

Laboratory and Field Studies on the Use of Artificial Mussels as a Monitoring Tool of Platinum Exposure in the Freshwater Environment.

Marelize Labuschagne

North-West University Potchefstroom Campus: North-West University <https://orcid.org/0000-0002-6215-9223>

Sonja Zimmermann (✉ sonja.zimmermann@uni-due.de)

North-West University <https://orcid.org/0000-0001-6929-7541>

Nico J. Smit

North-West University Potchefstroom Campus: North-West University

Johannes H. Erasmus

North-West University Potchefstroom Campus: North-West University

Milen Nachev

Universität Duisburg-Essen

Bernd Sures

Universität Duisburg-Essen

Victor Wepener

North-West University Potchefstroom Campus: North-West University

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Abstract

Background

The artificial mussel (AM) is a passive sampling device that was originally developed for monitoring metal concentrations in the marine environment but is also increasingly used in freshwater environments. The AM consists of a non-permeable Perspex tube, which contains Chelex-100 resin, water diffuses through the semi-permeable membranes where the metals bind to the Chelex-100. The AM allows for the determination of the dissolved, bioaccessible metal fraction in water bodies without killing organisms, as well as environments with unfavorable conditions for living bioindicators. In the present study, the use of the AM was adapted for the monitoring of platinum (Pt) in a freshwater ecosystem.

Results

Therefore, the elution of Pt from the Chelex-100 beads was optimized. Two modifications to the original method for the use of AMs are recommended, i.e. washing and separation of the beads through centrifugation and elution with a mixture of 4.5 mL HNO₃ and 0.5 mL HCl for approximately 2-3 hours to ensure the release of all Pt bound to the beads. Additionally, the uptake kinetics of the AM were determined under laboratory conditions over a wide exposure concentration range (0.1-1000 µg/L) showing highly correlated Pt accumulation in the AMs with the aqueous exposure concentration. For the tested Pt exposure concentrations of 0.1, 1, 10, and 100 µg/L, the Pt concentrations in the AMs increased during the exposure period of 6 weeks. At the highest exposure concentration of 1000 µg/L, the increase stagnated after 3 weeks. To validate the AM in the field, the Pt accumulation of the AM was assessed together with that of freshwater clams (*Corbicula fluminalis africana*), muscle and liver tissue of three fish species sharptooth catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*) and Mozambique tilapia (*Oreochromis mossambicus*), as well as water hyacinths (*Eichhornia crassipes*) at two sampling sites in the Pt mining area of South Africa.

Conclusion

Results from the present study showed that the AM is a promising tool to monitor Pt concentrations in the freshwater environment at contaminated sites.

Background

Monitoring of pollutants in the aquatic environment is necessary to fulfill legislative frameworks and directives. With the development of the Mussel Watch concept in the mid-1970s [1], the use of living organisms as biological indicators of environmental contaminants has become the mainstay of environmental monitoring. The use of bioindicators has several advantages since they can produce time-integrated results and are an indication of the bioaccessible fraction of metals in the environment [2,3]. However, the metal bioaccumulation in organisms can be affected by various factors, such as body weight, growth rate, age, reproduction and nutrition status [3,4]. Therefore the use of biological indicators for monitoring of e.g. metals in freshwater and marine environments has several limitations and disadvantages [2]. These include at first the need to use living organisms that have to be killed to receive information on the biological availability of selected pollutants.

Additionally, the variation in metal concentrations in the water column and sediment is affected by the particle size, organic content and redox conditions [2].

To overcome these challenges, passive sampling methods have successfully been used to monitor the aqueous concentrations of a wide range of pollutants [5]. All passive sampling devices are based on the principle that the analytes freely flow from the sampling medium to a receiving phase in the device [5]. The analytes are trapped in the device, which is subsequently analyzed to determine the biologically available fraction (the ecotoxicologically relevant fraction) within the water column. These devices are advantageous since they provide time estimated results as opposed to the snapshot view of traditional water sampling [6]. Most importantly, they could replace the use of living bioindicators and thus contribute to the fulfillment of the 3R principle [7] in reducing the number of animals killed if the results obtained with passive sampling devices correspond to accumulation data using living bioindicators.

Wu, Lau [4] tested the suitability of different polymer-ligands as a novel chemical device for the monitoring of metal pollution in the aquatic environment. They found that the use of Chelex-100 beads had many advantages over the other polymer-ligands and developed an artificial monitoring device in 2007 [2]. The artificial mussel (AM) was originally developed for use in the marine environment but has been applied in freshwater environments as well [8,6,9-12]. This device consists of Chelex-100 beads that are suspended in either artificial seawater (marine) or ultrapure water (freshwater) within a Perspex tube with two semi-permeable gel membranes that form a plug on both ends of the tube [2]. The semi-permeable membranes allow the passage of metal ions into the interstitial space where they bind to the chelating beads. The AM has been applied for the monitoring of a wide range of metals such as Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, V and Zn [2,3,13,14,9,15-18,8,6,10-12].

Among the metals monitored especially those that are potentially toxic deserve special attention. Platinum (Pt), which usually occurs only in extremely low concentrations in the environment [19], has been shown in recent years to not only increase in the environment due to anthropogenic activities but also to exert adverse effects on biota at certain concentrations [20-22].

South Africa is one of the largest producers of platinum in the world. In 2018 it was estimated that South Africa produced 73% of the world's platinum [23]. Recent studies have shown that the aquatic environments in the vicinity of Pt mines have elevated Pt concentrations in their sediments [24,25]. A recent field study in a river system of a Pt mining area in South Africa demonstrated that the AM can accumulate Pt from the water column, therefore it was necessary to further investigate the accumulation behavior of the AM for this metal [12]. This field study demonstrated clear Pt uptake together with other metals such as Cr, Ni, and Zn by the AM. This supported findings of other studies where significantly positive correlations between metal uptake in notably marine bivalve biological indicators and AMs were found [3,13,17,18].

Therefore, the present study aimed 1) to optimize the analytical extraction of Pt from the Chelex-100 beads, which is necessary for a reliable analysis of the Pt accumulation in the AM, 2) to determine the concentration-dependent Pt uptake kinetics of the AM in freshwater over a wide exposure concentration range under laboratory conditions, 3) to validate the AM under field conditions as a reliable tool for monitoring bioavailable Pt concentrations in freshwater environments by comparing the Pt uptake of the AM with that of living

organisms, i.e. different fish species and the water hyacinth, and 4) to comment on the appropriateness of the AM as an alternative to living organisms as biological indicators of metal exposure.

Materials And Methods

Validation of the efficiency of Chelex-100 beads to bind Pt

To determine if Chelex-100 beads can accumulate Pt from the ambient environment, a short-term laboratory study was done to test 1) the binding of Pt to the Chelex-100 beads and 2) the most effective method of Pt elution from the Chelex-100 beads. The loading, washing, and elution experiment was conducted to check the practicability and validity of the washing and elution procedure. To determine the validity (recovery and precision), Chelex-100 beads were loaded with a defined concentration of Pt to determine the loading of the beads. Furthermore, it was necessary to confirm that Pt binds to the beads in ultrapure water as it is used in the AMs.

Loading of Chelex-100 beads with Pt

To test the loading capability of the Chelex-100 beads, 200 mg beads (Sigma-Aldrich, sodium form, 50 - 100 mesh, dry) were placed in 15 mL polypropylene tubes. In total 20 polypropylene tubes were used, 10 of these tubes were loaded with 4.95 mL ultrapure water (loading solution 1) and 4.95 mL 0.5 M HCl (Merck, 37%, suprapure) in the other 10 tubes (loading solution 2). In each of these tubes, 50 μ L of 100 mg/L Pt standard solution (Sigma-Aldrich, Pt standard for ICP TraceCERT®, 1000 mg/L, Pt(IV) in hydrochloric acid, diluted with 1% HNO₃, sub-boiled from 65%; p.a. quality, Merck) were added resulting in a final concentration of 1 mg/L. Samples were taken from the supernatant in regular time intervals to determine the loading kinetics of the Chelex-100 beads and the Pt concentrations were immediately analyzed using a Perkin-Elmer model 4100ZL atomic absorption spectrometer (AAS) equipped with a Zeeman effect background correction system. During the loading of the beads, the tubes were continuously shaken on a mechanical shaker (VWR International, VWR 5000 Advanced digital shaker).

The samples were injected in a pyrolytic graphite furnace tube by the autosampler AS 70 and ran under the optimized operating parameters (Table 1). The calibration was performed by matrix adapted calibration where the concentrations in each sample were calculated by fitting a linear regression line to the points defined by the spiked concentration values.

Table 1: Optimized operating parameters for platinum analysis by AAS.

Temp (°C)	Ramp time (s)	Hold time (s)	Argon flow (psi)
110	1	5	250
140	10	60	250
600	20	1	250
1300	30	20	250
2200	0	3	0
2450	1	3	250

Washing of the Chelex-100 beads

Following the removal of the tubes from the shaker, the Chelex-100 beads went through three repeated washing steps. This was done to get rid of any unbound Pt that might have stayed in the tube or the loading solution. The supernatant in each of the tubes was carefully removed with a pipette, where after the Chelex-100 beads were resuspended in 5 mL ultrapure water. After centrifugation (2 minutes at 1000 g) the washing solution was removed. The centrifugation ensured that the beads settle at the bottom of the tube before removing the supernatant again, this was repeated two more times. The Pt concentrations in the supernatant of the washing steps were also analyzed by AAS as described above to determine any Pt loss that may occur. This is a new method for separating the beads from the solution as in former studies the separation of the beads was done by a more time-intensive filtration method [2].

Elution procedure

After washing the beads, the elution of the Pt from the Chelex-100 beads was tested with two different elution solutions. For the elution experiment, only the beads loaded in 0.5 M HCl were used due to better loading results. The first solution was used for 5 of the tubes consisting of 4.5 mL 6 M HNO₃ (Merck, sub-boiled from 65%; p.a. quality) and 0.5 mL 12 M HCl (Merck, 37%, suprapure). The second solution was used on the remaining 5 tubes consisting of 5 mL 8 M HCl (prepared from Merck, 37%, suprapure). Samples were taken from the supernatant in regular time intervals to determine the elution kinetics from the Chelex-100 beads and immediately analyzed by AAS as described above.

Optimization of the artificial mussel design

The artificial mussel was originally developed by Wu et al. [2] for marine environments but was adapted for the use in freshwater environments (Figure 1). This device consists of a non-permeable Perspex tube, which contains 200 mg Chelex-100 beads suspended in 5 mL ultrapure water. The water diffuses into the cavity that contains the Chelex-100 beads through two polyacrylamide gel layers (semi-permeable membranes).

The protocol for assembling the artificial mussel was as follows: The end of each Perspex tube was covered with parafilm to form a membrane to prevent the solutions from seeping out while the gel polymerizes. The gel was composed of three solutions: 15 g acrylamide (Sigma, Acrylamide for electrophoresis, 99% (HPLC) powder) and 0.5 g N,N-methylenebis-acrylamide (Sigma, BioReagent, suitable for electrophoresis, 99%) dissolved in 100 mL ultrapure water for the first solution, of this 4 mL was pipetted into the plastic tubing.

Where after 160 μL 10% ammonium peroxodisulfate (Sigma, Reagent grade, 98%) and 40 μL N,N,N',N'-Tetramethylethylenediamine (Sigma, BioReagent, for molecular biology, 99% (GC)) were added to form the semi-permeable gel membranes [12,10].

