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On mass dynamics in a closed ecological system, determined with a prototype vacuum mass comparator – a methodological validation study

Zur Massendynamik eines geschlossenen Ökosystems, gemessen mit einem Prototyp Vakuummassekomparator – eine methodische Validierungsstudie

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Abstract: The aim of this work was to validate a novel methodology for high-resolution, repetitive measurements of mass dynamics of biological processes and structures in a closed plant-earth ecosystem consisting of *Mammillaria vetula* and microorganisms. To perform these experiments, the living system was materially welded into a newly developed Titanium Weighing Hollow Body (TWHB) with a laser. Three non-vital, also hermetically welded and high-vacuum suitable, externally identical TWHBs, filled with sand, served as controls. All TWHBs were equipped with a feedthrough and integrated light source. LEDs generated continuous light in all four bodies, which drove the photobiological processes in the vital test body and allowed long-term growth. Mass differences of the TWHBs were measured with a vacuum mass comparator at four points in time three months apart against two stainless steel mass standards. The expanded measurement uncertainty of the mass increase of the vital TWHB was calculated according to the Guide to the Expression of Uncertainty in Measurement (GUM) in each of the three independent experiments. The mass gain of the vital over the three nonvital TWHBs over the total experimental period of 9 months was +18 µg with the expanded measurement uncertainty 30 µg. The resulting mass gain would have had to be >48 µg to be considered statistically significant with a confidence level of 97.7%; time intervals over three and six months were also not significant. The study validates for the first time a methodology capable of measuring mass dynamics of living matter over time, when statistically sound conclusions with measurement uncertainties in the microgram range are required. This opens up a new level of precision mass measurements, which makes the methodology a candidate, e.g., for the verification of the principle of mass conservation in the life-sciences.

Keywords: closed ecosystem; living matter; precision mass determination in the life sciences; principle of mass conservation in biology and medicine; titanium weighing hollow body.

Zusammenfassung: Ziel dieser Arbeit war die Validierung einer neuartigen Methodik zur hochauflösenden, wiederholt erfolgenden Messung der Massendynamik der biologischen Prozesse und Strukturen eines geschlossenen Pflanzen-Erde Modellökosystems, bestehend aus *Mammillaria vetula* und Mikroorganismen. Zur Durchführung der Experimente wurde das lebende System stoffschlüssig in einen neu entwickelten Titan-Wäge-Hohl-Körper (TWHK) mit einem Laser eingeschweißt. Drei nicht-vitale, ebenfalls hermetisch verschweißte und hochvakuumtaugliche, äußerlich identische TWHKs wurden mit Sand befüllt und dienten als Referenz. Alle TWHKs verfügten über eine Stromdurchführung und eine integrierte Lichtquelle. LED erzeugten in den vier Messkörpern Dauerlicht, das in dem vitalen Messkörper die photobiologischen Prozesse aktivierte und langzeitiges Wachstum ermöglichte. Die Massendifferenzen der TWHKs wurden gegen zwei Edelstahlstandards an vier Messzeitpunkten im Abstand von drei Monaten mit einem Prototyp eines Vakuummassekomparator gemessen. Die erweiterte Messunsicherheit der Massenzunahme des vitalen TWHK wurde in jedem der drei unabhängigen Experimente mit dem GUM (Guide to the Expression of Uncertainty in Measurement) berechnet. Der Massenzuwachs des vitalen gegenüber den drei nicht-vitalen TWHKs betrug in der gesamten neunmonatigen Beobachtungszeit +18 µg. Der resultierende Massenzuwachs hätte >48 µg betragen müssen, um bei einem

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Konfidenzniveau von 97.7% als statistisch signifikant zu gelten. Die in gleicher Weise untersuchten Massenzunahmen über drei und neun Monate waren ebenfalls nicht signifikant. Die Studie validiert zum ersten Mal eine Messmethodik zur Erfassung der Massendynamik von lebender Substanz, wenn statistisch fundierte Aussagen mit Messunsicherheiten im Mikrogrammbereich gefordert sind. Hierdurch werden Präzisionsmassemessungen von lebendem Material möglich, wie z.B. zur Verifizierung des Gesetzes der Massenerhaltung in den Life Sciences.

Schlüsselwörter: Lebende Substanz; geschlossenes Ökosystem; Prinzip der Massenerhaltung in der Biologie und Medizin; Titan Wägehohlkörper; Präzisionsmassebestimmung in den Life Sciences.

