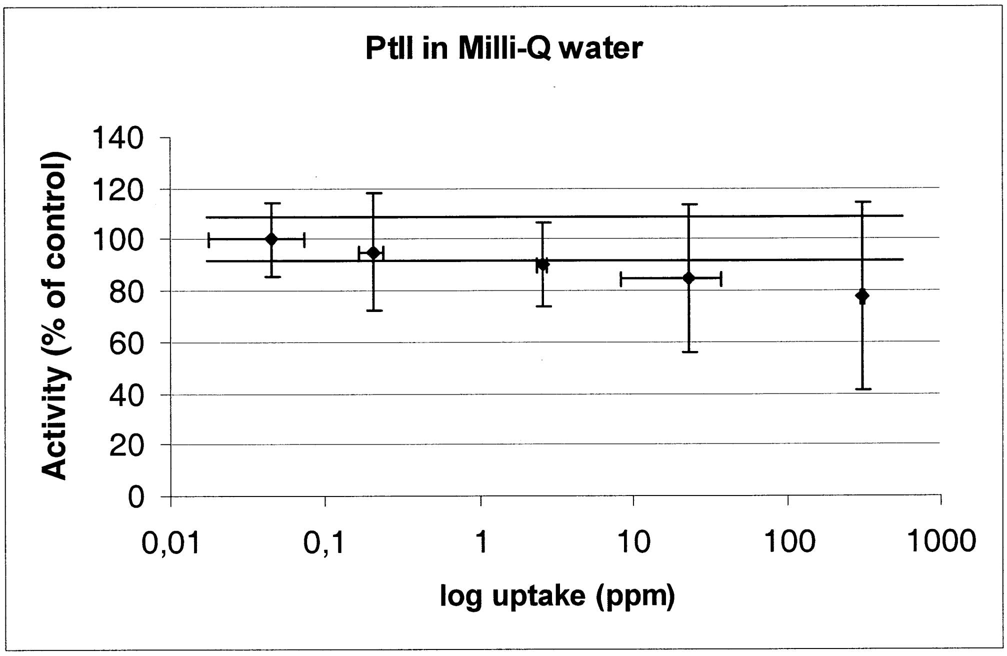


Figure 6. The effect on photosynthesis in periphyton in relation to uptake of Pt II in the Milli-Q water The unexposed sample is marked as 100% control. Dotted line indicates 'no effect region'.

