UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Processing

Earthworm
(*Lumbricus terrestris, Aporrectodea longa*)

Diana Teubner, Martin Paulus, Kathrin Tarricone, Roland Klein

Trier University, FB VI – Biogeography
Universitätsring 15, D-54286 Trier

Umwelt Bundesamt

Contents

1 German Environmental Specimen Bank 2

2 Objective of this Guideline 2

3 Function of the Specimen Type 2

4 Target Compartments 3

5 Predefinitions for the Sampling 3

5.1 Species Determination 3
5.2 Selection and Definition of Sampling Sites 3
5.3 Selection of Individuals and Sample Size 4
5.4 Sampling Period and Frequency 4
5.5 Area-Related Sampling Scheme 4

6 Sampling Procedure 5

6.1 Technical Preparation 5
6.2 Required Equipment and Cleaning Procedures 5
6.3 Sampling Technique 6

7 Biometric Sample Characterization 8

8 References 8

Appendices: Checklist to Prepare and Conduct the Sampling

Safety Guideline for the Capture of Earthworms with Electricity for the Environmental Specimen Bank

Specimen Data Sheets

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

Status: November 2018, V 2.0.1

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples and stores and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program. (Umweltbundesamt 2008, 2014)

The long-term storage is carried out under conditions which, as much as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigations of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based, standardized methods to avoid contamination and inhibit loss of chemical information. The need for an exceptionally high level of quality assurance results from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of the Quack *et al*. (2003) version.

Transport, further sample treatment and storage as well as chemical analysis have to be carried out according to the current guidelines of the ESB.

3 Function of the Specimen Type

As soil-dwelling organisms, deep-burrowing (anecic) earthworms, are involved in the complex processes of soil development in very different ways and, as saprophytes, play a central role in the conversion of organic matter and its associated energy (destruction). Of its numerous functions, only a few of the most important ones are listed here:

- They mechanically break down the annual litter and hydrolytically split the macromolecules.
- They work the crushed above-ground plant residue into the deeper soil layers and thereby increase the soil fertility.
- Through the forming of burrows and through their castings on the soil surface, they loosen the soil structure and improve the humus form, which increases the rootability of the soil.
- By forming a burrow system, they increase the water infiltration rate and thus reduce soil erosion.
- The organic-mineral compounds (clay-humus complexes) formed in the intestinal tract of the earthworms increase the stability of the soil.

The intensive participation in the nutrient cycles exposes them to all substances found in the ecosystem. This results in a great hazard potential for the food chains based thereon if toxic substances are present. Therefore, earthworms are often studied as accumulation indicators in the field and laboratory and are used in monitoring programs (Commission on Air Pollution Prevention VDI and DIN 2008, Calisi *et al*. 2013, Leveque *et al*. 2013, Rieder *et al*. 2013, Alvarez *et al*. 2014, Andrade *et al*. 2014).

For the purposes of the ESB, the deep-burrowing species are especially suitable because they feed particularly on dead organic substances and mix these with mineral soil by forming their burrows. Thus, they establish a relationship between the organic soil cover and the underlying soil horizons and demonstrate the pollution of the entire environmental medium soil. The two species *Lumbricus terrestris* and *Aporrectodea longa* were selected as suitable sample species for the ESB for the following reasons:

- They are the most widespread deep-burrowing species in Germany.
- They are the only representatives of destructors in terrestrial ecosystems that are available in sufficient biomass according to their body weight and abundance.
- As important soil formers, they occupy a central position in the energy flow and metabolism of ecosystems.
- As a food source for many predators, they occupy an exposed position in the terrestrial food web.
- They have a high location loyalty.

For the ESB, the two earthworm species represent the trophic level of the destructors in terrestrial ecosystems.

4 Target Compartments

Various studies show that metals and organic pollutants accumulate differently both in the individual organs of the worm body and within the organs themselves (Morgan and Morgan 1990, Bengtsson and Rundgren 1992, Sforzini *et al*. 2015). In addition, the dissection of certain worm parts is difficult to standardize because it is associated with the loss of blood and coelomic fluid and there is a risk of contamination. Thus, samples of the entire body are preferable.

The amount of food ingested by earthworms depends, among other things, on its quality (Hendriksen 1991) and the soil moisture and temperature (Curry and Schmidt 2007). In addition, the quantity of intestinal content correlates positively with the weight of the worm (Curry and Bolger 1984, Taylor and Taylor 2014). Thus, the quantity and qualitative composition of intestinal content is subject to individual, temporal and spatial variations, which result in a lack of sample comparability. Therefore, it is important to separate the intestinal content from the worm body.