1 Introduction

Many precise measurements have been made addressing the conservation of mass in different inorganic chemical reactions. In most of these experiments, vessels made of borosilicate glass were used [1], which were weighed in air. For example, Landolt [2], using comparators with double bowls, verified the conservation of mass within a measurement uncertainty of ±30 µg for 15 different redox reactions which took place under increasing entropy in closed systems made of borosilicate glass. The Law of Conservation of Mass is today one of the central theorems of modern chemistry. However, it has been verified experimentally [2] only within the uncertainty of measurement mentioned above. Nevertheless, even in its first formulation in the late 18th century, it was already extended – in our opinion insufficiently justified – from inorganic chemistry to the syntheses of organic compounds, and was finally declared to be a universal principle. Similarly, it was generalized from processes with short reaction times to processes of any reaction time. The only thing that has been verified experimentally is that the deviations from the law of conservation of mass for fast and inorganic reactions are smaller than ±30 µg. However, there have been no adequate attempts to measure the mass dynamics of cells, organisms, and complex living systems. Since a convincing experimental method to address this issue has been lacking, we decided to develop a suitable titanium hollow weighing body (TWHB) in order to satisfy biological and mass metrological requirements in equal measure. Since it was open whether the designed weighing body met all requirements, we decided to validate the new weighing body methodologically first. The mass-biological issues were to be dealt with and restricted to the main experiment which is intended to be executed on a much larger scale. Therefore, the only vital TWHB ($n = 1$) could just as well have been omitted from our validation study or replaced by a non-vital TWHB. Previously, hollow bodies were used in mass metrology as buoyancy standards [3]. However, in this validation study, we used a hollow precision weighing body with an integrated light source and a plant-earth-system analogous to a closed ecosystem for the first time. To reduce the uncertainties inherent in mass determination in air, the living system was put into a vessel suitable for high-vacuum. This allowed the use of a vacuum mass comparator, which has the highest resolution and precision in mass metrology today [4–6]. A second critical property needed was that the hollow body had to have a very high mass stability. First, we conducted a feasibility study [7] in which the ecosystem was hermetically sealed in vessels made of borosilicate glass, using elaborate cooling techniques. The lessons learned from that lead to a pilot study from late 2018 to late 2019 involving four quartz glass vessels. That study also involved our first use of a modified CCL1007 prototype vacuum mass comparator. High-vacuum mass comparators such as the CCL1007 are used to avoid the effects of errors caused by buoyancy, for example, when comparing the masses of samples with different densities [8]. However, it turned out that the mass stability of quartz glass was insufficient for our purposes. Therefore, we were compelled to consider stainless steel for the design of the experimental model. This allowed us at the same time to fulfil all the recommendations of OIML R 111-1 [9]. However, this also meant that we needed to construct an integrated internal light source for the weighing body with an electrical feedthrough, which required many preliminary tests. In the end, because of the risk of magnetization during turning, we decided not to work with stainless steel. The use of platinum-iridium had to be discarded for cost reasons. Titanium was ultimately chosen, because it has very low magnetic susceptibility and good machining properties. We expected that with the design of this novel Titanium Weighing Hollow Body (TWHB), we can meet all metrological and biological requirements simultaneously. Measurements involving four such TWHBs began in early 2021 and the results from those experiments are reported here.

2 Equipment

2.1 The vacuum mass comparator

All measurements in this work were performed using a modified, commercially available Sartorius CCL1007 1 kg prototype vacuum mass comparator [10], which was installed in a laboratory at the Technical University Ilmenau, Germany. The instrument allowed to intercompare four TWHBs and up to four mass standards placed on the turntable in one experiment (Figure 1). A precentering device ensured the repeatability of the positioning of the artifact when it was placed on the storage position on the turntable. The comparator was operated at a lab-temperature of about 25 °C during a measurement series, which lasted five days. A modified ABBA weighing cycle (in accordance with the OIML R 111-1 [9]) was performed in about 2 h. At the end of five days of operation, about 60 complete ABBA mass comparisons of each artifact were performed and recorded, out of which the mean of the last six ABBA cycles of each artifact was tanken as definite best result for the measured mass difference. Each measurement point corresponded to a contact time of about 200 s on the pan. The integration time itself was 29.75 s. The standard deviation, $\overline{\sigma(\Delta m_w)}$, averaged 0.55 µg for each of the 1 kg TWHBs and for the 1 kg stainless steel standards in the measurement series #01 through #04.

To achieve the required level of vacuum, which was always below $1 \cdot 10^{-5}$ mbar, the main vacuum chamber was continuously pumped down by an oil-free scroll pump in series with a magnetically levitated turbomolecular pump. Following the loading and the evacuation of the comparator a period of thermal stabilization was essential. The mandatory minimum times required were five days, provided that the difference between the initial temperature of the TWHBs and the temperature in the evacuated comparator was small. After this period, thermal equilibrium between the mass comparator, the TWHBs and the ambient conditions of the laboratory could be assumed. This was verified using the time series of the measured seven mass artifacts, which were stable. Table 1 summarizes the key features of the comparator.

Following each measurement series, the mass comparator was calibrated. For this purpose, the (second) standard on position 5, serial number 2821212, was additionally loaded with a "sensitivity" 500 mg E1 stainless-steel wire, serial number 1570819, and compared with the (main) standard, serial number 2811212, by ABBA comparisons in vacuum. This was necessary for the calibration of the electronics in the mass comparator at the end of each measurement series. The determined calibration factor, ($\varepsilon_{\mathrm{korr}}$), was used for the data analysis of the measurement data from the ABBA comparisons.

Table 1: The details of the modified Sartorius CCL1007 1 kg prototype vacuum mass comparator.

Capacity	1011 g
Resolution	0.1 µg
Typical standard deviation for mass comparison in vacuum (the mean of the last six ABBA comparisons after five days of operation of the comparator)	0.1 µg
Operation pressure for all measurements in high-vacuum	$<1 \cdot 10^{-5}$ mbar

2.2 The experimental artifacts

2.2.1 The stainless-steel mass standards

Two sets of stainless-steel weights of nominal mass 1 kg of cylindrical construction and a single 500 mg stainless-steel wire sensitivity weight, all manufactured in 2013 by Häfner in Germany to OIML Class E_1 specifications [9], were used for this work. Due to the long storage time, the mass standards were only cleaned with a bellows before the start of a series of measurements in order not to affect the mass stability. Their use was restricted to the experiments for this project. When not in use, both sets were kept in cases, which were stored in the laboratory. Three 1 kg mass artifacts served as working standards: The (main) standard, serial number 2811212 on position 7, the (second) standard, serial number 2821212 on position 5 and the (test) standard, serial number 2821212* on position 3. Initially, all the three mass standards were to be used in the experiments. However, the (test) standard, serial number 2821212, required ethanol purification and could no longer be used because the mass remained unstable. The 1 kg standard at position 1 served as a compensation mass for the turntable but was not included in the measurement.

2.2.2 The four 1 kg titanium weighing hollow bodies (TWHBs)

Four highly vacuum-tight, sealed TWHBs, which had an electrical feedthrough for the integrated, internal light source, were designed and built by ifw-Jena in Germany. Their nominal filled weight was 1 kg, Table 4 gives the absolute masses of all the four TWHBs. Figure 2 shows the details of the

Figure 1: The inner side of the main chamber of the modified Sartorius CCL1007 prototype vacuum mass comparator with the four TWHBs and the four stainless-steel mass standards (St), positioned on the turntable.