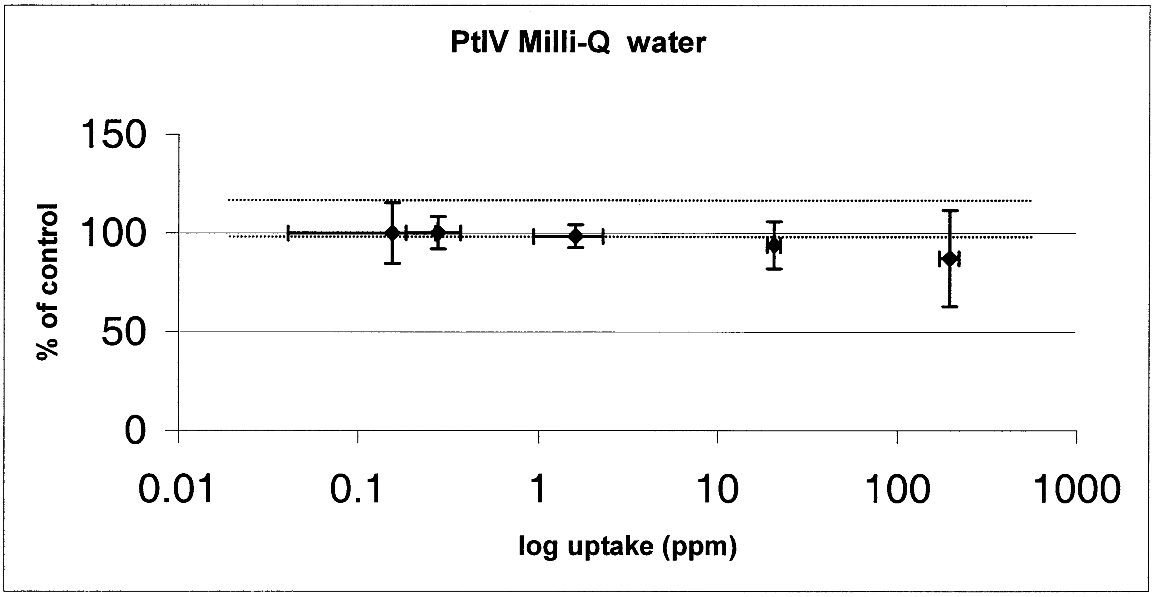


Fig 7. The effect on photosynthesis in periphyton in relation to uptake of Pt IV in the Milli-Q water. The unexposed sample is marked as 100% control. Dotted line indicates 'no effect region'.

As it was said above, the trend for In in Milli-Q water is visible (Fig 8.)

The similar tendency for In, but not as clear as in case of Milli-Q, can be seen in river water too. Moreover the results indicating changes registered at the two highest In uptake in river water posses the smallest SD. Those two points hardly overlap the lower part of the 'boarding region' (Fig 9)

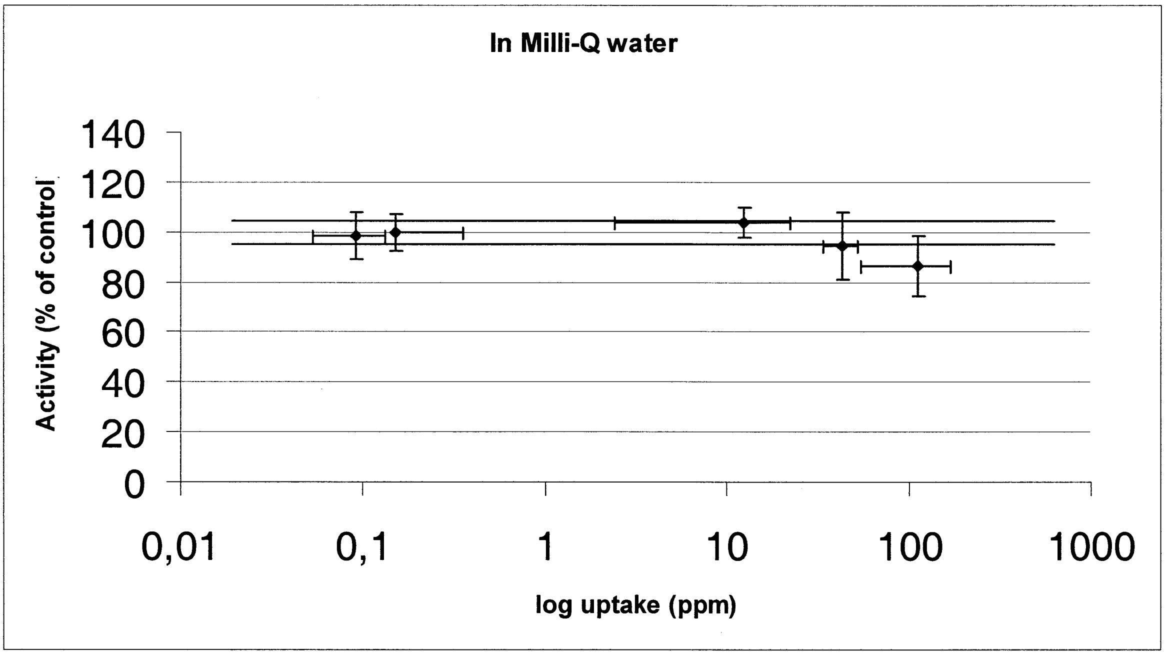


Figure 8. The effect on photosynthesis in periphyton in relation to uptake of Pt In in the Milli-Q water The unexposed sample is marked as 100% control. Dotted line indicates 'no effect region'.