As a biologically standardized soil extract, the excrement can be analyzed for substances that are not detectable or are hard to detect in the worm itself. These include, for example, polycyclic aromatic hydrocarbons (Paulus *et al*. 1994, Klein and Paulus 1995).

In the ESB, therefore, the entire body without intestinal content and earthworm excrement are obtained as separate target compartments.

5 Predefinitions for the Sampling

5.1 Species Determination

Determining the two species *Lumbricus terrestris and Aporrectodea longa* in the field with certainty requires practical experience. The macroscopic features of both target species are shown in Tab. 1.

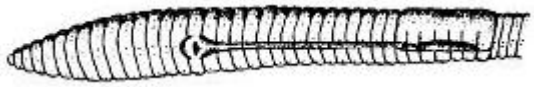
5.2 Selection and Definition of Sampling Sites

To determine the sampling sites and sample species, a screening must be performed before the first sampling. The aim is to investigate the availability of the two sample species, the dispersion range of pollutant contents as well as the spatial pattern of the pollutant burden.

For a screening, proceed as follows:

- All sub-sites that are potentially suitable for the two earthworm species with a sufficient minimum size and long-term use consistency are mapped.
- If either or both species are available on the mapped sub-sites, the sub-sites become screening sites, in which at least one of the two species can be obtained as a sample in sufficient quantity.
- The earthworms without intestinal contents and the earthworm excrement are homogenized and analyzed separately according to specimen types and screening sites.

Tab. 1: Important macroscopic features of the two target species

Lumbricus terrestri		*Aporrectodea longa*
		
dark brown-purple, under side lighter than upper side	**color**	smoky gray, strongly iridescent, underside ± same
90 – 300 mm	**length**	120 – 160 mm
6 – 9 mm	**diameter**	6 – 8 mm
large, the very prominent reproductive ducts extend into the 14th and 16th segment	**male pores**	large, restricted to the 15th segment
31st or 32nd - 37th segment, reddish colored	**clitellum**	28th - 35th segment, chocolate brown
33rd - 36th segment	**tubercula pubertatis**	32nd - 34th segment
bristles of the 25th, 26th and 27th segments on glandular papillae and converted to furrow bristles	**ventral bristles**	bristles of the 9th, 10th and 11th segments on glandular papillae

For determining the specimen type, availability is the most important decision criterion. *L. terrestris* is preferable to *A. longa* as a specimen type because it is more abundant in most ESB sampling areas. In addition, *L. terrestris* has been better studied for its accumulation behavior (Slizovskiy and Kelsey 2010, Lapied *et al*. 2011, Slizovskiy *et al*. 2011) than *A. longa* (Johnson *et al*. 2002, Tischer 2009, Qui *et al*. 2014).

The sampling site is determined by the selection of suitable sampling points. It is the sum of the selected sampling points. Appropriate sampling points are screening sites where the availability of the desired specimen type and the representativeness of the spatial pollution are present.

5.3 Selection of Individuals and Sample Size

For the ESB, the (age) group of sexually mature individuals is sampled. This can be recognized by the formation of the clitellum and can be determined in the field with certainty by means of external features that are easily identifiable with the naked eye (Tab. 1).

Several narrow catch strips can be selected per sampling point, for example strips approx. 5 m wide and 30 m long (Fig. 1). The earthworm mass to be collected per sampling point is determined in the area-related sampling scheme.

In order to obtain the ESB required sample amount of 1,100 g of earthworm body without intestinal content per sampling site, approximately 1,400 g of earthworms must be collected.

5.4 Sampling Period and Frequency

The earthworm sampling is carried out from October to mid-December. During this period there is the greatest likelihood of having a high proportion of sexually mature individuals. In addition, the worms are in the ecologically-physiologically stable (activity) period.

After summers with little rain and, consequently, dry soils, the earthworms are still in their resting state in early autumn. Then they cannot be driven to the ground surface using electricity. In this case, sampling can only be carried out after rainfall.

5.5 Area-Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area-related sampling scheme. These include, but are not limited to:

- location and demarcation of the sampling sites,
- required sample size,
- sampling period,
- appropriate authorities,

- site property owners.

Here it is important to consider how to ensure long-term sampling continuity. If changes are made, the document must be updated.