Following the gel polymerizing (usually within 5 minutes), the tubes with the gels were placed in a container filled with ultrapure water for 1 hour, this allows for the gels to swell. After 1 h of swelling, the gels were carefully moved to the desired position within the tube by cautiously rubbing in a circular motion on the gel surface until it moved. A 1 cm Perspex spacer was then placed within the tube with 200 mg Chelex-100 beads and 5 mL ultrapure water. The second gel was then carefully transferred into the tube until it was firmly positioned against the spacer, making sure that no air bubbles were trapped in between the two gel layers. After assembly, the AMs were stored in ultrapure water until needed.

Long-term exposure of the artificial mussels to a series of Pt concentrations

Experimental design

To validate the Pt uptake by the AM, a static exposure study with a series of increasing dissolved Pt concentrations was performed. The AMs were divided into six experimental groups (Table 2). The nominal Pt concentrations in the tank water were 0 $\mu\text{g/L}$, 0.1 $\mu\text{g/L}$, 1 $\mu\text{g/L}$, 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$ and 1000 $\mu\text{g/L}$. Platinum standard solution (Sigma-Aldrich, Pt standard for ICP TraceCERT[®], 1000 mg/L, Pt(IV) in 2 M hydrochloric acid), as well as HCl (2 mol/L), was added to the exposure medium according to Table 2.

Table 2: Experimental layout for Pt exposures.

	Experimental group	# AMs/ sampling	# AMs/ tank	addition of	
				Pt standard	HCl (2 mol/L)
Tank 1	Control	7	42	none	10 mL
Tank 2	0.1 $\mu\text{g Pt/L}$	7	42	1 μL	10 mL
Tank 3	1 $\mu\text{g Pt/L}$	7	42	10 μL	10 mL
Tank 4	10 $\mu\text{g Pt/L}$	7	42	100 μL	9.9 mL
Tank 5	100 $\mu\text{g Pt/L}$	7	42	1 mL	9 mL
Tank 6	1000 $\mu\text{g Pt/L}$	7	42	10 mL	None

Six plastic tanks (300 x 750 mm) lined with polypropylene (PP) bags (Sarstedt, disposable bags, autoclavable, 600 x 780 mm) were filled with deionized water to rinse the bags and to check for any leakages [22]. By lining the tanks with PP bags it is possible to reuse the plastic tanks. The tanks were aerated, which also allowed for the continuous movement of the water and a permanent mixing of Pt within the tank.

After 24 hours the water in the PP bags were discarded and replaced with 10 L reconstituted freshwater [22]. The Pt stock solution was added to the tanks as indicated in Table 2. The bags were pre-conditioned to saturate the surfaces of the PP bags with Pt before the exposure commenced. This pre-conditioning step was done to minimize the loss of Pt due to adsorption processes on the bag surfaces during the exposure.

Following the pre-conditioning period of 24 hours, the water was replaced with new exposure medium and Pt stock solution, as described above. After another 24 h of pre-conditioning, a total of 42 AMs were added to each tank.

Water sampling and removal of AMs

Water samples were taken from each tank at different intervals: 1) before the addition of the Pt, 2) 10 min after the addition of the Pt standard solution, 3) before the addition of the AMs, and 4) subsequently weekly before AMs were removed. The procedures were as follows: 10 mL tank water was removed and acidified with 10 μ L HNO_3 (Merck, sub-boiled from 65%; p.a. quality). Additionally, artificial mussel samples ($n = 7$) were taken once every week over six weeks. The AMs were plugged at both ends with cotton soaked in ultrapure water, placed in plastic bags, marked and stored at room temperature for metal analysis.

Platinum analysis

For the determination of the Pt accumulated in the AMs, the Chelex-100 beads were removed from the AMs by gently moving the gel layer to one end of the AM by carefully rubbing with the finger against the opposite gel layer until the interspace moves. For metal analysis, the content (including the Chelex-100 beads) of each individual AM was emptied into an acid pre-washed 15 mL polypropylene tube. These samples were then centrifuged (2 minutes at 1000 g), the supernatant was removed and the beads were rinsed with 5 mL ultrapure water. The supernatant was removed and the beads were eluted with 4.5 mL 6 M HNO_3 (sub-boiled from 65%; p.a. quality, Merck) and 0.5 mL HCl (Merck, 37%, suprapure). The beads were placed on a mechanical shaker to eluate the Pt from the Chelex-100 beads in the acid solution for approximately 2 hours. This was based on the results obtained from the elution experiment, to ensure that all bound metals would be released from the beads. The supernatant was removed and placed in new polypropylene tubes for further analysis.

Accordingly, Pt concentrations in the water and AMs were determined by a quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS) system (Perkin Elmer, Elan 6000) with an autosampler system (Perkin Elmer, AS-90). For ICP-MS analysis, the wash time was set to 30 s with 2% HNO_3 to avoid contamination. After every 10 samples, a Pt standard solution (10 μ g/L) was measured to check the accuracy and stability of the measurements. Before ICP-MS measurement, samples were diluted 1:10 with an internal standard solution, consisting of 1% HNO_3 and 10 μ g/L thulium (Merck, Certipur[®]). Calibration of the instrument was performed using a series of 11 dilutions of Pt standard solution (Sigma-Aldrich, Pt standard for ICP TraceCERT[®], 1000 mg/L). With this, the concentrations of the sample analytes were calculated using regression lines with a correlation factor of ≥ 0.999 for the Pt-194 mass line.

Interferences occur and can have an effect on the Pt measured by the analytical instruments. To check for Hafnium (Hf) oxide interferences at that mass line, a series of 5 dilutions of increasing Hf concentrations were measured. The Hf interferences on Pt-194 were below 2%, thus mathematical correction was not necessary. The detection limit for the AMs in the long-term laboratory exposure was 0.47 ng/AM, for the water in the laboratory the detection limit was 0.045 μ g/L.

Field study on the Pt accumulation in artificial mussels in comparison with living organisms

In addition to the laboratory investigations, field sampling of living organisms was combined with active monitoring using AMs (described in detail in Labuschagne et al. [12]) at two impoundments in the Pt mining area of South Africa in March 2018. In Olifantsnek Dam, the Pt exposure only originates from geogenic sources, while Bospoort Dam is downstream of various anthropogenic activities such as intensive Pt mining and refining activities, industrial and urban effluents (see Erasmus et al. [26] and Labuschagne et al. [12] for more detail). *In situ* water parameters including electrical conductivity (EC), pH and temperature (ExStik II EC500, Extech Instruments), as well as dissolved oxygen (DO) (ExStik II DO600, Extech Instruments) were measured at the two impoundment sites.

Water samples were collected in triplicate in pre-cleaned polyethylene containers for inorganic nutrient and metal analyses. The water samples were analysed for nutrients (NH_4^+ , NO_3^{2-} , NO_2^- , PO_4^{3-}) and other chemical water quality variables (Cl^- , SO_4^{2-} and turbidity) using a spectrophotometer (Spectroquant Pharo 300, Merck) and Merck Spectroquant test kits. For metal analysis, 10 mL water was taken and acidified with 10 μL HNO_3 (sub-boiled from 65%; p.a. quality, Merck, Darmstadt, Germany) and stored in polypropylene tubes at room temperature. Sediment samples were collected in triplicate from the upper 10 cm of the substratum in pre-cleaned polyethylene containers for organic content (OC) and metal analysis. The sediment samples were prepared for metal analysis following the methods described in Erasmus et al. [24]. Water and sediment samples were analysed for Pt concentrations as described for the laboratory exposure samples.

During the exposure of the AMs in the two impoundments over 6 weeks, living bioindicator organisms, i.e. freshwater clam (*Corbicula fluminalis africana*), the fish species sharptooth catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*) and Mozambique tilapia (*Oreochromis mossambicus*), were sampled from the same sites as Labuschagne et al. [12] and Erasmus et al. [26]. Muscle and liver of the fish were analyzed by ICP-MS after microwave digestion according to Erasmus et al. [24,26]. At Bospoort Dam, i.e. the impoundment impacted with anthropogenic activities, water hyacinth (*Eichhornia crassipes*) was also assessed as this plant is known to accumulate Pt [27,28].

Statistical analyses

Statistical analyses were performed using GraphPad Prism[®] 7 software. Platinum uptake of the AM in the lab experiments are expressed in ng/AM and the field study in $\mu\text{g/g}$ related to 200 mg Chelex for a better comparison with the fish tissues. The correlation of Pt uptake by the AM (mean of seven AMs per sampling time) and the respective exposure concentrations was determined by using linear regression. The water quality data from March 2018 (from Labuschagne et al. [12]) contained one outlier value which was discarded for statistical reasons for this study. For both water and sediment, Shapiro-Wilk normality test indicated that data were distributed normally, where significant differences in Pt concentrations between Olifantsnek and Bospoort Dam were determined by using Welch's t-test. To evaluate the usefulness of the AM a two-way ANOVA was performed to compare only the bioindicator organisms from the same impoundment, followed by Tukey's multiple comparisons test. To compare the concentrations in the organisms between the two impoundments a two-way ANOVA was performed, followed by Sidak's multiple comparisons test. Significance was set at $p < 0.05$ for all comparisons.

Results

Binding and elution of Pt from Chelex-100

The loading experiment indicated that Pt was bound in both loading solutions to the Chelex-100 beads as there was a significant loss of Pt in the loading solutions over time (Figure 2). However, the Chelex-100 loading kinetics differed for the two different loading solutions. The Pt concentration of loading solution 1 (ultrapure water) was relatively stable during the loading period and contained about 45% of the initially added Pt concentration after 200 min. In contrast, the Pt concentrations of loading solution 2 (0.5 M HCl) decreased over time and contained only 13% of the initially added Pt concentration after 200 min. Thus, it can be assumed that in 0.5 M HCl a much higher part of the Pt was loaded to the Chelex beads than in ultrapure water.

The washing of the beads through centrifugation was very effective. Following the first washing step, only 1% of the added Pt was found in the wash solution. Following the second and the third washing step, no Pt was detectable in the wash solution. For the elution experiment, only the beads loaded in 0.5 M HCl were used due to better loading results. Immediately after the elution solution was added to the beads, the Pt concentrations in the supernatant started to increase, whereas the highest Pt concentrations were reached after 120 min (Figure 3). The elution rate of the mixture of 4.5 mL 6 M HNO₃ and 0.5 mL 12 M HCl was 98% and thus much higher when compared with the elution rate of 68% of 8 M HCl.

Concentration-dependent Pt uptake kinetics by the artificial mussel

During the long-term exposure, Pt uptake was found in all Pt-exposed AMs (Figure 4). However, for the 0.1 µg/L group the Pt mass measured in the AMs (i.e. the Pt mass bound to 200 mg Chelex-100 beads) exceeded the detection limit of 0.47 ng Pt/AM only after 6 weeks of exposure. In all higher exposed groups, the Pt mass increased already after one week of exposure (Figure 4). The Pt masses in the AMs increased throughout the entire six-week exposure period in all Pt exposed tanks, except for the 1000 µg/L group. In this tank, the Pt masses increased during the first 3 weeks of exposure, followed by a slight decrease during the next 3 weeks (Figure 4 E).

The analyzed Pt concentration in the tank water and the Pt uptake by the AMs were positively correlated, which indicated that the Pt mass in the AMs increased with increasing exposure concentration (Figure 5).

Pt accumulation in artificial mussels in comparison with living organisms under field conditions

The Pt accumulation in AMs and living organisms, i.e. different fish species, were determined under field conditions at two different impoundments of a Pt mining area. To better characterize these impoundments water quality parameters (Table 3) and the Pt concentrations in water and sediment samples (Figure 6) were analyzed.