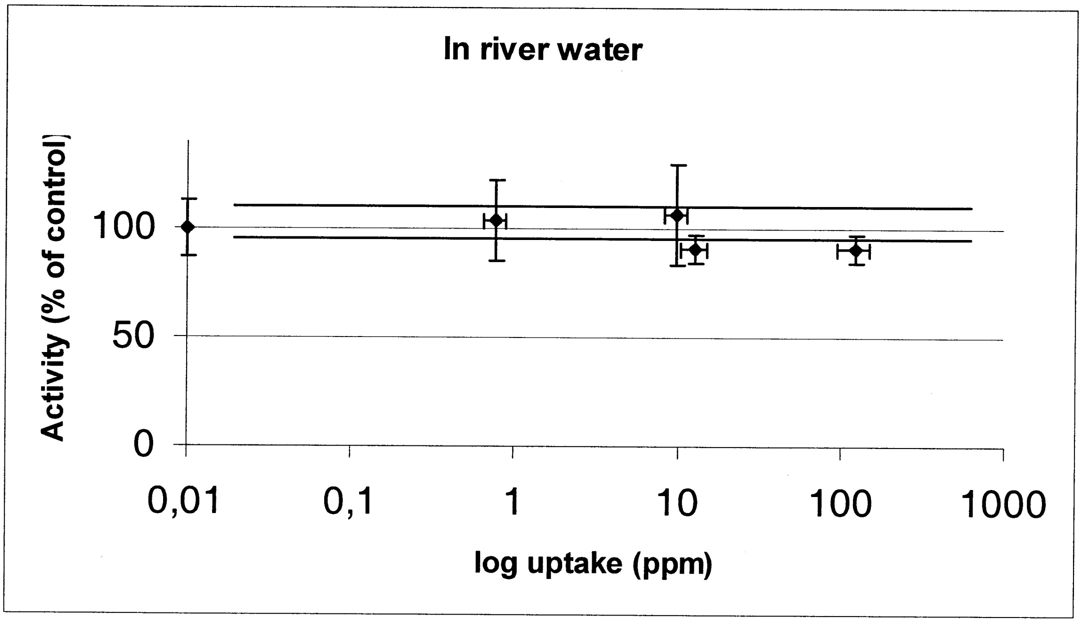


Fig 9. The effect on photosynthesis in periphyton in relation to uptake of In in the river water
The unexposed sample is marked as 100% control. Dotted line indicates 'no effect region'.

Ag and Pd toxicity graphs show no specific trend. The percentage of control almost doesn't change along uptake.

To interpret toxicity tests one must remember that the exposure concentrations used were relatively low and they were to imitate the realistic water concentrations of analyzed metals. Additionally, the tests consisted of short term exposure (few hours) only. There is a low risk that short term exposure can cause a drastic drop in percentage of control.

Taking under consideration above conditions no clear toxic effect could be expected.

5.3.Laser ablation results

5.3.1Pt

The laser ablasion was performed under the same conditions (speed and spotsize) for periphyton samples exposed to both forms of platinum.

The analysis of platinum samples resulted in the higher and clearer signal for Pt II than for Pt IV in the Milli-Q water. Cobalt signal was comparable and, possible to some extends, the correlation between Co and Pt peaks could be found (Fig. 10). A few characteristic peaks appeared when laser beam passed spots suspected as algae colonies on the sample.