6 Sampling Procedure

All data collected during sampling and biometric sample characterization must be documented in the corresponding specimen data sheets (see appendix). In addition, a protocol must be prepared for each sampling with the following information:

- persons that participated in the sampling,
- chronological sequence of the sampling,
- the underlying version of the sampling guideline and the area-related sampling scheme for the current sampling as well as,
- deviations from the sampling guideline and the area-related sampling scheme.

6.1 Technical Preparation

The earthworms are captured using alternating current. This requires special safety precautions set out in the "Safety Guideline for the capture of earthworms with electricity for the Environmental Specimen Bank" (see Appendix). The sampling leader is required to inform each person involved in the sampling about:

- the dangers of capturing earthworms using electricity,
- how to handle the electric earthworm trap to be used and
- the safety precautions set out in the "Safety guideline for the capture of earthworms with electricity for the Environmental Specimen Bank."

6.2 Required Equipment and Cleaning Procedures

Field Work

- generator (unit) (230 V) with 4-stroke gasoline engine,
- ammeter, emergency stop switch and protection fuse,
- electrodes with connecting cable,
- extension cable (at least 50 m),
- connecting cable from electrode rows to the ammeter (app. 2 x 50 m),
- portable voltmeter incl. two electrode stakes for measuring the step voltage,
- barrier tape with rods and warning signs for public areas,
- portable pasture fence for occupied pastures,
- electric lawnmower, scythe or electric lawn trimmer including rake,
- safety boots and safety gloves with insulating properties tested up to 1,000 volts,
- insulated stainless steel tweezers,
- conditioning equipment including borosilicate petri dishes with lids (Fig. 2),
- collection vessels made of borosilicate or stainless steel,
- specimen data sheets,
- scale (weighing range at least 4 kg, reading 1 g),
- absorbent laboratory paper and water-resistant marker,
- powder-free disposal gloves,
- air and soil thermometer.

Laboratory:

- clean bench with particle and activated carbon filtration,
- specimen data sheets,
- stainless steel pincers,
- powder-free disposal gloves,
- cooling device for the process of intestinal evacuation (8°– 12°C),
- chest freezer (at least -20°C) to kill the worms,
- cooling device for immediate deep freezing and transport of the samples in the gas phase above liquid nitrogen (LIN),
- liquid nitrogen,
- protective clothing for handling liquid nitrogen,
- stainless steel containers (1.5 l and 5.5 l) with lids and fasteners.

Sample containers and all equipment are cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90 – 95°C) rinsing, neutralization using 30% phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure, the containers are dried in a cabinet dryer at 130°C (± 10°C) for a minimum of an hour

(sterilization). Subsequently, the vessels cool in a closed drying oven. Sterilization is not applied to synthetic materials.

6.3 Sampling Technique

According to the diagram in Fig. 1, the generator, the control box with ammeter and emergency stop switch as well as the electrode rows are arranged on the catch strip. The generator is placed in such a way that the catch strips and the samples are not contaminated by exhaust gases. The wind direction must therefore be checked regularly to see if the placement of the device needs to be changed. The attached safety guideline is to be observed.

The two electrode rows are inserted in a zigzag pattern, which increases the catch area per row compared to arranging the electrodes in a straight line. The spacing of the rows depends on the site conditions.

After switching on the generator, the step voltage is measured and the current is read on the built-in ammeter. The measurement of the step voltage by means of a voltmeter is carried out on two electrode stakes which are inserted directly into the ground at the electrode rows at a distance of one step. A suitable voltage cannot be precisely specified because, depending on the conductivity of the soil, earthworms can also be captured easily at low voltages.

Both electrode rows are moved by repositioning the electrodes either parallel in one direction or towards each other across the surface.

As the sampling is conducted with alternating current, the earthworms appear on both electrode rows close to 30 – 50 cm of the soil surface. The collection always takes place on both electrode rows (Fig. 1).

During transfer there is a risk of the sampler tapping voltage on the bare areas of the electrodes. Therefore, only one electrode should ever be touched and transferred at a time (see safety guideline). The frequency and rate of transfer depend on the length of time that it takes the earthworms to appear on the surface of the soil.

The individuals of the target species appearing at the electrodes who have formed a clitellum are collected from the ground with insulated stainless steel tweezers. The worms may only be grasped with the tweezers after they have completely left their burrows, otherwise they may be injured. Injured individuals are not collected. The worms are cleaned of adhering soil particles or grass residue by removing the particles with a tweezers and they are then collected in a sufficiently high vessel made of borosilicate or stainless steel. Approximately 10 individuals are transferred to previously labeled Petri dishes (approximately 9 cm in diameter) and weighed. This number ensures that the worms do not dry out during the five-day storage in the dishes.