Olifantsnek Dam (geogenic Pt source) had higher dissolved oxygen, turbidity, suspended solids and pH in the water, as well as organic content in the sediment than Bospoort Dam. In contrast, Bospoort Dam had very high electrical conductivity (EC) values during the field survey, as well as anions (Cl⁻, SO₄²⁻) and nutrients (NH₄⁺, PO₄²⁻). The chloride concentration was 20 times higher in Bospoort Dam than in Olifantsnek Dam. However, Olifantsnek Dam had higher nitrate (NO₃²⁻) and nitrite (NO₂⁻) values.

Table 3: Water quality parameters (mean \pm SD) measured in Olifantsnek and Bospoort Dam during the field exposure.

	Code	Olifantsnek Dam	Bospoort Dam
pH	pH	8.1 \pm 0.22	7.4 \pm 0.35
Electrical conductivity (μ S/cm)	EC	200 \pm 14.8	1356 \pm 277.2
Temperature ($^{\circ}$ C)	Temp	23.8 \pm 0.71	25.7 \pm 3.7
Dissolved oxygen (mg/L)	DO	8.02 \pm 3.56	5.53 \pm 3.66
Turbidity (FAU)	Turb	38 \pm 5	9 \pm 2
Ammonium (mg/L)	NH ₄ ⁺	0.02 \pm 0.01	0.13 \pm 0.01
Nitrate (mg/L)	NO ₃ ²⁻	0.32 \pm 0.04	0.14 \pm 0.05
Nitrite (mg/L)	NO ₂ ⁻	0.007 \pm 0.004	0.004 \pm 0.001
Phosphate (mg/L)	PO ₄ ²⁻	0.04 \pm 0.01	0.20 \pm 0.01
Chloride (mg/L)	Cl ⁻	12 \pm 1	240 \pm 3.8
Sulphate (mg/L)	SO ₄ ²⁻	86 \pm 4.7	233 \pm 3.8
Suspended solids (mg/100mL)	SS	3.8 \pm 2.8	1.9 \pm 1.1
Organic content (%)	OC	3.4 \pm 1.4	2.3 \pm 0.5

Platinum concentrations in the water taken at the sampling site of the AMs and the fish, respectively, within each impoundment were significantly higher in Bospoort Dam than Olifantsnek Dam (Figure 6A). In contrast, the sediment samples, which were only taken at the fish sampling site, showed an opposite pattern between the two impoundments (Figure 6B).

Although there were significant differences in the Pt concentrations of the water and sediment between both impoundments, significant differences between Pt accumulation in the fish and AM occurred only between the two impoundments for the muscle and liver of *C. carpio* and *C. gariepinus* and always with higher Pt concentrations at Bospoort Dam than at Olifantsnek Dam (Figure 7). For the AMs and the muscle and liver of *O. mossambicus* similar Pt concentrations were found at both impoundments.

There was no significant difference between the Pt accumulation in the AM and the different fish species from Olifantsnek Dam, while in Bospoort Dam muscle and liver of *C. carpio* and liver of *C. gariepinus* showed significantly higher Pt concentrations than those measured in the AM. In Bospoort Dam, *E. crassipes* were also analyzed and separated in the different sections of the plant: root, stems and leaves. The roots of *E. crassipes* contained significantly higher Pt concentrations than all the other organisms tested in the field study (Figure 7). The Pt levels in the freshwater clam from both impoundments were below detection limits [12].

Discussion

Platinum uptake by AMs was demonstrated in a recent field study by Labuschagne et al. [12]. However, the suitability of this form of passive sampling to monitor trace levels of platinum group elements has never been validated and hence the need for this study. A requirement for the Pt accumulation in the AM is the ability of Pt to bind to Chelex-100 beads which were demonstrated by Samczyński et al. [29]. In the present study, two solutions were used to test the loading of the beads. The ultrapure water was selected since in previous studies the Chelex-100 beads were suspended in ultrapure water within the AM [6,10]. The second loading solution was a diluted hydrochloric acid solution (0.5 M HCl) since metals forming stable chloride complexes, including Pt, show the highest affinity towards Chelex-100 in diluted (0.1-1.0 M) HCl solutions [29].

In the loading experiment, the decline of the Pt concentration in the loading solutions can be attributed to the binding of Pt to the Chelex-100 beads as respective controls without Chelex-100 beads showed no decline in the Pt concentration over time (data not shown). For the ultrapure water, approximately half of the initially added Pt remained in the loading solution for the duration of the experiment. Based on these results it can be concluded that in ultrapure water only about 50% of the Pt had bound to the Chelex-100 beads. As the loading of Pt to the Chelex-100 beads were more effective in a 0.5 M HCl solution, only the tubes from this loading were used for the elution experiment. However, about 13% of the initially added Pt remained in solution when equilibrium conditions were reached. In the present study, ultrapure water was used as a suspension medium for the Chelex-100 beads in the AMs according to previously published studies (Table 4).

Table 4: Previous studies using artificial mussels (AMs) in marine and freshwater ecosystems.

Marine	Study area	Elements monitored	Bioindicator organism (BO)	Exposure period	Patterns	Concentrations
	Marine environments (developed) [2]	Cd, Cr, Cu, Pb, and Zn	<i>Perna viridis</i>	8 weeks	Laboratory patterns are similar while field patterns were differential	BO accumulated higher concentrations in the laboratory, in the field the AMs showed higher Cd, Cr and Pb, while Zn higher in BO
	Scotland and Iceland [3]	Cd, Cu, Cr, Pb, and Zn	<i>Mytilus edulis</i>	94 days	Differential patterns	BO contained significantly higher concentrations
	South African coastline [13]	Cd, Cr, Cu, Pb, and Zn	<i>Perna</i>	6 weeks	Differential patterns	Differential concentrations
	Portuguese Coast [14]	Cd, Cr, Cu, Pb, and Zn	<i>Mytilus galloprovincialis</i>	4 months	Similar patterns at different sites between different months sampled	Zn higher in BO, Pb higher in AM
	China coastline [9]	Cd, Cr, Cu, Hg, Pb, and Zn	<i>Mytilus edulis</i> and <i>Perna viridis</i>	30 days and 90 days	Similar patterns	<i>M. edulis</i> showed correlations for Cd, Cr, Cu and Pb. <i>P. viridis</i> showed correlations for Cr, Cu, Pb and Zn
	Bay of Bengal, Bangladesh [15,16]	Cd, Cu, Fe, Mn, Ni, Pb, U and Zn	Only AMs were used	4 weeks	-	-
	Estero de Urias Lagoon, Gulf of California [17]	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, and Zn	<i>Crassostrea palmula</i> and <i>Mytella strigata</i>	6 months	Differential patterns	Concentrations of Cd, Fe, Zn, Ni and Co in AMs were consistently lower than in the BO, Hg and U were only detectable in AMs.

	Port Phillip Bay, Victoria, Australia [18]	As, Cd, Cr, Cu, Hg, Pb, Se and Zn	<i>Mytilus galloprovincialis</i>	4 weeks	Similar patterns for Pb, Cu and Cr	Significant correlation between Cr, Cu and Pb, lower correlations with As, Hg and Zn
Freshwater	Goulburn-Murray catchment, Victoria, Australia [8]	Cd, Cu, Hg, Pb, and Zn	Only AMs were used	4 weeks	-	-
	Koekemoer Spruit, South Africa [6]	As, Cd, Cr, Co, Cu, Pb, Mn, Ni, U, V and Zn	<i>Melanoides tuberculata</i>	30 days	Differential patterns	Most of the concentrations are similar, only Cd, Mn and Pb show significant differences
	Nyl River floodplain, South Africa [10]	Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, and Zn	Spot water samples	4 weeks	Differential patterns	Differential concentrations
	Sarıçay Stream, Turkey [11]	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, and Zn	<i>Unio crassus</i>	4 weeks	Differential patterns	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn concentrations higher in BO
	Hex River System, South Africa [12]	As, Cd, Co, Cr, Ni, Pb, Pt, V, and Zn	<i>Corbicula fluminalis africana</i>	6 weeks	Differential patterns	Significantly higher Cr, Co, Cd and Zn concentrations in BO, AM accumulated Pt

For the analysis of the metals taken up by the AM, the Chelex-100 beads need to be removed from the AM, followed by washing steps and the elution of the metals from the beads. In the present study, the Chelex-100 beads went through several washing steps by means of centrifugation. There were detectable (but low) Pt concentrations in the first washing solution, whereas no Pt was detectable in the second and third washing solutions, indicating that washing the beads by means of centrifugation is efficient. Since the original development of the AM, some modifications regarding the elution of metals from the Chelex-100 beads have been made. The earlier methods involved emptying the contents of each AM into a sintered glass filter followed by eluting twice with 12.5 mL 6 M HNO₃. The elutriant was then made up to a known volume with ultrapure water [2,3,8,9,15,16,11] while Degger et al. [13] rinsed the beads with ultrapure water several times before eluting with 6 M HNO₃.

Another adapted version of this method consisted of rinsing the gel membrane and Chelex-100 beads with ultrapure water three times and then adding 10 mL 6 M HNO₃ [14]. Another method consisted of emptying the contents of each AM into a sintered glass filter, where after the Chelex-100 beads were placed in glass conical

flasks in 20 mL 6 M HNO₃. These flasks with the content were then mixed on a shaker plate for 24 h [6,10]. The most recent method entailed that the resin was placed on a glass funnel covered by filter paper where after the resin was rinsed 3 times with ultrapure water and washed with 10 mL 70% HNO₃ [18]. In comparison, the washing method of the present study is more practical and less time-consuming when compared to the traditional filtration method.

According to literature, all AM studies (except for Labuschagne et al. [12]) used the same elution solution (i.e. 6 M HNO₃). However, because Pt forms stable Pt chloro-complexes in HCl solution, HCl was added to the HNO₃ in the first elution solution [29]. The second elution solution (8 M HCl) was used since it was shown in former studies that 8 M HCl solution eluted 95 ± 2% of the Chelex-bound Pt [29]. In the present study, the mixture of HNO₃ and HCL as elution solution revealed an elution rate of 98% and was clearly more effective as the second elution solution (8 M HCl) with only 68%. The elution experiment demonstrated that an elution period of approximately 2-3 hours is sufficient, as afterward, the Pt concentration remained constant in the eluent. In comparison, in former studies, the metals were eluted in nitric acid over a period that ranged from a few seconds to 24 hours (see references in Table 4). This is the first study that gives information regarding the efficiency of the elution period or elution rate of the metals from the Chelex-100 beads

The second aim of the present study was to obtain a better understanding of the accumulation behavior of Pt in the AM. To this end, the concentration-dependent Pt uptake kinetics of the AM over a wide exposure concentration range were determined under laboratory conditions. Except for the highest exposure group (1000 µg/L), the Pt concentrations in the AMs increased over the whole 6-week exposure period and steady-state conditions were not reached indicating that the binding capacity of the 200 mg Chelex-100 beads was not reached. However, at an exposure concentration of 1000 µg/L, which is far above any environmentally relevant Pt concentrations, the capacity of the Chelex-100 beads seems to be saturated or the exposure period should be extended. Nevertheless, the Pt accumulation by the AM showed a strong linear correlation with the Pt concentration in the exposure medium. This relationship is important when using the AM for monitoring purposes as it makes it possible to draw conclusions about the environmental Pt contamination, exactly the dissolved, bioaccessible Pt concentration in the water phase, via the Pt accumulation in the AM. In Wu et al. [2] the uptake kinetics and depuration of the AM were compared to those of *Perna viridis* at different salinities, even though there was a difference in the concentrations between the AM and *P. viridis* the patterns are the same.