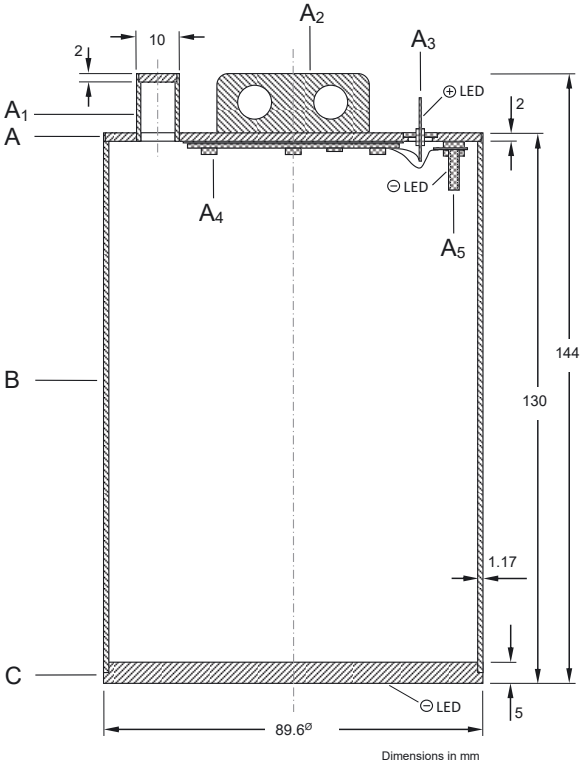

Figure 2: The details of the titanium weighing hollow body (TWHB). A: cover. A_1 hose connection for final leakage test; A_2 handle; A_3 single wire feedthrough with molybdenum core (+pole), housed in a ceramic insulator; A_4 module with nine LEDs; A_5 screw to fasten the (– pole). B: seamless-rolled tube. C: base plate (– pole).

construction, and Table 3 gives the technical specifications and results of the preparatory measurements that were taken. All the semi-finished products, made of unalloyed titanium grade 2 (DIN 3.7035), were supplied and polished by Ankuro International in Germany. All parts were seamlessly welded by laser beam welding. A finite element analysis was performed to optimize the thickness and stiffness of the base plate C in Figure 2 so that its stiffness and properties do not differ from the standard stainless-steel weights used. This was the case, as shown in the results and discussion section.

Each TWHB was equipped with a LED-light module inside, which was operated continuously during the three storage times. Resistors required for these modules were placed outside the storage vessel to prevent ferromagnetic effects during weighing as well as energy transfers into the storage vessel. The three biologically inactive, avital probes, TWHB 1, TWHB 2 and TWHB 4, filled with sterile sand, were used as controls. The biologically active, vital probe TWHB 3 was equipped with a materially closed, energetically open vegetation-soil-atmosphere ecosystem. The biological model used (shown in Figure 3) consisted of 26 cactuses of the *Mammillaria vetula* ssp. gracilis (shown in Figure 3) variety (about 12 g) and microorganisms and mineral nutrients in the soil substrate (about 167 g). The water supply enclosed (about 192 g) allowed the living system to exist for a decade.

The electrical energy to each LED-module was supplied through a single wire current feedthrough with molybdenum core, housed in a ceramic insulator (see Figure 2, A_3). It was manufactured by IPT Albrecht in Germany. The

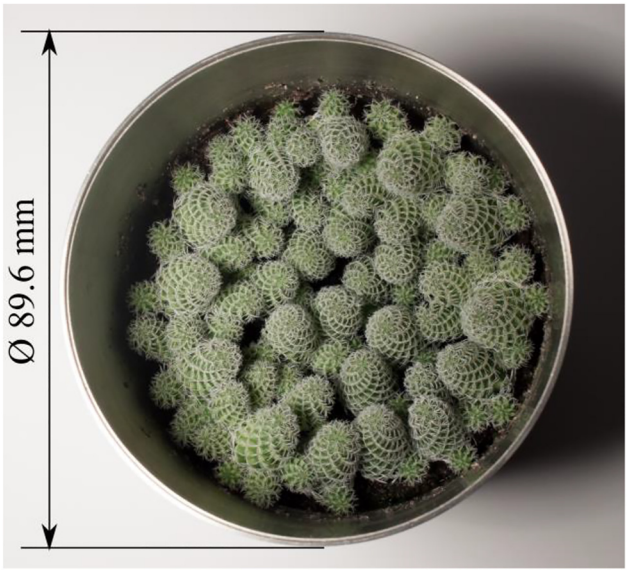

Figure 3: *Mammillaria vetula* ssp. gracilis, the photosynthetic active part of the complex ecosystem in the TWHB prototype, which was not welded.

baseplate of the vessel, connected to the baseplate of the container, served as the return path. 213 g of tungsten were needed to bring the mass of the vital TWHB 3 to the nominal weight of 1 kg. The power provided to each LED module was 0.92 W (0.12 A at 7.68 V). This means that over a full year the electrical energy provided to each TWHB is equal to about $2.90 \cdot 10^7$ Ws (0.92 W for $3.15 \cdot 10^7$ s). Given that for typical LEDs only about 30% of the electrical energy is converted into light, most of the electrical energy supplied to each TWHB is converted into heat, which raised the temperature of the TWHB covers to about 35 °C. Only about $8.70 \cdot 10^6$ Ws ($2.90 \cdot 10^7$ Ws $\cdot 0.3$) is converted into light energy. This energy could be converted into mass by some, yet unknown, process related to photosynthesis, which converts energy into mass according to the well-known equation, written in the form it was originally presented, $\Delta E/c^2 = \Delta m$. If all the light energy was converted into mass this way, it would result in a yearly increase in mass of the TWHB of $9.68 \cdot 10^{-11}$ kg ($8.70 \cdot 10^6$ Ws$/9 \cdot 10^{16}$ m^2/s^2). The total annual increase is about 0.1 µg/year. Such an increase would be within the resolution of the prototype vacuum mass comparator and thus be measurable. However, this rough estimate is only valid if the overall uncertainty of the measurement method is small.