The number of earthworms per dish and their weight are written down in the data sheets. The Petri dish is placed in the conditioning equipment for intestinal evacuation (Fig. 2). When this is filled with all Petri dishes, it is closed so that the worms cannot press up and open the lids of the dishes.

All captured individuals remain in the conditioning equipment for 5 days for intestinal evacuation in the (dark) refrigerator at 8 – 12°C. Experiments at different temperatures have shown that this temperature range provides the best results in terms of intestinal evacuation without there being a risk of dehydration of the worms (at sufficiently high numbers of individuals per Petri dish), and without the pollutants contained in the worm bodies being remobilized (Klein and Paulus 1995).

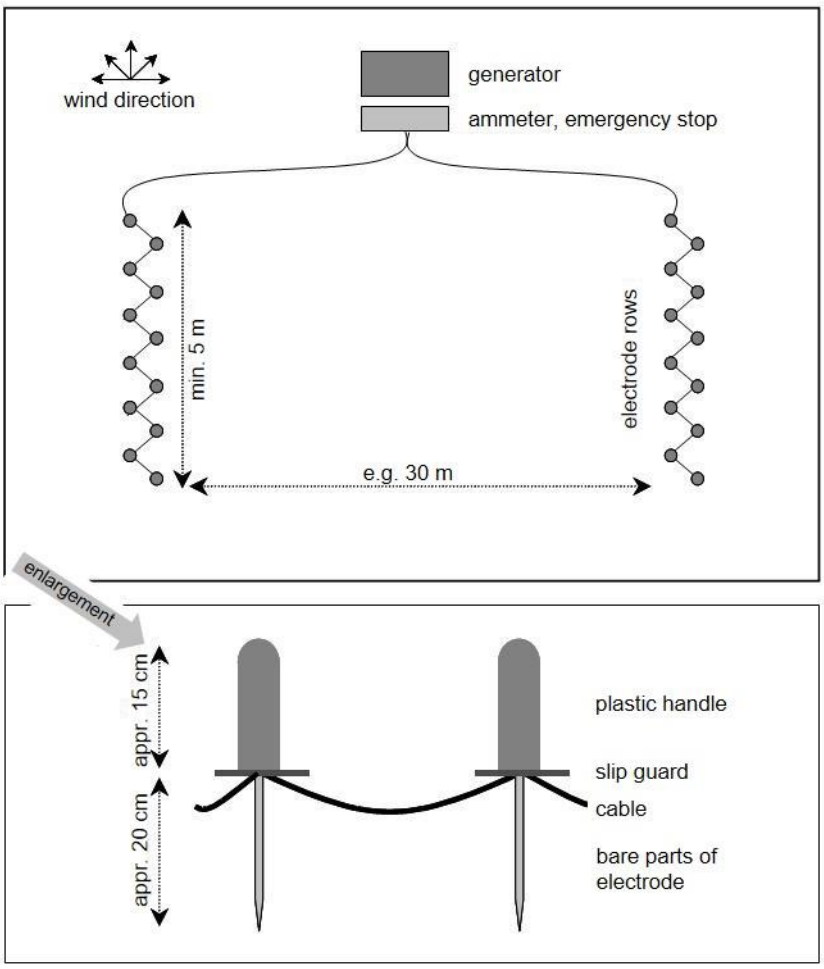


Fig. 1: Example of capture equipment

After the first conditioning day, the excrement is collected under clean air conditions with stainless steel tweezers from each Petri dish and put into a stainless steel container. The excrement from all petri dishes is weighed and stored over liquid nitrogen. Damaged or dead earthworms are removed and their number recorded.

After five days of conditioning, the collection and weighing of the excrement is repeated. Damaged or dead earthworms are removed and their number recorded. After the excrement has been collected, the earthworms are transferred, in the conditioning equipment for intestinal evacuation, to a freezer at min. -20°C. As a result, they release more excrement and are killed at the same time. After about 1 to 2 days, the earthworms are frozen through. The duration depends on, among other things, the total biomass, the number of petri dishes and conditioning equipment in the freezer.

The frozen earthworms put into storage as quickly as possible via liquid nitrogen. To do this, they are taken out of the Petri dishes in the deep-frozen state under clean air conditions, separated from the excrement with stainless steel tweezers and transferred to stainless steel containers pre-cooled with liquid nitrogen.