The main purpose of the field survey was to determine the efficiency of the AM to accumulate Pt under field conditions in comparison to living test organisms. The AMs took up Pt at both sampling sites. However, Pt concentrations of the AMs from the two impoundments were similar, although the two selected sampling sites for this fieldwork differed significantly in the Pt concentrations of the water and sediment, and also in various physicochemical parameters. Bospoort Dam showed higher Pt concentrations in the water than Olifantsnek Dam whereas the sediment demonstrated an opposite pattern. This could be explained by the different sources of Pt in the two impoundments. Olifantsnek Dam is located upstream of the mining activities so that the Pt concentrations are only of geological origin explaining the rather low Pt concentrations in the water and higher concentrations in the sediment. In contrast, Bospoort Dam collects all the runoff and wastewater from the Pt mines explaining the higher Pt concentrations in the water as compared with Olifantsnek Dam.

Therefore, as the AMs are only able to take up dissolved metals, higher Pt accumulation in the AMs of Bospoort Dam as compared with Olifantsnek Dam would be expected. However, besides the Pt concentrations in the water phase, other factors such as physico-chemical water parameters (e.g. dissolved organic matter, inorganic ions, salinity, source of Pt) can influence the bioavailability of Pt [30,31]. Notable were the chloride concentrations, which were 20 times higher in Bospoort Dam than in Olifantsnek Dam. Chloride is of particular interest as Pt can form chloro-complexes with chloride, which may be not able to cross the polyacrylamide gel layer of the AM.

Another aim of the field study was the comparison of the Pt accumulation in the AM with that of living organisms. Labuschagne et al. [12] could not detect any Pt in transplanted clams (*C. fluminalis africana*) exposed at the same sampling sites and during the same sampling period. In contrast to the active monitoring study of Labuschagne et al. [12], in the present study, naturally occurring organisms, i.e. different fish species, were collected at the sampling sites. There are significant differences between the metal uptake mechanisms of AMs and fish. With AMs, biomagnification, the metal fraction which is bioaccumulated via food, is not taken into account. Additionally, AMs are fixed at one point, while fish can move around the impoundment integrating the Pt concentrations over a wider area.

Furthermore, the AMs were deployed in the impoundments for a period of 6 weeks, while fish has been exposed, depending on their age at capture, for a couple of months to a couple of years. These differences in metal uptake routes and exposure period can account for the differences in the Pt bioaccumulation between AM and fish. This may also explain why, in contrast to the AMs, two of the three fish species, i.e. the common carp and sharptooth catfish, showed higher Pt concentrations in the muscle and liver at Bospoort Dam as compared with Olifantsnek Dam and thus the same pattern as the aqueous Pt concentration. These Pt concentrations at Bospoort Dam were significantly higher in the fish tissues as compared with the AMs. The different Pt accumulation patterns of the three fish species and the clam in the study of Labuschagne et al. [12] could be explained by differences in their feeding behaviors and preferred habitats, as well as the chemical form in which Pt is present in these systems. It should be noted that the differences between AMs and other test organisms would also occur should different species of organisms be used and compared with one another [13].

This and all previous AM studies (see Table 4), expressed the Pt accumulation in the AM as $\mu\text{g/g}$ related to the 200 mg Chelex-100 beads to enable a comparison with the fish tissues. However, such a comparison should be handled with care as these Pt accumulation values of the AMs are highly dependent on the mass of Chelex-100 beads used in the AM. Nevertheless, in the present study, significant differences between AM and fish were only found at Bospoort Dam demonstrating higher Pt concentrations in muscle and liver of the common carp and liver of the sharptooth catfish, but not for the tissues of the tilapia. This can be explained by the fact that both the common carp and sharptooth catfish are more associated with the sediment and actively feed in and on it [32,33].

In a previous study completed by Erasmus et al. [26], the bioconcentration factors and biota-sediment accumulation factors indicated that both the common carp and sharptooth catfish accumulated Pt more readily from the sediments compared to the dissolved fraction in the water. On the other hand, in another study (unpublished data) the tilapia accumulated Pt concentrations from the dissolved water fraction rather than

from the sediments. These three fish species also occupy different trophic levels and utilize different feeding strategies, the common carp and sharptooth catfish are both omnivores, whereas the tilapia is an algaevore and detritivore (Skelton, 2001). Another possible explanation is that common carp and sharptooth catfish specimens were more mature compared to the tilapia specimens, which can attribute to a longer exposure period where the first two species can accumulate higher Pt concentrations compared to the tilapia specimens.

A hyacinth bloom coincided with the AM deployment period in Bospoort Dam. Since the water hyacinth is a floating macrophyte and there is no contact between the roots and sediments, metal uptake can only take place via the water phase. The results showed that Pt was taken up with the highest concentrations in the roots. This supports the laboratory-based findings of Farago, Parsons [27], Farago, Parsons [28], which demonstrate that water hyacinth is effective bioaccumulators of Pt.

The AM device has successfully been used in many marine and freshwater monitoring studies for a wide range of different metals concurrently with many bioindicator organisms (Table 4). Some of these studies confirmed that the patterns of metal accumulation are similar between the AM and the bioindicators [9,14,2], while for several studies differential patterns were observed. However, it should be noted that in most studies the test organisms contained higher concentrations than those measured in the AMs. In contrast, Labuschagne et al. [12] found that the AMs accumulated Pt concentrations that could be quantified while the transplanted clams were all below the detection limit. Platinum bound to high organic content and suspended solids could be bioavailable to e.g. filter-feeding organisms, however, not to the AMs as these particles would not be able to diffuse through the gel membranes [31].

Additionally, the present laboratory study demonstrated a clear correlation of the Pt concentration in the AM with that in the exposure medium, which has not been shown in other studies for a wide range of concentrations (see references in Table 4). The laboratory uptake experiments revealed that at the lowest exposure concentration (0.1 µg Pt/L), which reflects environmentally relevant Pt concentrations (pg/L to ng/L range), the six-week exposure period was insufficient to allow for any Pt uptake. Further studies should focus on decreasing the AM detection limit by optimizing the surrounding medium of the Chelex-100 beads to increase the Pt accumulation in the AM. Nevertheless, for hot spots like Pt mining areas the AM was successful in indicating environmental exposure. However, it is recommended to extend the exposure period to increase the Pt concentration taken up by the AM as the accumulation plateau was not reached after six weeks.

This study demonstrated that AMs are promising tools to assess spatial and temporal trends in metal exposure in freshwater ecosystems. Additional advantages of AMs are that they allow the monitoring of environmental metal exposure without the need to kill organisms and in environments with unfavorable conditions for living bioindicators [6]. Their use might therefore directly address the first aspects of the 3R principle as living organisms can be replaced by AMs. Further advantages include the possibility of continuous monitoring, high cost-effectiveness, no need for power or energy supply as well as simple handling, deployment, recovery, and analysis [15]. Additionally, these devices are advantageous since they provide time estimated results as opposed to the snapshot view of traditional water sampling [6].

Conclusions

Thus far the AM has only been validated for Cu, Cr, Pb and Zn in the marine environment. In the present study, the use of the AM was adapted for the monitoring of Pt under freshwater conditions. Two modifications to the original method are therefore recommended, i.e. washing by centrifugation (it is more practical and less time-consuming) and using a mixture of HNO₃ and HCl for the elution of Pt from the Chelex-100 beads. The field exposures demonstrated that the AM is a promising tool for monitoring Pt in the freshwater environment as it accumulates Pt in a concentration-dependent manner. However, for monitoring of very low Pt concentrations in freshwater environments, the exposure periods should be increased (> 6 weeks) and further efforts should be made to improve the accumulation of Pt by the AM and to decrease the detection limit for Pt. The AM can only represent the dissolved bioavailable metal fraction and does not consider, e.g. particle-bound metals which can be taken up by living organisms via the food. Thus, it is recommended to combine active monitoring using living bioindicators with AMs to obtain insight into the role of dissolved bioavailable metal fractions and particulate bound metals in metal exposure in aquatic ecosystems.

Abbreviations

AAS – Atomic absorption spectrometer

Al – Aluminium

AM – Artificial mussel

As – Arsenic

BO – Bioindicator organism

Cd – Cadmium

Cl⁻ - Chloride

Co – Cobalt

Cr – Chromium

Cu – Copper

DO – Dissolved oxygen

EC – Electrical conductivity

Fe – Iron

HCl – Hydrochloric acid

Hf – Hafnium

Hg – Mercury

HNO₃ – Nitric acid

ICP-MS – Inductively coupled Plasma mass spectrometer

LOD – Limit of detection

Mn – Manganese

NH_4^+ - Ammonium

Ni – Nickel

NO_2^- - Nitrite

NO_3^{2-} - Nitrate

OC – Organic content

Pb – Lead

PO_4^{2-} - Phosphate

PP – Polypropylene

Pt – Platinum

SO_4^{2-} - Sulphate

SS – Suspended solids

Turb – Turbidity

U – Uranium

Zn – Zinc

Declarations

Ethics approval and consent to participate

The necessary permits (HO 09/03/17-125 NW; HO 20/02/18-057 NW, North West Province, Department of Rural, Environmental and Agricultural Development) and ethical clearance (NWU-00282-17-A5. NWU-AnimCare) were obtained prior to sampling.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ML and JHE contributed towards the conception of study, acquisition, analysis and interpretation of data, and drafting of manuscript. MN contributed with acquisition, analysis and interpretation of data and substantively revised the work. SZ and VW contributed with the conception of the study, acquisition, analysis and interpretation of data and substantively revised the work. NJS and BS contributed to the interpretation of data and substantively revised the work. All authors read and approved the final manuscript.

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Figures

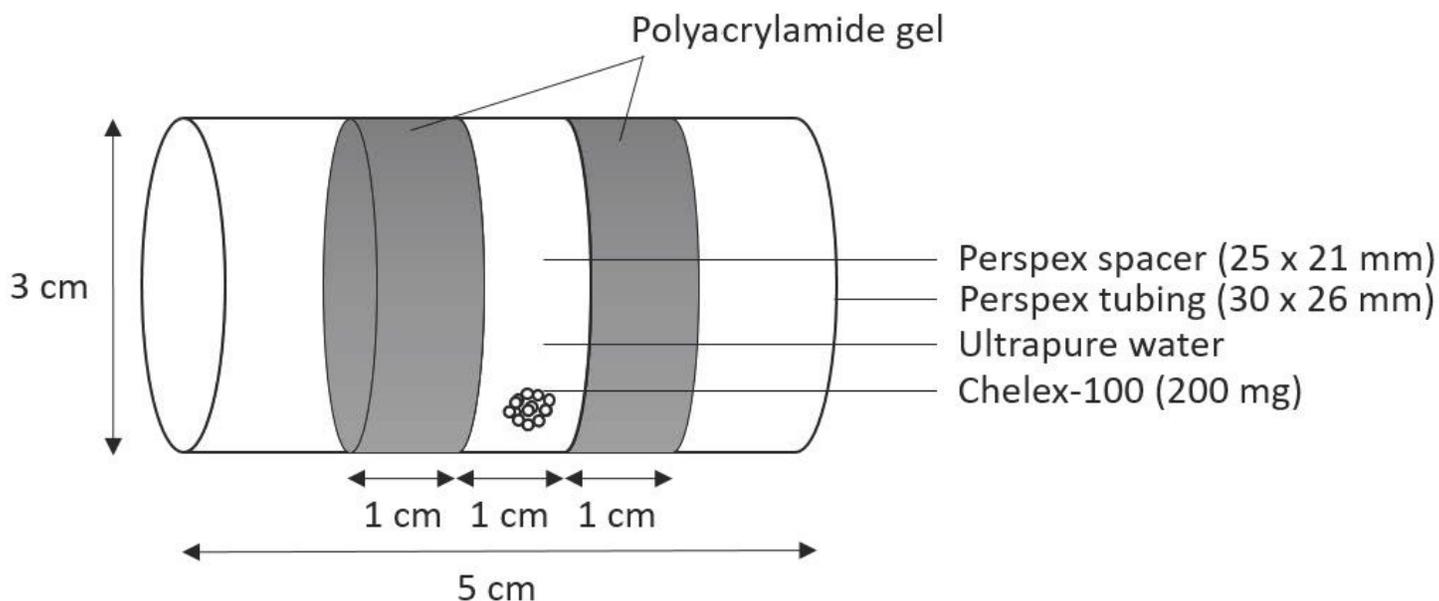


Figure 1

Graphical representation of the artificial mussel [12]