2.2.3 The type-1 glass storage vessel, filled with ambient air and molecular sieve 0.4 nm

The type-1 glass storage vessel was used in the first three experiments to store the four TWHBs altogether for nine months (for details see Table 3). It consisted of a lime-soda glass cover with a fluorocarbon rubber seal and a stainless-steel baseplate. A flanged electrical feedthrough with four copper cores served to operate the light modules of the stored four TWHBs inside the storage vessel. A cross bar with two screws held the cover in place. The vessel was filled with dry ambient (laboratory-) air at room pressure and was situated 2 m away from the comparator to shorten the transfer time in air while loading and unloading the comparator or the storage vessel.

All TWHBs stored in the type-1 storage vessel exhibited a significant mass increase (see Figure 5 and Table 4), presumably due to contamination by airborne short-chain hydrocarbons desorbed from the various materials in the Type 1 storage vessel. Table 3 gives a presentation of the different materials in the type-1 storage vessel. The contaminations were influenced by the temperature of about 30 °C in the storage vessel. Molecular sieve MiniPax® 0.4 nm in dust-tight Tyvek® bags, was used to maintain the value of relative humidity (RH) in the type-1 storage vessel close to a fixed relative humidity of 0%RH. A data logger (Mostra-Log®) was used to measure the relative humidity (%RH) and the temperature (°C) in the storage vessel continuously (see Figure 4). The Sensirion® SHT15 maximal sensor tolerance of the data logger is specified as ±4%RH at 25 °C near to 0%RH and ±0.4 °C near to 30 °C. While the molecular sieve adsorbed water vapor, the pressure in the container decreased by approximately 13 mbar.

3 Experimental procedure

3.1 Initial experiments

Initial experiments involved four Quartz Glass Weighing Bodies (QGWBs) made from amorphous silicon dioxide (purity of 99.995%), filled with sterilized sand. Using for the first time the modified CCL1007 vacuum mass comparator we investigated the mass stability of these QGWBs which were stored initially in the described manner in the type-1 glass storage vessel for a period of 39 days. During this time each QGWB (nominal weight of 600 g, volume of 591 cm^2, surface area

Figure 4: The type-1 glass storage vessel filled with ambient (laboratory-) air at room pressure and molecular sieve 0.4 nm.

of 337 cm^2), gained an average mass of about 500 µg, which corresponds to an adsorbed surface layer of 1.5 $\mu g/cm^2$. The effect of an adsorbed surface layer in a transition from 0%RH to 50%RH would be an order of magnitude smaller for vessels made from platinum-iridium, or stainless-steel [11]. This observed mass instability of quartz glass vessels motivated us to switch to vessels made from metal. We decided to use titanium, grade 2, because it can be easily welded with a laser beam with low heat input, suitable for high vacuum applications.

3.2 Preparatory measurements and experimental protocol

Table 2 gives the results of the preparatory measurements. Each joined assembly group, except the last one, was leak-tested separately by a Pfeiffer Vacuum ASM 310 leak detector, which gave a helium total leakage rate of less than $5 \cdot 10^{-12}$ mbar $\cdot$ l $\cdot$ s^{-1} (vacuum inside the assembly group or the whole TWHB). For the final leakage test the detector was connected to the hose connection (see Figure 2). The helium leakage rate of the entire TWHB was always measured to be less than $5 \cdot 10^{-7}$ mbar $\cdot$ l $\cdot$ s^{-1}. We accepted this result, and the nozzle to which the leak detector was connected was welded, too. The manufacturer of the 1-pole feedthrough gave a helium leakage rate of less than $1 \cdot 10^{-9}$ mbar $\cdot$ l $\cdot$ s^{-1}. After equipping one weighing hollow body with the ecosystem and the other three with sterile sand, the hose connection was welded, and the target weight 999.4 g was tared with platinum wire, corresponding to 1 kg in vacuum. All taring was performed in air. The TWHBs were cleaned in the vibratory trough of an ultrasonic cleaning unit with a capacity of 5 L in ethanol >99.8%. Each measuring body was treated for 15 min with 35 kHz. A Mex-tech Surface Roughness Tester SRT-6200 was used to measure the R_a -roughness of the cylindrical parts of the TWHBs. The mean of 12 points was 0.12 µm. Accordingly 12 points of the ground- and the cover-plate were measured which gave 0.13 µm. OIML R 111-1 [9] recommends the limits of magnetic susceptibility, <0.02 for E_1 weights >20 g. A Sartorius YSZ02C-Susceptometer was used to measure the magnetic susceptibility of the semifinished titanium grade 2 products. The magnetic susceptibility, χ, of a titanium test sample with a diameter of 15 mm and a length of 30 mm, having a mass of approximately 10 g, was measured to be 0.00023. Since the biological process in the vital TWHB 3 could not be visually observed, we fabricated seven hermetically sealed, equal-volume hollow glass test specimens that were matched in properties to the experimental system. Each of these received a *Mammillaria* soil ecosystem illuminated by a type-matched LED module (see Table 2, Figures 2 and 3). Condition and state of *M. vetula* ssp. *gracilis* was observed simultaneously to the current experiments over nine months and was found to be truly excellent. In the future, growth in the vital TWHBs will be monitored by a device for measuring the center of gravity height from [13]. The device measures the height shift of the center of gravity due to growth processes in the TWHBs. As the 9 LEDs converted a power of approx. 0.9 W to light and heat, each TWHB heated up. Therefore we conducted after disconnecting the 36 LEDs a first period of thermal stabilization which took place for 24 h (for details see Section 2.2.2). Following the transfer and the evacuation of the mass comparator, a second period of thermal stabilization began in the comparator chamber, lasting another five days. At the end of both periods of time an equilibration of temperature of the comparator, the four TWHBs and the ambient temperature conditions of the laboratory was to be assumed. The stability of the mass comparator was checked and verified using the time histories of the measured values. The first series of measurements on four TWHBs in the mass comparator began on April 22nd in 2021.