Subsequently, the total weight of the earthworms without intestinal content is determined and the containers are brought into the gas phase over liquid nitrogen. The excrement excreted in the freezer is discarded because it thaws very quickly. All data collected is written down in the corresponding specimen data sheets.

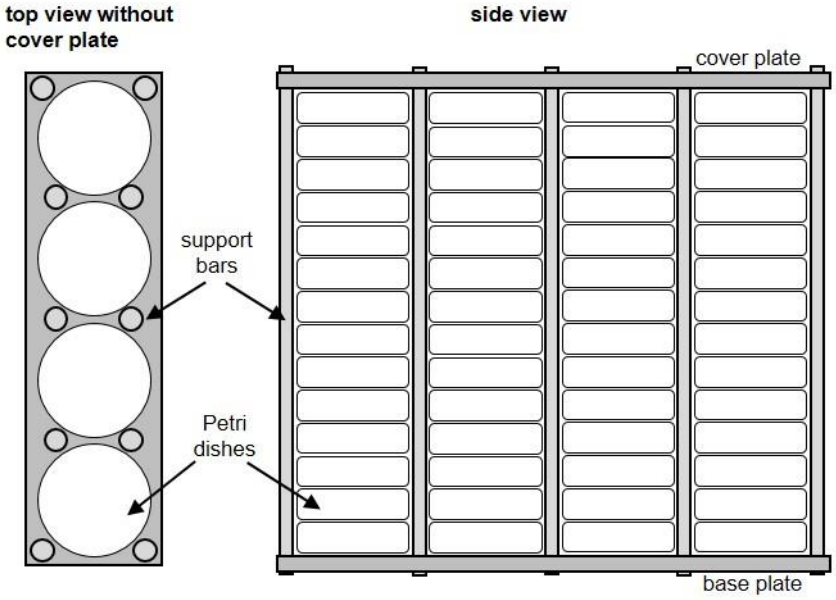


Fig. 2: Conditioning equipment for intestinal evacuation

7 Biometric Sample Characterization

For the sample characterization of the collected earthworms, the following parameters are used during sample preparation (see Chap. 6.3):

- total weight of worms without intestinal content (reading 1 g),
- total weight of the excrement (reading 1 g),
- number of individuals in the sample without intestinal contents.

From this it is possible to calculate the average worm weight per individual and the average amount of excrement delivered per individual.

8 References

Alvarez C.R., Moreno M.J., Bernardo F.J.G., Martin-Doimeadios R.C.R. and Nevado B.J.J. (2014): Mercury methylation, uptake and bioaccumulation by the earthworm *Lumbricus terrestris* (Oligochaeta). *Applied Soil Ecology*, 84, 45-53

Andrade N.A., Centofanti T., McConnell L.L., Hapeman C.J., Torrents A., Nguyen A., Beyer W.N., Chaney R.L., Novak J.M., Anderson M.O. and Cantrell K.B. (2014): Utilizing thin-film solid-phase extraction to assess the effect of organic carbon amendments on the bioavailability of DDT and dieldrin to earthworms. *Environmental Pollution*, 185, 307-313

Bengtsson G. and Rundgren S. (1992): Seasonal variation of lead uptake in the earthworm *Lumbricus terrestris* and the influence of soil liming and acidification. *Archives of Environmental Contamination and Toxicology*, 23, 198-205

Calisi A., Zaccarelli N., Lionetto M.G. and Schettino T. (2013): Integrated biomarker analysis in the earthworm *Lumbricus terrestris*: Application to the monitoring of soil heavy metal pollution. *Chemosphere*, 90(11), 2637-2644

Curry J.P. and Bolger T. (1984): Growth, reproduction and litter and soil consumption by *Lumbricus terrestris* L. in reclaimed peat. *Soil Biology & Biochemistry*, 16(3), 253-257

Curry J.P. and Schmidt O. (2007): The feeding ecology of earthworms – A review. *Pedobiologia*, 50, 463-477

Hendriksen N.B. (1991): Gut load and food-retention time in the earthworms *Lumbricus festivus* and *L. castaneus*: A field study. *Biology and Fertility of Soils*, 11, 170-173

Johnson D.L., Jones K.C., Langdon C.J., Piearce T.G. and Semple K.T. (2002): Temporal changes in earthworm availability and extractability of polycyclic aromatic hydrocarbons in soil. *Soil Biology & Biochemistry*, 34, 1363-1370