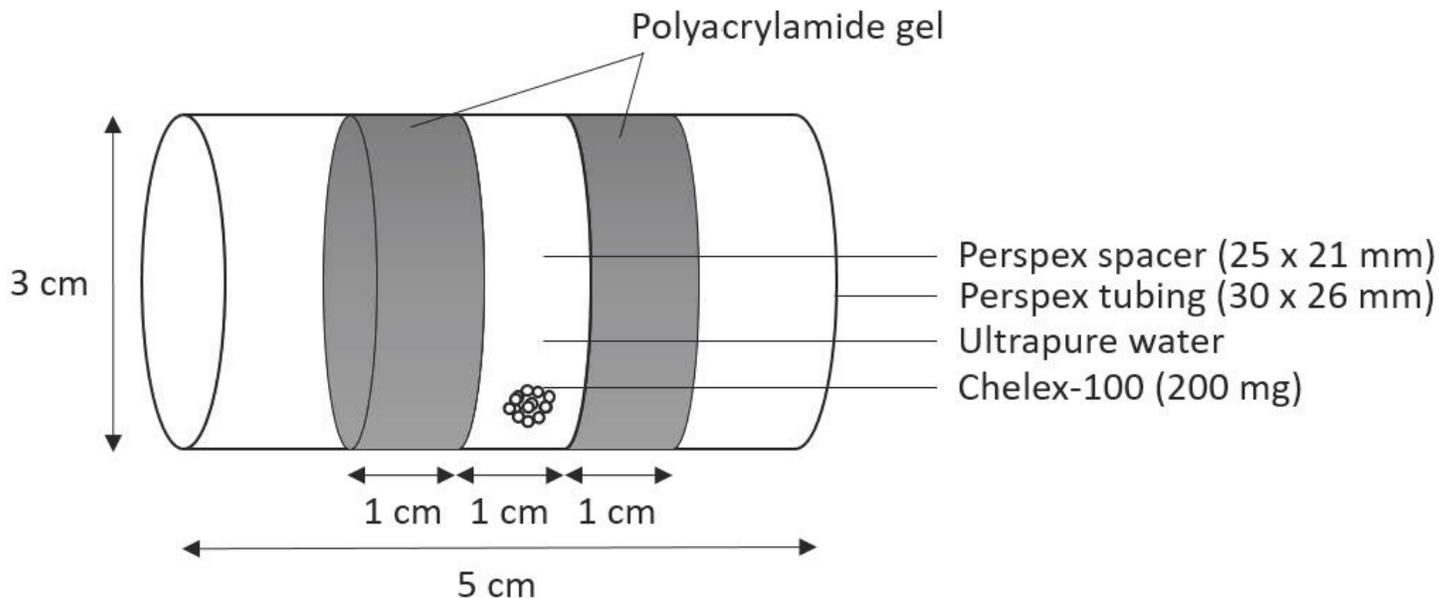


Figure 1

Graphical representation of the artificial mussel [12]

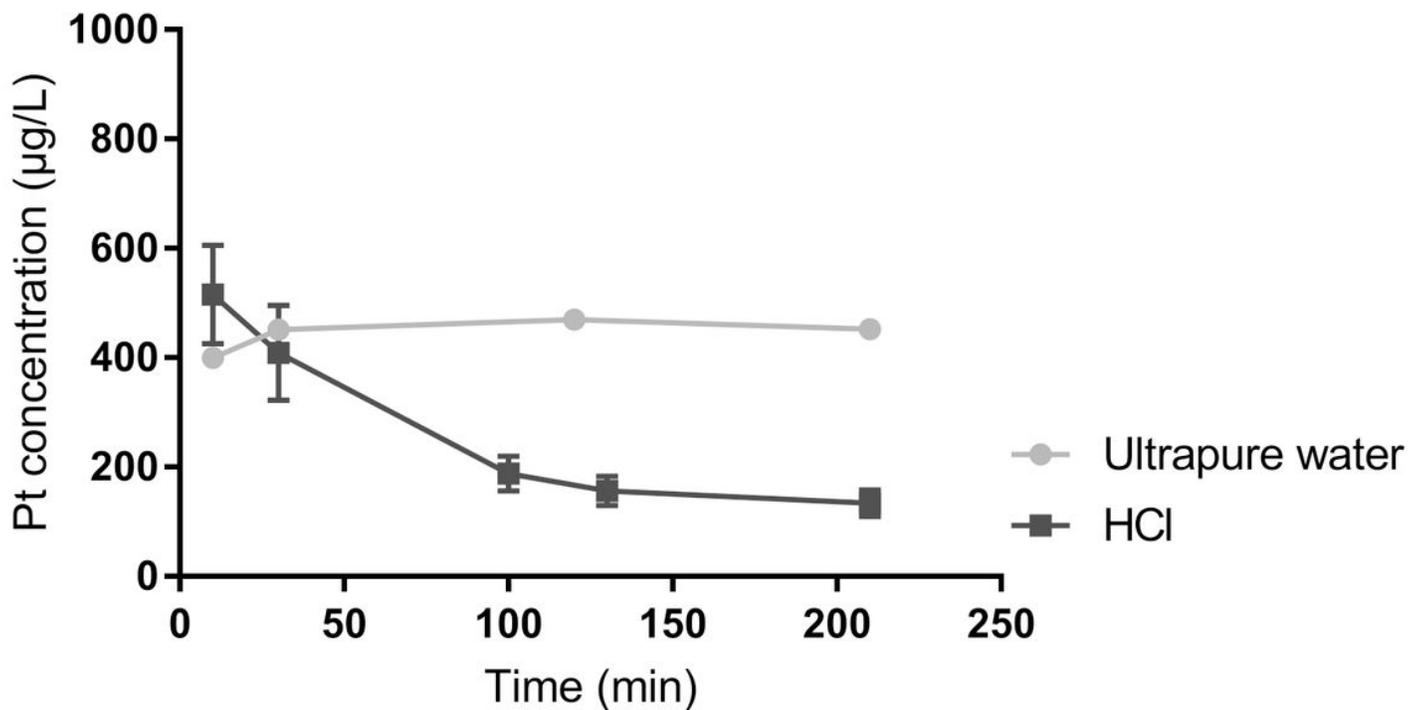


Figure 2

Platinum concentrations (mean \pm SEM, $n = 10$) in the loading solutions, ultrapure water and 0.5 M HCl, with increasing loading time of the Chelex-100 beads. The initially added Pt concentration was 1000 $\mu\text{g/L}$.