Table 2: The technical specifications of the four TWHB and the results of their preparatory measurements.

Nominal mass	1 kg
Outer surface area	507.0 cm^2
Outer volume	781.2 cm^{-3}
Density	1.280 g cm^{-3}
Helium leackage rate of the 1-pole feedthrough	$<1 \cdot 10^{-9}$ mbar $\cdot$ l $\cdot$ s^{-1}
Helium leakage rate of the total TWHB	$<1 \cdot 10^{-7}$ mbar $\cdot$ l $\cdot$ s^{-1}
Internal pressure of the four TWHBs	≈1013 mbar
Outer surface roughness (R_a)	<0.13 µm
Magnetic susceptibility of the titanium grade 2 ($\chi_{Ti,grade2}$)	$2.3 \cdot 10^{-4}$
Light module with nine LEDs	6 red ($\lambda = 630$ nm)3 blue ($\lambda = 470$ nm)

Table 3: Specifications of the type-1 storage vessel itself.

Dimensions	Internal height Baseplate diameter	222 mm 300 mm
Materials	Cover Baseplate Four-pole feedthrough Sealing	Lime-soda glass Stainless-steel Stainless-steel, ceramic, copper Fluorocarbon rubber
Volume of the glass cover		≈0.01 m^3
Storage gas		Dry (laboratory) air at room pressure
Relative air humidity		0%RH ± 4%RH
Temperature		30 °C ± 0.4 °C
Date in operation		April 2021 to January 2022

4 Experimental procedure

OIML R 111-1 specifies stainless-steel of a density of 8000 kg $\cdot$ m^{-3} for the manufacture of weights of the highest accuracy class with a mass >100 g [9]. As expected, a literature search using the search terms “titanium” and “weights” yielded no results. So far, hollow bodies have

Table 4: The upper part of the table contains the results of the calculated absolute masses of TWHB 1 to 4 with standard deviations, σ, at the four measurement times T_1, T_2, T_3, and T_4. The lower part gives the mass changes to the respective earlier measurement during the measurement series $T_2 - T_1$, $T_3 - T_2$, and $T_4 - T_3$ determined for the very last 6 measuring points.

Results of the calculation of the absolute masses					
		TWHB 1	**TWHB 2**	**TWHB 3**	**TWHB 4**
T_1	m in g	1000.204683627	1000.142345005	1000.244341601	1000.200041788
	σ in g	0.000000849	0.000000903	0.000000349	0.000000628
T_2	m in g	1000.204693195	1000.142363989	1000.244364368	1000.200064966
	σ in g	0.000000680	0.000000403	0.000000343	0.000000820
T_3	m in g	1000.204712249	1000.142389010	1000.244396599	1000.200085862
	σ in g	0.000000675	0.000000228	0.000000210	0.000000478
T_4	m in g	1000.204724325	1000.142409754	1000.244414617	1000.200101186
	σ in g	0.000000707	0.000000531	0.000000539	0.000000347
Mass changes relative to the respective earlier measurement					
		TWHB 1	**TWHB 2**	**TWHB 3**	**TWHB 4**	$\overline{\textbf{TWHB}}$
$T_2 - T_1$	Δm in µg	9.568	18.984	22.768	23.178	18.624
$T_3 - T_2$	Δm in µg	19.055	25.021	32.230	20.896	24.301
$T_4 - T_3$	Δm in µg	12.075	20.745	18.018	15.324	16.541
$T_4 - T_1$	Δm in µg	40.698	64.749	73.016	59.398	59.465

Table 5: The upper part of the table contains the results of the calculated absolute masses of the steel standards with standard deviations, σ, at the four measurement times T_1, T_2, T_3, and T_4. The lower part gives the mass changes relative to the respective earlier measurement during the measurement series $T_2 - T_1$, $T_3 - T_2$, and $T_4 - T_3$ determined for the very last 6 measuring points.

Results of the calculation of the absolute masses				
		1 kg* E1 2821212	**1 kg E1 2821212 + 500 mg E1 1570819**	**1 kg* E1 2811212**
T_1	m in g	1000.000122989	1000.500000177	1000.000000000
	σ in g	0.000000816	0.000000473	0.000000000
T_2	m in g	1000.000023152	1000.500000169	1000.000000000
	σ in g	0.000000491	0.000000452	0.000000000
T_3	m in g	1000.000039830	1000.500000194	1000.000000000
	σ in g	0.000000597	0.000000411	0.000000000
T_4	m in g	1000.000050992	1000.500000810	1000.000000000
	σ in g	0.000000167	0.000000987	0.000000000
Relative mass changes to the respective previous measurement				
		1 kg* E1 2821212	**1 kg E1 2821212 + 500 mg E1 1570819**	**1 kg* E1 2811212**	$\overline{\textbf{Steel}}$
T_2-T_1	Δm in µg	−99.837	−0.008	0.000	−33.282
T_3-T_2	Δm in µg	16.678	0.025	0.000	5.568
T_4-T_3	Δm in µg	11.162	0.616	0.000	3.926
T_4-T_1	Δm in µg	−71.997	0.632	0.000	−23.788

rarely been used in mass metrology, and known applications concern merely the experimental determination of air density and the construction of liquid scales [14]. Given that, the designed TWHBs had to be considered novel. This meant that performance testing was required first, and we chose mass stability as the main criterion. Other properties of titanium, such as its biocompatibility and low temperature oxidation, appeared less critical. We included one vital TWHB in the first set of experiments because a statistical evaluation strategy also had to be developed and tested at an early stage as well. In the following, we report the results of the three independent validation experiments, which form the core of this work.