Klein R. and Paulus M. (1995): Terrestrische Probenarten - Regenwürmer. In: Klein R. and Paulus M. (Ed.): *Umweltproben für die Schadstoffanalytik im Biomonitoring - Standards zur Qualitätssicherung bis zum Laboreingang*, p. 183-202. Jena, Gustav Fischer Verlag

Kommission Reinhaltung der Luft im VDI und DIN (2008): Biologische Verfahren zur Erfassung der Wirkung von Luftverunreinigungen (Bioindikation) – Passives Biomonitoring mit Regenwürmern als Akkumulationsindikatoren. In: Verein Deutscher Ingenieure (Ed.): *VDI/DIN-Handbuch Reinhaltung der Luft, Band 1a: Maximale Immissions-Werte*, VDI 4230, Blatt 2, 26 pp, Düsseldorf

Lapied E., Nahmani J.Y., Moudilou E., Chaurand P., Labille J., Rose J., Exbrayat J.-M., Oughton D.H. and Joner E.J. (2011): Ecotoxicological effects of an aged TiO(2) nanocomposite measured as apoptosis in the anecic earthworm *Lumbricus terrestris* after exposure through water, food and soil. *Environment International*, 37(6), 1105-1110

Leveque T., Capowiez Y., Schreck E., Mazzia C., Auffan M., Foucault Y., Austruy A. and Dumat C. (2013): Assessing ecotoxicity and uptake of metals and metalloids in relation to two different earthworm species (*Eiseina hortensis* and *Lumbricus terrestris*). *Environmental Pollution*, 179, 232-241

Morgan J.E. and Morgan A.J. (1990): The distribution of cadmium, copper, lead, zinc and calcium in the tissues of the earthworm *Lumbricus rubellus* samples from one uncontaminated and four polluted soils. *Oecologia*, 84, 559-566

Paulus M., Altmeyer M., Klein R., Hildebrand A., Ostapczuk P. and Oxynos K. (1994): Biomonitoring und Umweltprobenbank - II. Aufbau flächenrepräsentativer Probenahmen von Umweltproben zur Schadstoffanalytik am Beispiel der Regenwürmer in landwirtschaftlich genutzten Räumen. *Umweltwissenschaften und Schadstoff-Forschung*, 6(6), 375-383

Quack M., Bartel M., Klein R., Neitzke M., Nentwich K., Paulus M. and Wagner G. (2003): Guideline for Sampling and Sample Treatment Earthworm (*Lumbricus terrestris*, *Aporrectodea longa*), www.umweltprobenbank.de

Qiu H., Peijnenburg W.J.G.M., van Gestel C.A.M. and Vijver M.G. (2014): Can commonly measurable traits explain differences in metal accumulation and toxicity in earthworm species? *Ecotoxicology*, 23(1), 21-32

Rieder S.R., Brunner I., Daniel O., Liu B. and Frey B. (2013): Methylation of mercury in earthworms and the effect of mercury on the associated bacterial communities. *Plos One*, 8(4), e1215

Sforzini S., Moore M.N., Boeri M., Benfenati E., Colombo A. and Viarengo A. (2014): Immunofluorescence detection and localization of B[a]P and TCDD in earthworm tissues. *Chemosphere*, 107, 282-289

Slizovskiy I.B. and Kelsey J.W. (2010): Soil sterilization affects aging-related sequestration and bioavailability of p,p '-DDE and anthracene to earthworms. *Environmental Pollution*, 158(10), 3285-3289

Slizovskiy I.B., Kelsey J.W. and Hatzinger P.B. (2011): Surfactant-facilitated remediation of metal-contaminated soil efficacy and toxicological consequences to earthworms. *Environmental Toxicology and Chemistry*, 30(1), 112-123

Taylor A.R. and Taylor A.F.S. (2014): Assessing daily egestion rates in earthworms: using fungal spores as a natural soil marker to estimate gut transit time. *Biology and Fertility of Soils*, 50(1), 179-183

Tischer S. (2009): Earthworms (Lumbricidae) as bioindicators: the relationship between in-soil and in-tissue heavy metal content. *Polish Journal of Ecology*, 57(3), 513-523

Umweltbundesamt (Ed.) (2008): Concept of the German Environmental Specimen Bank (As of: October 2008); www.umweltprobenbank.de

Umweltbundesamt (Ed.) (2014): Concept of the German Environmental Specimen Bank (As of: October 2014); www.umweltprobenbank.de