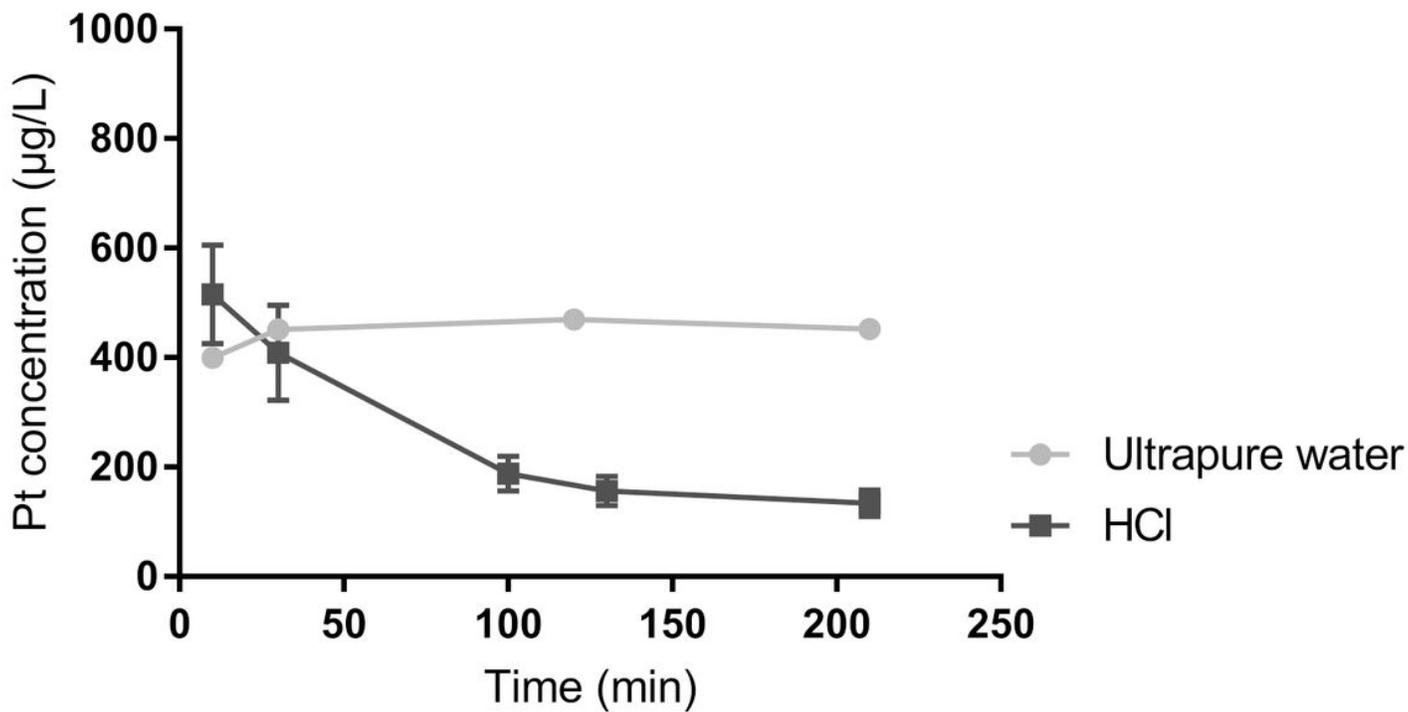


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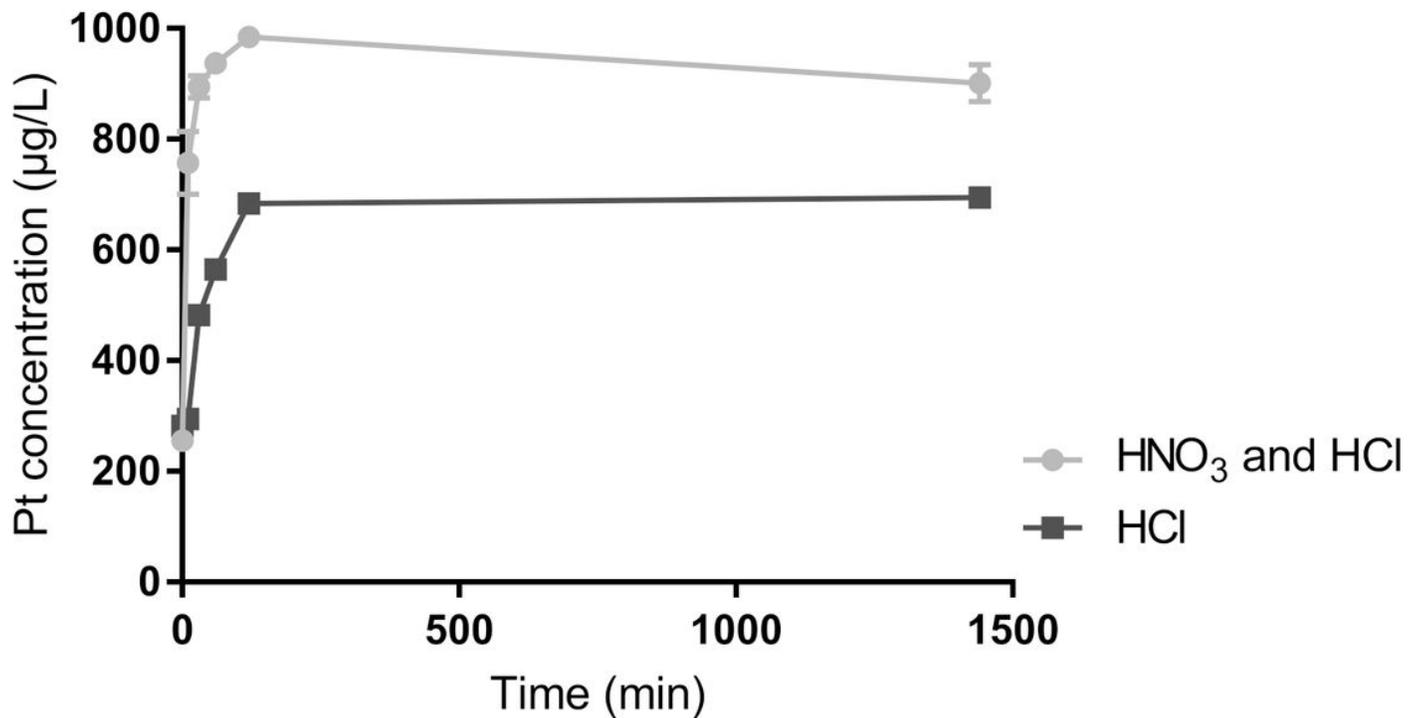


Figure 3

Platinum concentrations (mean \pm SEM, n = 5) in two different elution solutions, i.e. 8 M HCl and a mixture of 4.5 mL 6 M HNO₃ and 0.5 mL 12 M HCl, with increasing elution time.

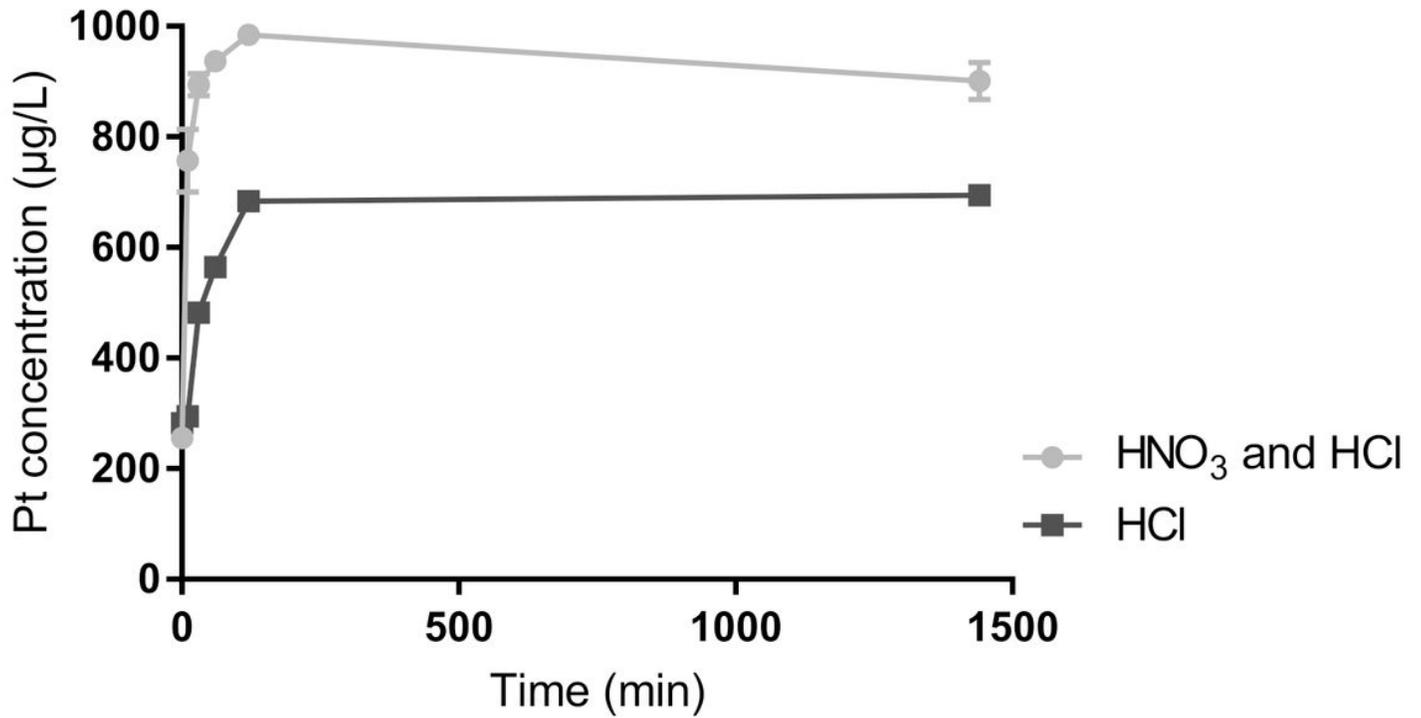


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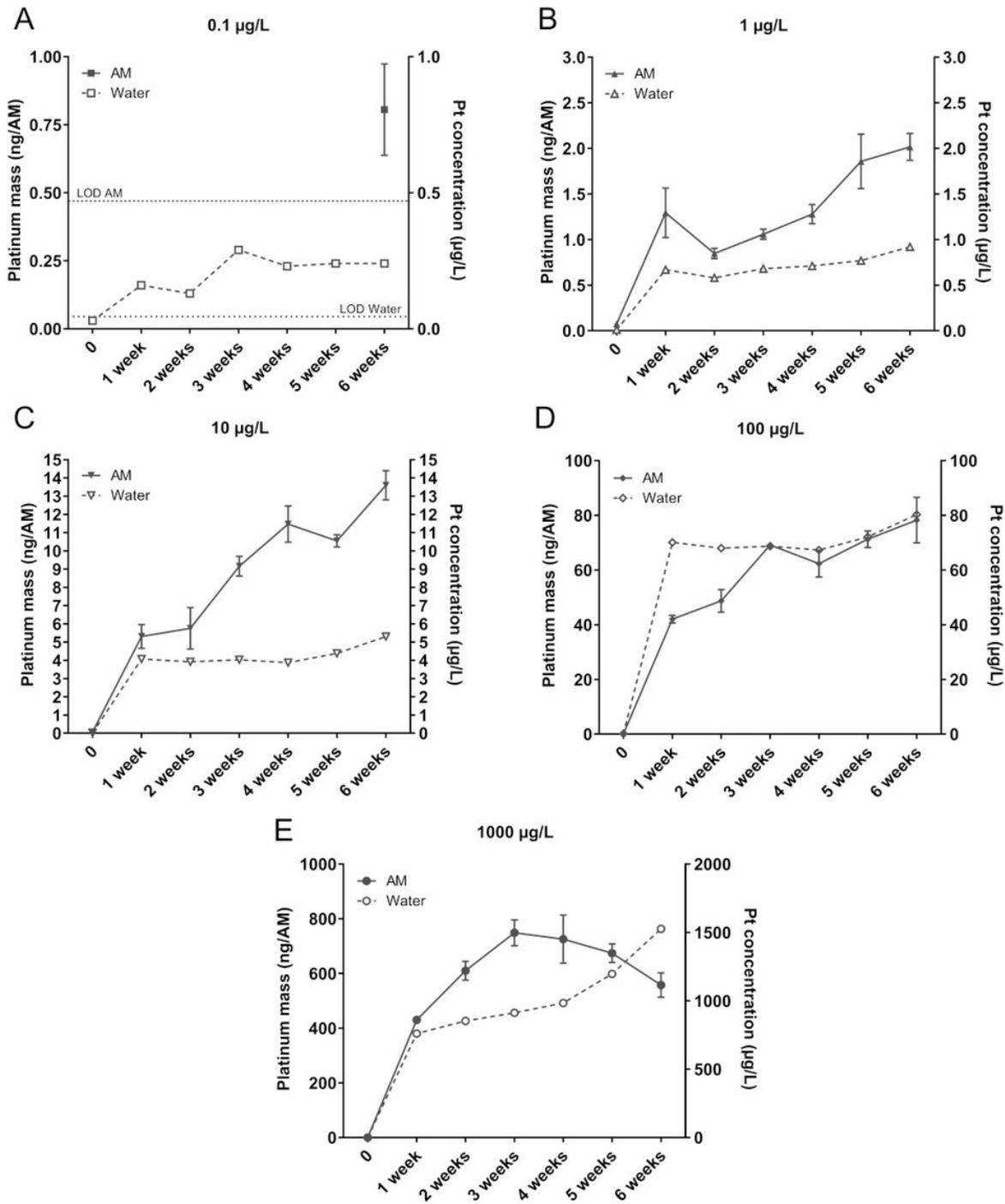


Figure 4

Kinetics of the Pt uptake by the artificial mussels (AM) expressed as ng/AM (mean \pm SEM, n = 7) and the Pt concentrations measured within the water expressed as $\mu\text{g/L}$ during the six-week exposure period. A: Pt masses of the AMs of week 0 - 5 were below the limit of detection (LOD) (LOD of AMs = 0.47 ng/AM and LOD of water = 0.045 $\mu\text{g/L}$).