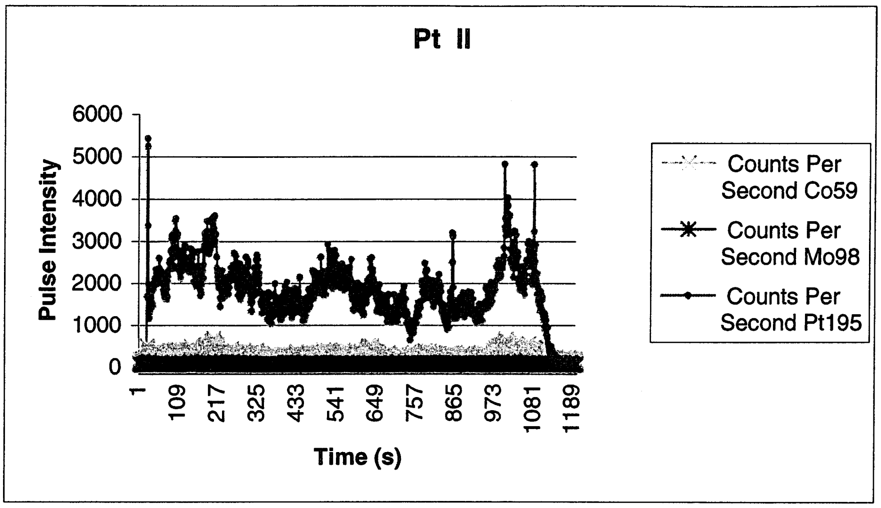


Figure 10. Laser ablation of periphyton community after 24 h exposure to 1000nM Pt II solution. Milli-Q water was used as an exposure medium.

5.3.2 Pd

Ablation of Pd samples resulted in the lowest signal in comparison to other metals. The cobalt signal was at the same level as in other samples but Pd signal was rather 'flat' with some very sharp peaks that might appeared according rather to very high sensitivity than to real increase in concentration of palladium in the sample.

5.3.3 Ag

The level of cobalt signal registered in periphyton samples exposed to silver was similar to other samples exposed in Milli-Q water. The analysis of Ag sample in river water resulted in not so clear signal of Ag but a relation of cobalt and carbon could be possibly seen.

Silver signal obtained during ablasion of Milli-Q water samples resulted in some peaks but they were not related to specific algae colonies (Fig.11).

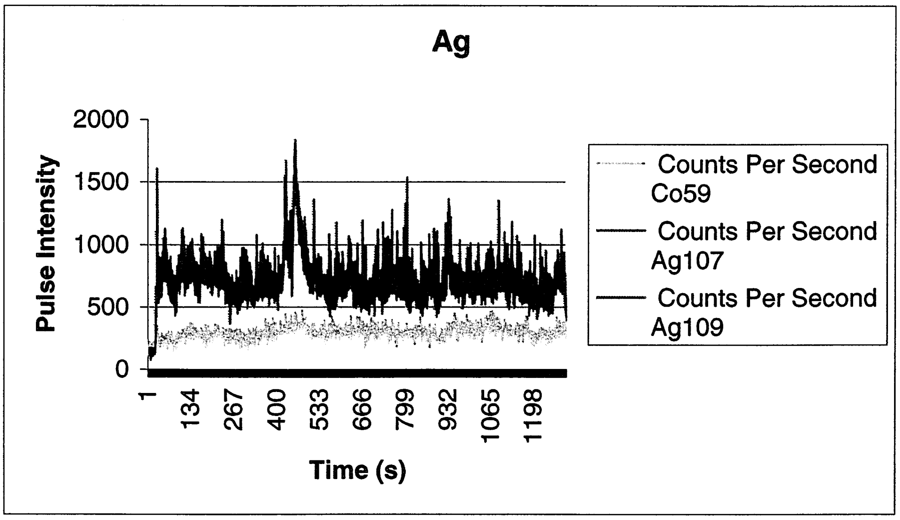


Figure 11. Laser ablation of periphyton community after 24 h exposure to 1000nM Ag solution. Milli-Q water was used as an exposure medium.

5.3.4 In.

The ablation of In focused the most attention during analysis. The relation of Pt and Co was firstly suspected during Pt samples analysis but it was much more clear seen in case of samples exposed to indium. Results of indium and cobalt were correlated (Fig.12).
During the ablation of samples exposed to indium it was seen that signal of indium was followed by signal of iron.