Checklist to Prepare and Conduct the Sampling

Specimen Type	**Earthworm (*Lumbricus terrestris* / *Aporrectodea longa*)**
Target compartment	earthworms without intestinal content, earthworm excrement
Individual specimens	adult earthworms
Sample number	at least 50 individuals per sampling point
Sample quantity for the ESB	for a sample quantity of 1,100 g it is recommended to remove approx. 1,400 g per sampling site
Sampling period	October through mid-December
Sampling frequency	one sampling annually
Required equipment for field work	• specimen data sheets • capture equipment and accessories • safety boots and gloves • insulated stainless steel tweezers for collecting the worms • collection vessel made of borosilicate or stainless steel • waterproof marker for labeling Petri dishes and stainless steel containers • scale (reading 1 g) and test weights • conditioning equipment for intestinal evacuation incl. Petri dishes • air thermometer, soil thermometer • transport vehicle including clean bench with activated carbon and particle filtration
Sampling packing until sample processing	• stainless steel containers (1.5 l and 5.5 l) • storage of the earthworms until complete intestinal evacuation in the Petri dishes of the conditioning equipment
Transport and interim storage	• cooling device for temporary storage of earthworms in the conditioning equipment (at 8 – 12°C) • cooling device to kill the worms (at least -20°C) • cooling device (dewar) for immediate deep freezing and transport of the samples in the gas phase above liquid nitrogen (LIN)
Required equipment for laboratory work	• specimen data sheets, • stainless steel pincers • powder-free disposable gloves and lab clothing • scale (reading 1 g) • clean bench with particle and activated carbon filtration • protective clothing for handling liquid nitrogen
Biometric sample characterization	• total weight of worms without intestinal content (reading 1 g) • total weight of the excrement (reading 1 g) • number of individual worms without intestinal content

Safety guideline for the capture of earthworms with electricity for the Environmental Specimen Bank

as an appendix to the Guideline for Sampling and Sample Processing "Earthworm", Status: August 2018

Basics

The capture of earthworms using electricity is only carried out by a system certified by a qualified electrician and provided with an inspection sticker. The system must be tested once a year for safety and functionality. If the system is faultless, it will receive an inspection sticker with a validity of one year.

Only capture systems with a valid inspection sticker may be used.

The current version of ESB's Sampling Guideline for earthworms describes the exact procedure for catching earthworms with electricity.

Employee safety

Before starting the operation of the electric worm capture system, the persons involved in the sampling must be trained by the responsible sampling leader.

When operating the electric worm capture system, there must always be at least 2 persons present who are trained in first aid. The last first aid training must not be more than two years old. The responsible sampling leader and at least one assistant must be familiar with the rescue measures described below.

The electric worm capture system may only be operated by the responsible sampling leader together with at least one trained assistant. It must be ensured that potential dangers for third parties are recognized beforehand. If danger arises, the system must be switched off immediately.

When operating the electric worm capture system, unauthorized persons must be appropriately warned of dangers and, if necessary, be directed out of the danger zone.

Operating the electric worm capture system

All persons involved in sampling must use safety gloves and boots that are tested for electrical insulation up to 1,000 volts during operation of the electric worm capture system. The handles of the stainless steel tweezers for taking hold of the earthworms must be insulated with insulating tape or heat-shrink tubing.

The insulating protective clothing must be inspected by the user for obvious damage before each use. After use, the insulating protective clothing must be cleaned and dried.

Moving cables should be handled gently and protected against damage from edges, heavy loads and the like.

The connection devices – including plug-in devices – for the electrode lines must be voltage-free when making or breaking the connection. Before the electric worm capture system is put into operation, protection against direct and indirect contact with live parts must be ensured. Before each start-up, the electric worm capture system, especially its supply line, must be thoroughly inspected for external damage. Devices and system components that are dangerously damaged must not be used.

The danger zone shall be defined, blocked off and indicated by warning signs in a suitable manner. The switch for switching off the electrode supply lines of the electric worm capture system may only be switched on at the order of the responsible sampling leader. After turning on the electric worm capture

system, the step voltage near the electrode rows should be measured immediately and checked regularly. If the step voltage is more than 50 volts, the electrode rows must be moved further apart until it is less than 50 volts. If this is not successful, the system should be dismantled and, if necessary, rebuilt elsewhere.

The electrodes should only be touched by the insulated handle. Even when wearing protective clothing, touching the bare end is prohibited. A person involved in the sampling may only pick up one electrode at a time.