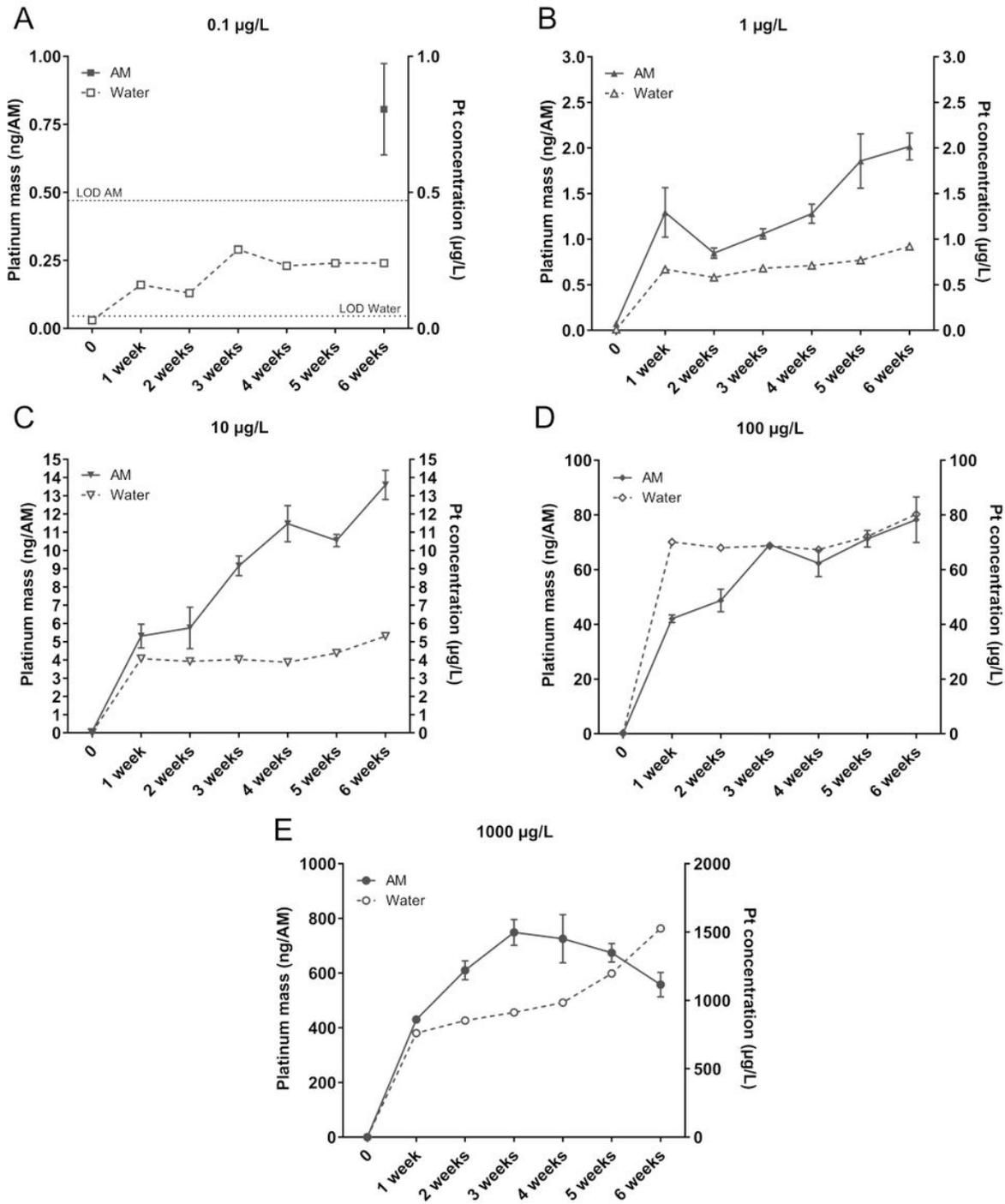


Figure 4

Kinetics of the Pt uptake by the artificial mussels (AM) expressed as ng/AM (mean \pm SEM, n = 7) and the Pt concentrations measured within the water expressed as $\mu\text{g/L}$ during the six-week exposure period. A: Pt masses of the AMs of week 0 - 5 were below the limit of detection (LOD) (LOD of AMs = 0.47 ng/AM and LOD of water = 0.045 $\mu\text{g/L}$).

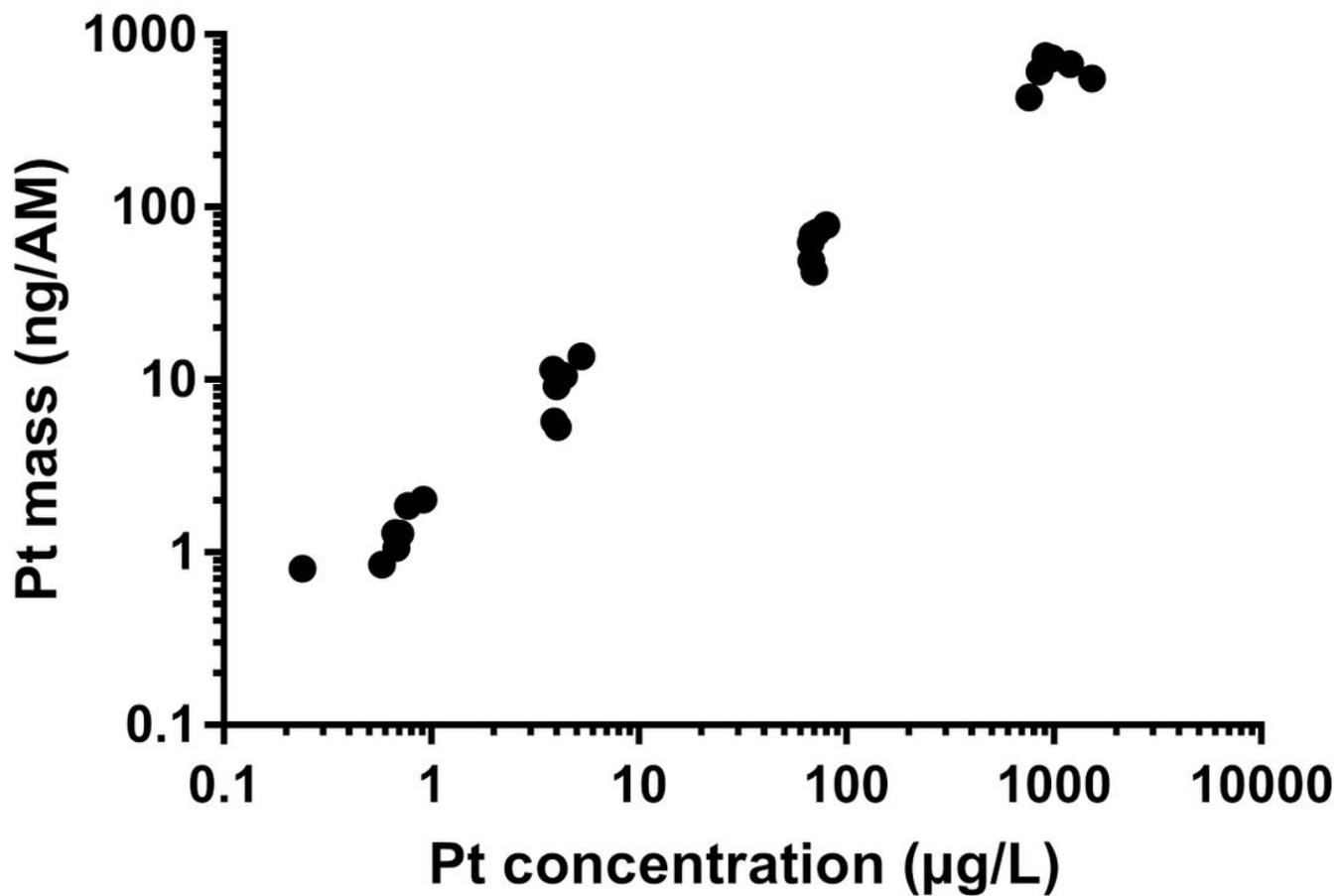


Figure 5

Correlation of Pt uptake by the AM and the determined exposure concentrations in the tank water. The x- and y-axis are presented in logarithmic scale. Linear regression analysis revealed for the regression line the following equation: $y = 0.5502x + 20.13$; $R^2 = 0.8915$.

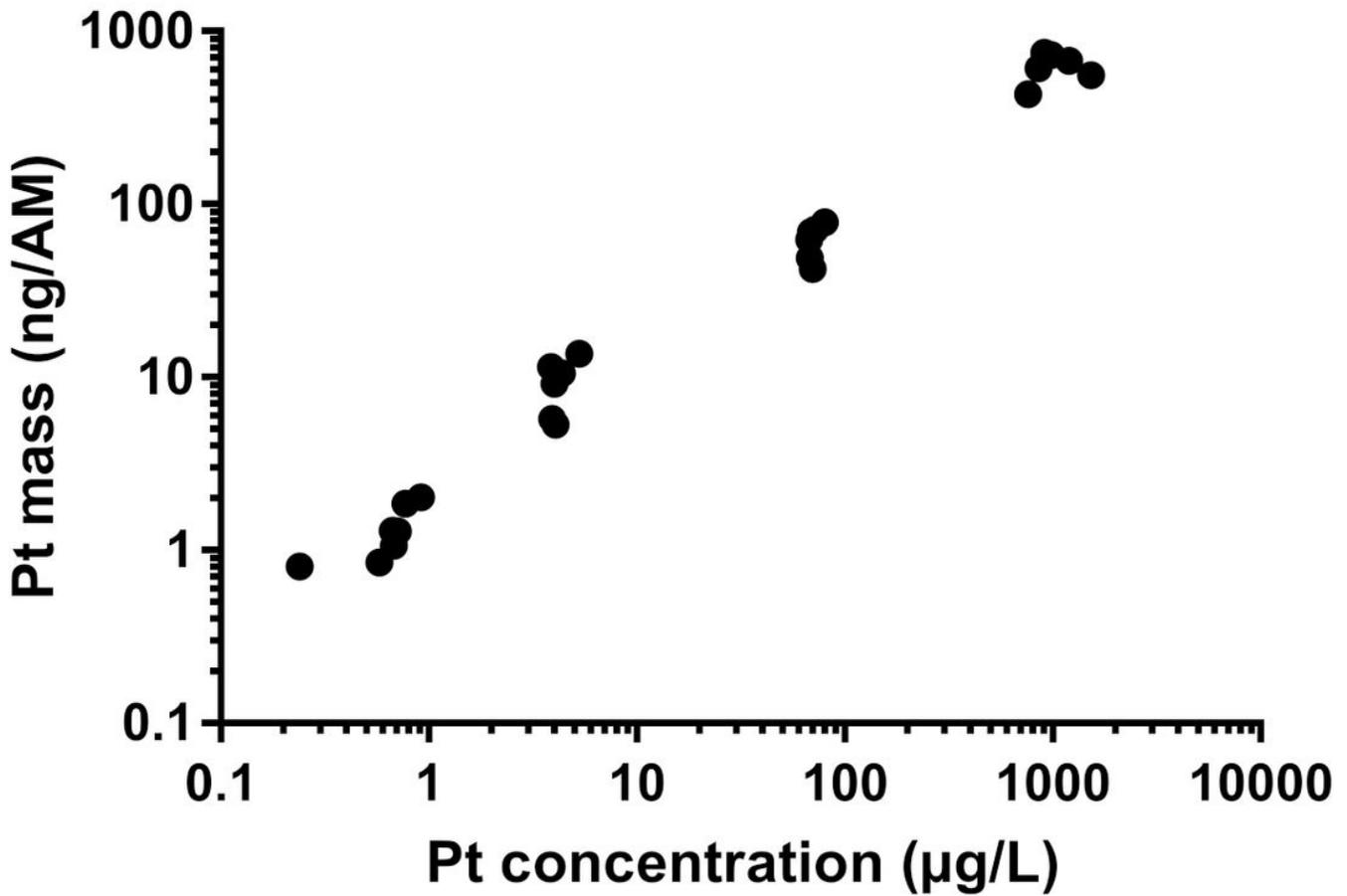


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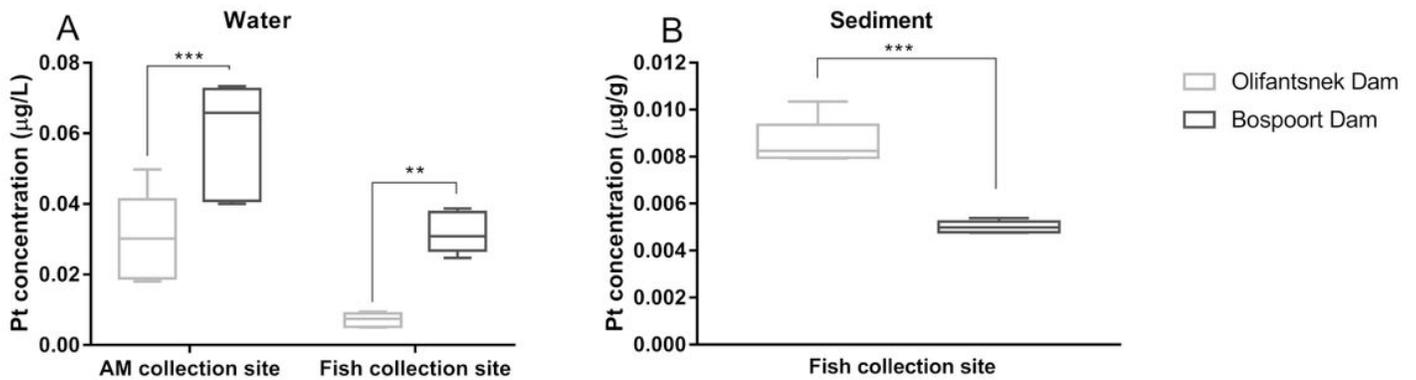