Averaged over all four measurement points T_1 to T_4, the standard deviations of the last six measurements, $\overline{\sigma(\Delta m)}$, of both, the four TWHBs (see upper part of Table 4) and the two stainless-steel mass

standards (see upper part of Table 5) had a similar standard deviation of 0.55 µg. The agreement can be taken as confirmation that the measurement device treated the two geometrically very different targets in exactly the same way and also measured them in the same way. Figure 5 shows the four time series of mass gain, Δm in µg, of the four investigated TWHBs at the four measurement time points, T_1, T_2, T_3, and T_4. The total experiment duration was 272 days. During this period of about 9 months, the first, original experiment, $T_2 - T_1$, was replicated twice under identical conditions in the independent experiments $T_3 - T_2$ and $T_4 - T_3$. The quantitative results of all three measured mass increases agreed well in the three experiments carried out as identical, independent replications. In Figure 6 the mass changes are shown compensated for the average mass gain from Figure 5. Table 4 gives the mean mass change of each TWHB in the three experiments over about 9 months as +59.47 µg, corresponding to an average of $\overline{\Delta m_{T4-T1}} = 59.465/3 = 19.82$ µg in each of the three individual experiments. The mass dynamics due to oxidation processes on smooth titanium surfaces is well studied [15]. At room temperature, the growing oxide layer limits the kinetics after a few hours [15]. The mass occupancy of the stable oxide layer is about 1.8 µg/cm^2 [16].

The molecular sieve used in the storage vessel maintained a constant very low humidity during the storage at about 0%RH (see Figure 4). The table below lists all materials in the container that may have given off sorbates during storage. The most important specifications of the content inside the type −1 storage vessel are listed below:

– 5 g MiniPax® molecular sieve (synthetic zeolites), pore opening size 0.4 nm, welded in five Tyvek®-bags made of high-density polyethylene (HD-PE), dustproof according to DIN 55473:2021-07.

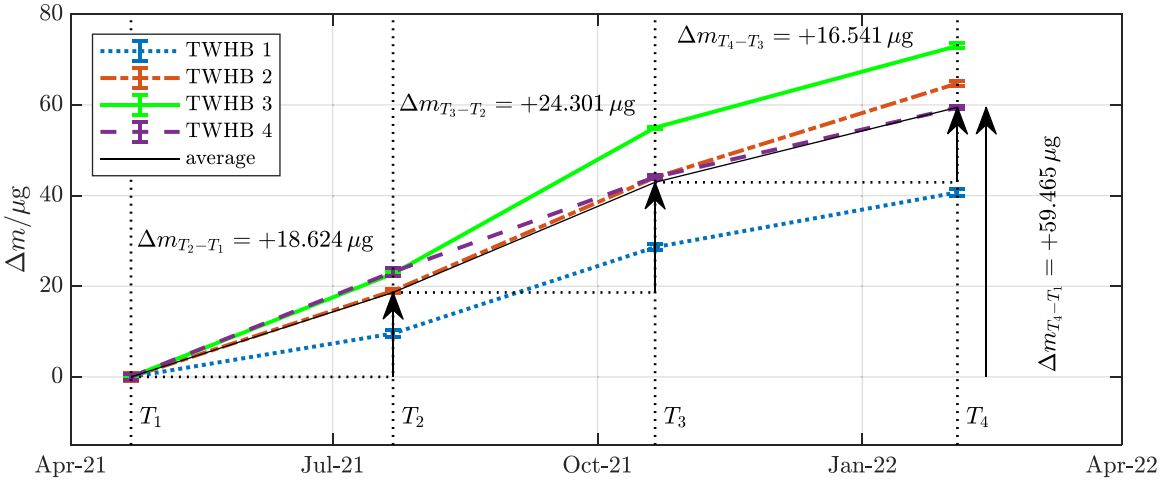

Figure 5: Temporal relative mass changes of the non-vital (nv) artifacts, nvTWHB 1, nvTWHB 2 and nvTWHB 4, and of the vital (v) artifact, vTWHB 3, with standard deviation, σ, at the four measurement time points, T_1, T_2, T_3, and T_4 in all three identical experiments under high vacuum conditions determined for the very last 6 measuring points.

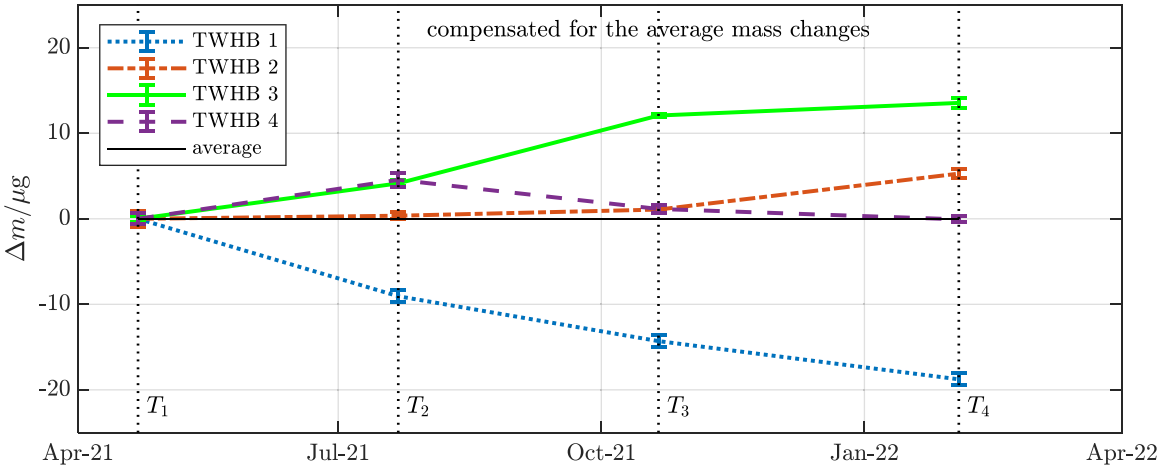

Figure 6: Temporal mass changes, compensated for the average, of the non-vital (nv) artifacts, nvTWHB 1, nvTWHB 2 and nvTWHB 4, and of the vital (*v*) artifact, vTWHB 3, with standard deviation, σ, at the four measurement time points, T_1, T_2, T_3, and T_4 in all three identical experiments under high vacuum conditions determined for the very last 6 measuring points.