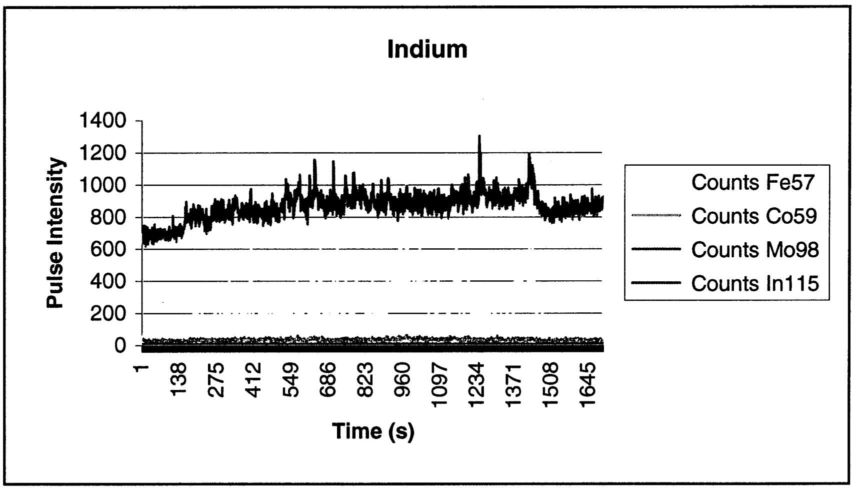
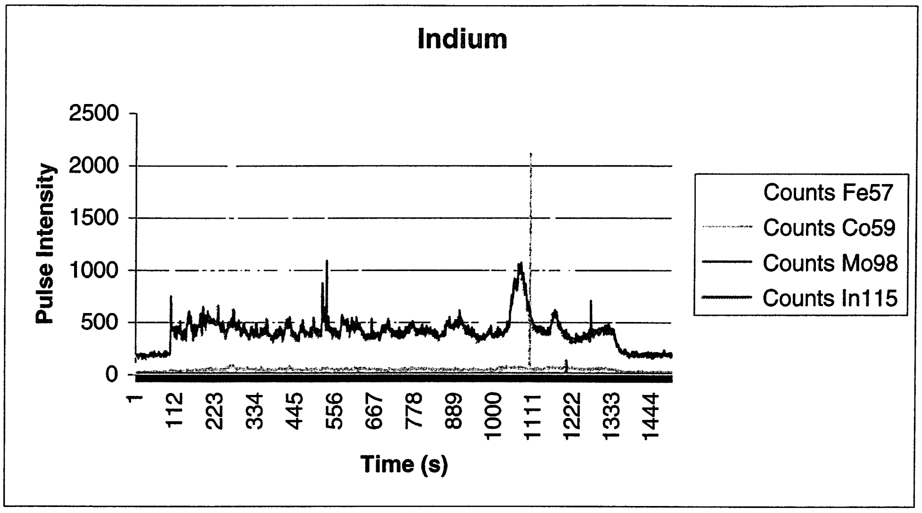


Figure12. Laser ablation of periphyton community after 24 h exposure to 1000nM In solution. Milli-Q water was used as an exposure medium.

Bigger spot and higher speed resulted in bigger area ablated and thus signal was much stronger. However it might be seen that proportions between different signals of elements are changed. During ablation, with spot size 5 and speed equaled to 10 um/s, signal for In was stronger than for Fe and ablation with bigger spot size (7) and higher speed (50 µm/s) presented just opposite relation. By using bigger spot size it is more difficult to track just algae and rest of material is also ablated. It was suspected from previous studies on bacteria that might be a relation between Fe and In. In the tests Fe reduced the toxicity and bacteria excreted indium bound to phosphate.
Despite the fact that spot size and speed was changed during ablation the In/Co signal ratio seemed to be constant.

Comparison of perifyton sample and clean disc exposed to solution 1000nM Milli-Q water solution

Together with perifyton samples a clean glass disc with no biofilm was exposed to In solution in Milli-Q water. The same exposure conditions were provided to notice any changes in signal during LA-ICP-MS analysis (Fig. 13). It was done to test the possible adsorption mechanism of elements to the glass disc. The background signal could be estimated in that way. Moreover, the elements associated with biota were possible to track The absence or just a trace of some elements on the clean discs could be potential prove that they are typical for biota.