Figure 6

Platinum concentrations in the water (A) and sediment (B) from Olifantsnek and Bospoort Dam. Data presented in the graphs are from the following articles: water from the AM collection site is from Labuschagne et al. [12], water and sediment from the fish collection site are from Erasmus et al (2020b). Box-plots indicate minimum, 25th percentile, median, 75th percentile and maximum values. Stars show significant differences between the two impoundments (**: $p < 0.002$, ****: $p < 0.0002$)

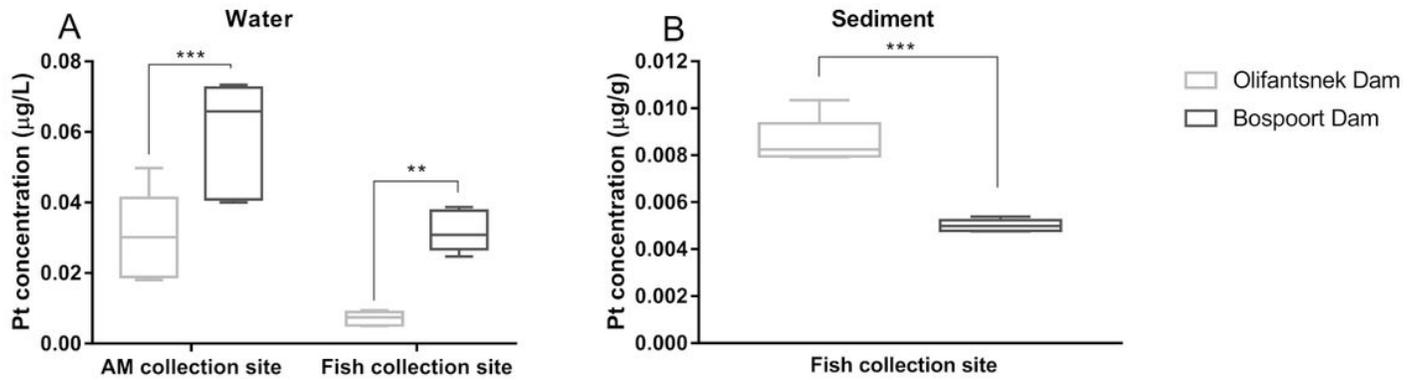


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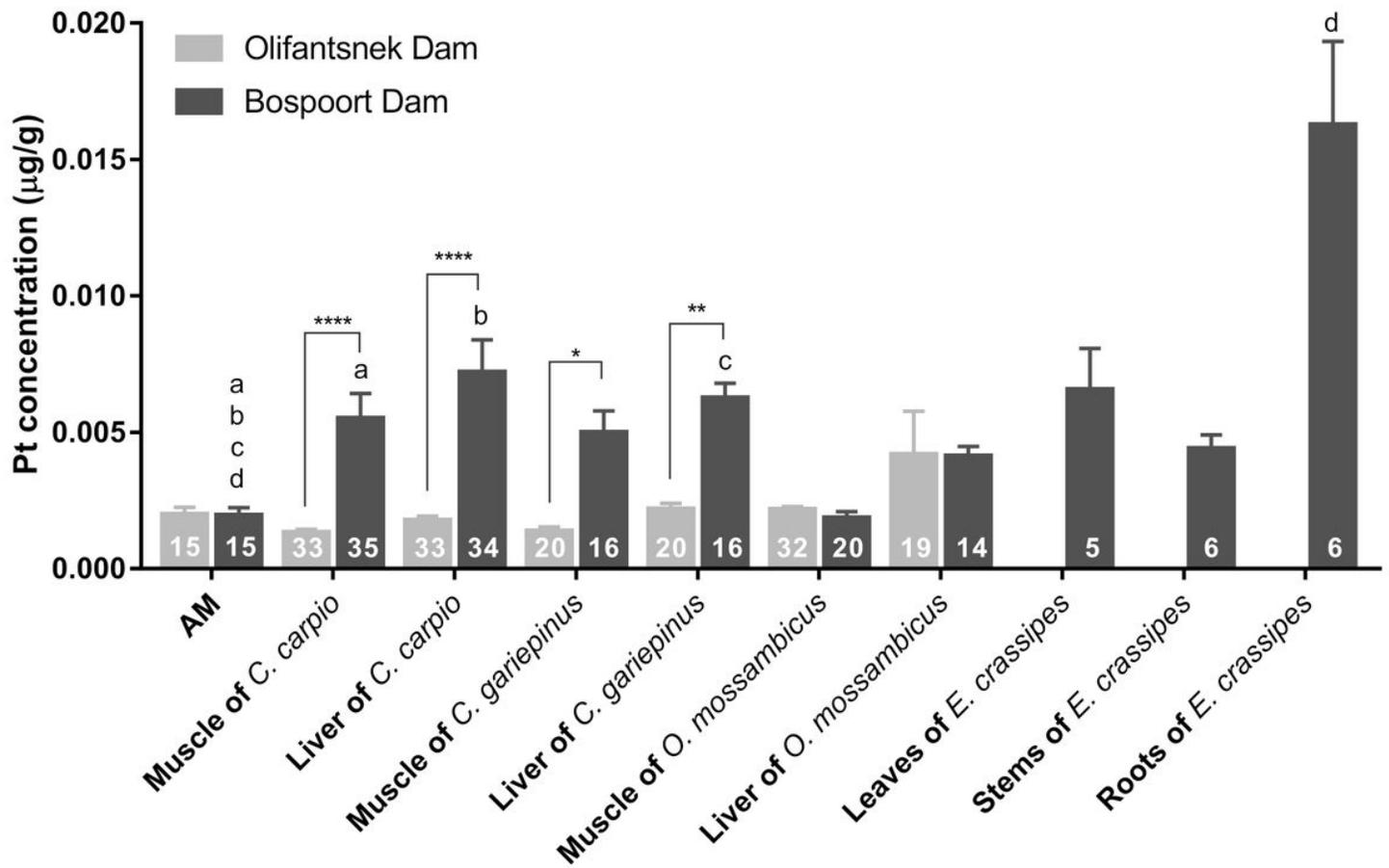


Figure 7

Platinum concentrations (mean \pm SEM) measured in AMs (in $\mu\text{g/g}$ related to the Pt mass accumulated on 200 mg Chelex-100 beads; data from Labuschagne et al. 2020) and muscle and liver of three different fish species and the water hyacinth, from two impoundments (Olifantsnek Dam and Bospoort Dam) of a Pt mining area in South Africa. Bars with common letter superscript represent a significant difference from AM ($p < 0.05$), stars show significant levels between differences of the two impoundments (*: $p < 0.03$, **: $p < 0.002$, ****: $p < 0.0001$). Numbers within the bars represent the number of replicates (n).

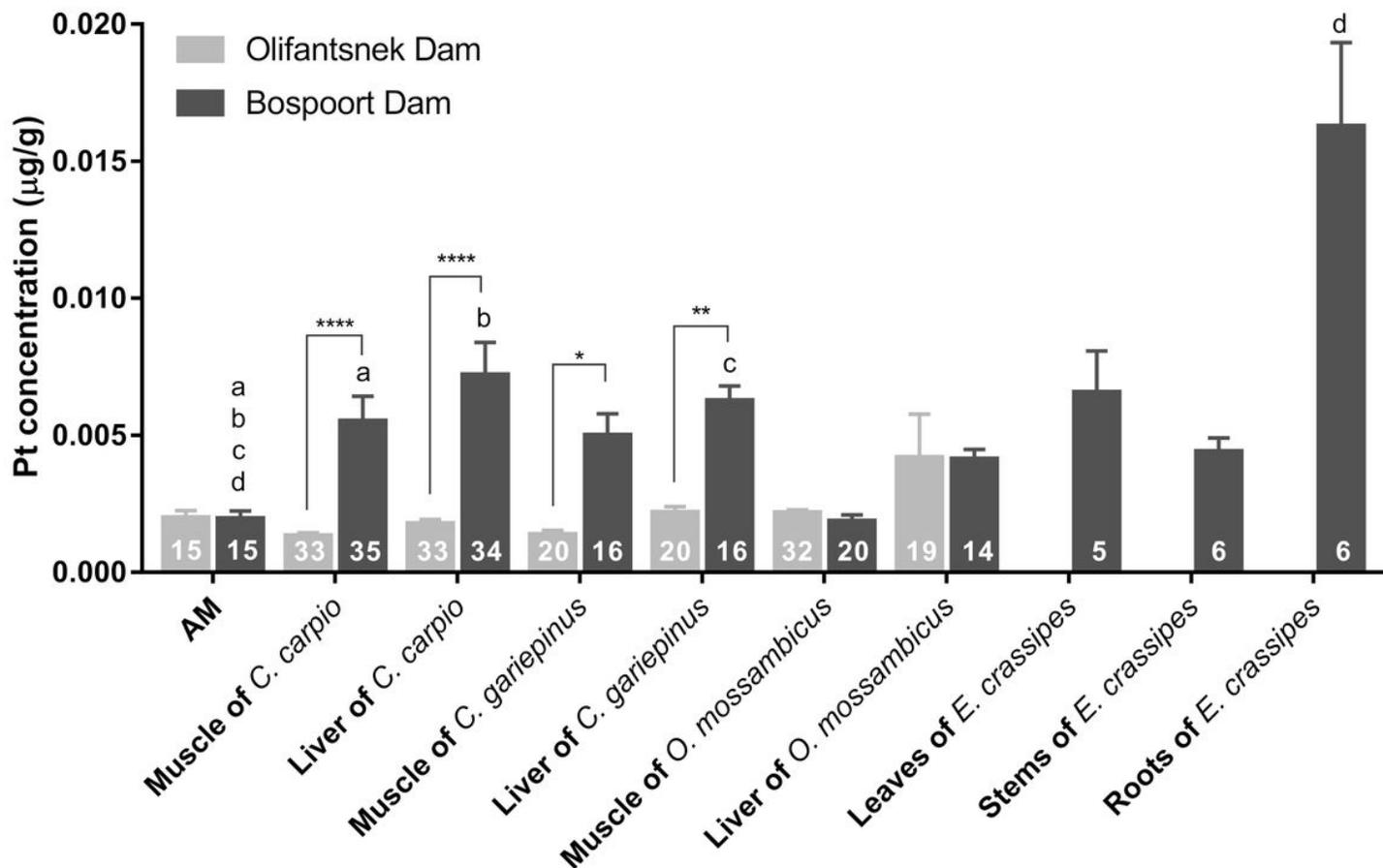


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