- The humidity and temperature data logger No. 2 (Mostra-Log®), equipped with two AAA batteries.
- 4 flexible wires, coated with polyvinyl chloride (PVC).
- 4 Hirschmann® miniature clamps (KLEPS 064), made of polyphenylene ether (PPE), to connect the single-pole current feedthrough of each TWHB with 12 VDC.
- A Viton® seal of the glass dome, made of fluorocarbon rubber (FKM/FPM).

Sorption processes can also occur during transfer in (laboratory-) air. During one transfer for loading the type-1 vessel, each TWHB had a contact time with air of 25 min. During the transfers for loading the comparator, the air contact time per TWHB was 45 min, as positioning on the turntable was time consuming. From the first measurement time point, T_1, to the last one, T_4, the air contact times of all performed transfers added up to about 3.5 h for each TWHB. To shorten the transfer time in air, it is planned in future to fill the comparator chamber and the storage container with argon of quality 6.0 as a protective gas, as no transfer system is available.

Davidson compared sorption processes on the two 1 kg platinum-iridium national prototypes, artifact No. 81 and No. 82, gravimetrically [12]. No. 82 was kept in vacuum alone. No. 81 was transferred between air and vacuum. Both prototypes increased their masses over 9 months. However, the transferred No. 81 kg increased significantly its amount of sorbate by about 33% compared to the vacuum storage kilogram No. 82. The results are only partially transferable to the TWHBs because these were stored at about 30 °C. In addition, some materials in the storage vessel of the TWHBs are suspected of having released sorbates during storage. The earlier listing contains all suspected materials. Davidson [12] also investigated platinum-iridium surface samples that were stored alongside No. 81 and No. 82. Using X-ray photoelectron spectroscopy (XPS), he found a typical stratification of the top layer of sorbed molecules in both modalities.

The depth of the contamination overlayer on the transferred samples increased most, and there was good correlation between the determination of overlayer accretion by surface analysis and that measured by weighing. The typical stratification of the overlayers of the both samples was differentiated into three fractions: Oxides (O) directly above the metal substrate with C=O and C−OH carbonaceous contamination above this and hydrocarbon C−H as the uppermost layer. The author concluded that the regular transfer of mass standards between the two media significantly influences their mass stability, mainly due to the increase in hydrocarbon contamination, absorbed from the surrounding air.

As sorption studies on titanium grade 2 are currently lacking, long-term mass stability cannot be predicted with certainty. Options for optimizing storage and transfer conditions that can be used have already been mentioned in the text. Following the GUM method at least allows an accuracy assessment of the experimental methodology validated here for high-resolution measurements of mass dynamics of biological processes and structures according to a defined method. This reduces misinterpretations and creates transparency of the optimization processes. The GUM model equation of the actual mass increase of the vital sample, m_{vP}, and the measured mass difference of the vital sample, Δm_{vP3}, as well as the measured mean mass change of the three non-vital samples, $\Delta m_{\mathrm{nvP1,2,4}}$, is $m_{\mathrm{vP}} = \Delta m_{\mathrm{vP3}} - \Delta m_{\mathrm{nvP1,2,4}}$. GUM first calculates the standard uncertainties of the two individual observations, Δm_{vP3} and $\Delta m_{\mathrm{nvP1,2,4}}$. Since only one vital sample was measured, its standard deviation, σ, and the standard deviation of the three non-vital samples were set equal.

The appendix of the article contains the following calculated measurement uncertainty budgets for the three input variables. For the actual mass increase of the vital sample, m_{vP3}, of +18 µg, GUM calculated an expanded measurement uncertainty of 30 µg. This estimate expresses the range in which the true measured value lies with a probability of 95%. The expanded measurement uncertainty was based on the values of the input variables and the calculation of the measurement uncertainty budgets of the variables $\overline{\Delta m_{\mathrm{vP3}}}$, $\overline{\Delta m_{\mathrm{nvP1,2,4}}}$ and $\overline{m_{\mathrm{vP3}}}$. Formulated as a probability statement, the result of the mass increase of the vital sample in the experimental period over about 9 months is as follows: The resulting mass increase would have had to be greater than 48 µg for the extension factor $k = 2.87$ and the coverage probability $p = 95\%$ to be considered statistically proven with a certainty of 97.7%.

The appendix contains the three GUM protocols for the three observation periods studied: 1st experiment alone; 1st and 2nd experiment; 1st, 2nd and 3rd experiment combined. The associated probability statements are also included there.

5 Conclusions

We validated a novel methodology for high-resolution measurements of the mass dynamics of biological processes and structures, that makes it possible for the first time to measure mass dynamics in a closed plant-soil model ecosystem *in vivo* repetitively over very long periods of time, when statistically reliable statements with measurement uncertainties in the microgram range are required. To achieve this, a novel weighing hollow body was designed, constructed and characterized here which allowed the use of a very precise vacuum mass comparator. Except for the material titanium grade 2, from which the new TWHBs were constructed, all specifications followed the recommendations of OIML R 111-1 for a weighing artifact of the highest quality class. Some features of titanium grade 2 even surpass the properties of stainless steel.

A critical factor was the lack of prior experience with cleaning and washing of the new building material used to construct the TWHBs. The four new TWHBs tested turned out to be just as high-vacuum compatible in the high vacuum of the mass comparator as the two 1 kg E_1 stainless steel cylinder weights used as standards. The different geometries of the artifacts also had no influence on the result of the mass measurements.