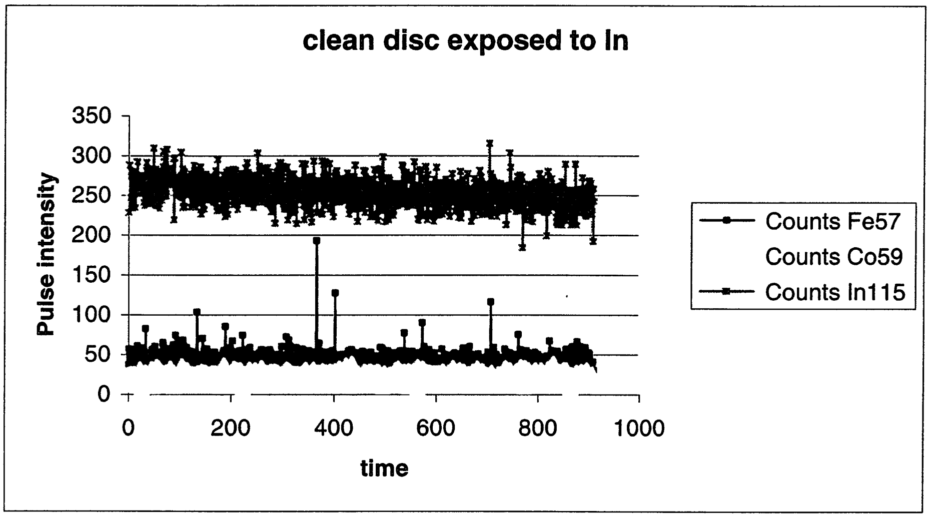


Figure 13. Laser ablation of clean disc (above) and periphyton community (below) after 24 h exposure to 1000nM In solution. Milli-Q water was used as an exposure medium in both cases.

The analysis of clean disc determined the In signal at the maximum level of 3000 pulse intensity. Under the same ablation conditions (spot size 5, speed 10μm/s) the analysis of pherifyton indicated that indium signal was at higher level than that one identified on clean disc.

The mean iron signal level on the clean disc oscillated around pulse intensity 400 and on the perifyton disc around pulse intensity 6 000. This indicated that iron is associated with biota. Cobalt signal was around twice higher for exposed disc than clean disc and this might prove that Co is associated to living cells.

Molibdenum signal was very low to be taken into analysis

6.Conclusions and recommendations

During the analysis it turned out that all metals analyzed were taken up by periphyton however the uptake was individual to each metal. Generally, to different extent, there is a linear relation between exposure and uptake noticed. The highest uptake was associated with exposure to Pt and In and the lowest to Pd.
Generally, the uptake in Milli-Q water is higher than in river water and it may results from metals binding to organic matter.

The exposure concentrations used are not toxic to periphyton communities and there might be at least three reasons for that. Either the concentrations are not high enough to cause any toxic effect or the exposure time was too short. Another explanation might be that metals were just absorbed on microorganism cells and were not transported inside. Finally, the test parameter chosen is questionable. It is possible that metals, which were studied in these experiments, belong to the toxicants that are hazardous to algae but photosynthesis is not inhibited in the short-term tests.

In analysis of In and Pt a trend could be seen that indicates that further increase of exposure concentration might cause a toxic effect.

The LA-ICP analysis indicated that concentration of metals is not uniformly distributed on sample surface. This indicates that metals are absorbed or bound to nonliving elements of periphyton or are taken up by microorganisms. In the first case signal was usually lower and in the second one was higher.

Cobalt signal was at the similar level in all of exposed samples, independently of the metal solution they were exposed to, that were ablated by spot size 5 and speed 10 um/s.
Only analysis of clean disc resulted in lower and stable signal thus indicating that cobalt is associated with biota.

Like cobalt, iron seems to be characteristic for biota only. Not only the signal during ablation of periphyton sample was higher than in case of clean disc but also a correlation between Fe and In was observed.

Molibden, regarded as characteristic for biota, could not be used as biota indicator due to relative small signal. Iron and cobalt were more useful probably because their natural concentration in algae is higher.

It is recommended to increase exposure concentration and exposure time in future study. Because of promising LA-ICP-MS results, this set of instruments could be used to bigger extend in this type of study. Some more specific approaches could be suggested like evaluation of element signal ratio characteristic for biota. The ablation of different algae colonies, previously indicated by microbiologists would lead to bioavailability comparison study.

Also a variety of test parameters, besides photosynthesis, could be used in toxicity tests.

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web ref 6 http://www.ritox.vet.uu.nl/mtxgen.html

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