The biological model, illuminated by LEDs integrated in the TWHB, remained photosynthetically active and showed growth throughout the entire duration of the experiment. In about 9 months, the total mass increase of each TWHB was +59.47 µg on average. In the period of three months, a mean mass increase of +19.82 µg was found in each of the three independent experiments. Much longer lasting experiments

seem possible as well, which could allow to measure very small mass dynamics cumulatively. The mass increase of the vital compared to the three non-vital TWHBs was 18 µg with the expanded measurement uncertainty 30 µg in the total experimental period. The resulting mass gain wolle have had to be greater than 48 µg for a coverage factor, k, with $k = 2.87$ and a coverage, P, with $P = 95\%$ in order to prove it with the experiment with 97.7% certainty.

In retrospect, the introduction of various plastic materials into the type-1 storage vessel was questionable, because the observed mass dynamics of all four TWHBs were almost certainly affected by these materials. The mass increase of weighing artifacts due to adsorbates also depends on the number and duration of air-vacuum transfers performed, as Davidson showed [12]. Storage exclusively in air or in a vacuum leads to mass stability or at least smaller changes [12]. Therefore, by increasing the measuring intervals or reducing the number of transfers between air and vacuum, a long-term improvement in the mass stability of the weighing artifacts can be achieved in all likelihood. A shortening of the contact time with air during transfers should also have a positive effect. Based on the molecular sizes of the adsorbates on weighing artifacts measured by Davidson in the typical stratifications [12], we modelled their kinetic diameters as impact- or motion-relevant molecular dimensions to select a suitable molecular sieve. In the future, the molecular sieve with a pore size of 0.4 nm used so far will be replaced by a molecular sieve with a pore size of 1.0 nm. The reader will find an outlook on improvements to the design in the main experiment in Table 6 in the Appendix of this paper. These modifications should increase the evidence of a mass increase of the model ecosystem. Since comparative sorption studies on titanium grade 2 are lacking, the measured mass drift with sorbates of 0.12 $\mu g \cdot cm^{-2}$ on average, which formed on the TWHBs over about 9 months, cannot be reliably assessed at present. Therefore, specific sorption studies of the titanium material are desirable. The applied GUM methodology will also permit an accuracy assessment of individual improvement measures carried out in the future. The validated methodology can thus be considered a promising measurement procedure for the verification of the law of conservation of mass in life sciences, when statistically well-founded conclusions with measurement uncertainties in the microgram range are required. Since the issue of biological mass conservation is fundamental, further research is needed.

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References

[1] I. Freund, *The Study of Chemical Composition: An Account of its Method and Historical Development, with Illustrative Quotations*, Cambridge, University Press, 1904.

[2] H. H. Landolt, "Über die Erhaltung der Masse bei chemischen Umsetzungen," in *Königlich Preußische Akademie der Wissenschaften*, Berlin, Physik.-Math. Classe, 1910.

[3] K. Lehrmann and F. Härtig, "Substitutionsmessung von Massenormalen unter Verwendung neuartiger Transferkörper," *TM - Tech. Mess.*, vol. 88, no. s1, pp. s14—s16, 2021.

[4] M. Gläser, "Response of apparent mass to thermal gradients," *Metrologia*, vol. 27, pp. 95—100, 1990.

[5] M. Gläser, "Effect of free convection on the apparent mass of 1 kg standards," *Metrologia*, vol. 30, pp. 67—73, 1993.

[6] M. Gläser, "Change of the apparent mass of weights arising from the temperature differences," *Metrologia*, vol. 36, pp. 183—197, 1999.

[7] T. Lintzen, *A Method to Determine Changes in the Amount of Mass in a Closed Model Ecosystem to High Accuracy — A Feasability Study*, Research Gate, 2019. Available at: https://www.researchgate.net/publication/330497710.

[8] F. Härtig, D. Knopf, and K. Lehrmann, "Das Kilogramm im neuen SI," *TM — Tech. Mess.*, vol. 87, no. 4, pp. 237—247, 2020.

[9] OIML. International recommendation OIML R 111-1 2004 weights of classes E1, E2, F1, F2, M1, M1-2, M2, M2-3 and M3; part 1: metrological and technical requirements.

[10] M. Borys, T. Fehling, T. Fröhlich, D. Heydenreich, Mecke, and R. Schwarz, "Design and performance of the new Sartorius 1 kg vacuum mass comparator at PTB," in *XVIII Imeko World Congress*, Rio de Janeiro, Brazil, 2006.

[11] M. Kochsiek, "Measurement of water adsorption layers on metal surfaces," *Metrologia*, vol. 18, pp. 153—159, 1982.
[12] S. Davidson, "Characterization of the long-term stability of mass standards stored in vacuum by weighing and surface analysis," *Metrologia*, vol. 49, pp. 200—208, 2012.
[13] M. Pabst and T. Fröhlich, "Novel method for determining the center of gravity height of mass standards," in *Sensoren und Messsysteme*, 2022, pp. 204—207.
[14] M. Gläser, R. Schwartz, and M. Mecke, "Experimental determination of air density using a 1 kg mass comparator in vacuum," *Metrologia*, vol. 28, pp. 45—50, 1991.
[15] M. Mindel and S. Pollack, "The room temperature oxidation of titanium thin films," *Acta Metall.*, vol. 17, no. 12, pp. 1441—1448, 1969.
[16] A. Szokefalvi-Nagy and E. Fromm, "Experimental evidence of the enhancement of the low-temperature oxidation rate of titanium films by the Mott potential," *Philos. Mag. Lett.*, vol. 79, no. 5, pp. 289—295, 1999.
[17] G. Girard, *The procedure for cleaning and washing platinum-iridium kilogram prototypes used at the Bureau International des Poids et Mesures, Sèvres*, BIPM Monographie, Sévres 142, BIPM, 1990.

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