If dangers or irregularities occur, the electric worm capture system must be switched off immediately.

In addition, the electric worm capture system should not be operated during times of precipitation, especially rain and snowfall.

What to do in case of accidents

In general, the schema of the first aid rescue chain must also be observed here and it is essential to pay attention to self-protection when providing assistance. The following are especially important:

- To save the injured, first make sure that there is no voltage on the system. Systems and devices must be disconnected from the power supply with the emergency stop button or the fuse and the generators must be switched off. Simply switching off the device or the line does not guarantee an absence of voltage.
- For unconscious persons, priority is given to ensuring respiration and cardiovascular function after switching off the power supply. If necessary, cardiopulmonary resuscitation (CPR) is to be used immediately. For safety reasons, a defibrillator is always to be brought with for earthworm sampling. This is to be operated according to the voice instructions. An emergency call can be placed with the number 112.
- If a person has suffered an electric shock, they must go to a doctor immediately even if they seem well, in order to rule out cardiac damage. The person must be supervised until they are under medical care.

German Environmental Specimen Bank

Specimen Data Sheet 1: Sampling Point(s)

Earthworm (*Lumbriucs terrestris* / *Aporrectodea longa*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

	Specimen Type
	Specimen Condition
	Collection Date (MM/YY)
	Sampling Area (SA)
	Sampling Region (SR)
	Sampling Site (SS)
	Additional information

Sampling Site (plaintext)

Sampling Point (number) _____

Sampling Point (plaintext) _____

Sampling Leader

Remarks _____

_____

_____

_____

_____

_____

Notes _____

_____

_____

_____

_____

_____

_____

_____

German Environmental Specimen Bank

Specimen Data Sheet 2: Weather Conditions

Earthworm (*Lumbriucs terrestris / Aporrectodea longa*)

Identification: __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Sampling Point: _____

Last precipitation date preceding the sampling: __ __ . __ __ . __ __

Type of precipitation: _____ (see table below)

Start of Sampling		**End of Sampling**
__ __ . __ __ . __ __	sampling date	__ __ . __ __ . __ __
__ __ : __ __	time	__ __ : __ __
__ __	air temperature at 1.5 m height (°C)	__ __
__ __	soil temperature at 10 cm depth (°C)	__ __
__ / 8	cloud covering	__ / 8
__	type of clouds (see table below)	__
__ __ __	wind direction	__ __ __
__	wind force in Beaufort (see table below)	__
__	type of precipitation (see table below)	__

Type of Clouds:

0 = unclouded
1 = cirrus
2 = stratus
3 = cumulus
4 = fog
5 = high fog
6 = stratocumulus

Cirrus

Stratus

Cumulus

Stratocumulus

Type of Precipitation:

0 = no precipitation
1 = rain
2 = drizzle
3 = snow
4 = dew
5 = rime
6 = torrential rain
7 = hail

Wind Force (according to Beaufort):

0 = calm
1 = very slight breeze
2 = slight breeze, moves leaves
3 = light breeze, moves twigs
4 = moderate breeze, moves thin branches
5 = bright breeze, move medium sized branches
6 = strong wind, moves thick branches
7 = stiff wind, shakes trees

German Environmental Specimen Bank

Specimen Data Sheet 3: Collection Results

Earthworm (*Lumbriucs terrestris / Aporrectodea longa*)

Identification: __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Sampling Point: _____

No.	Weight of earthworms __ __ g	Number of worms collected	Number of worms discarded	Number of worms stored	Remarks

German Environmental Specimen Bank

Specimen Data Sheet 4: Sampling Technical Details and Storage

Earthworm (*Lumbriucs terrestris / Aporrectodea longa*)

Identification: __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Sampling Point: _____

Capture day 1: dish no. from __ __ to __ __

	capture start	capture end = start of intestinal evacuation	end of intestinal evacuation
date			
time			

Capture day 2: dish no. from __ __ to __ __

	capture start	capture end = start of intestinal evacuation	end of intestinal evacuation
date			
time			

Sampling:

Total catch strip area in the sampling point __ __ __ m²

Number of catch strips in the sampling point __ __

Step voltage in volts from __ __ to __ __

Current strength in Amperes from __ , __ to __ , __

Collection results: ☐ ***Lumbricus terrestris*** ☐ ***Aporrectodea longa***

__ __ __ g	weight of excrement collected after 24 hrs	_____ Date / Signature
__ __ __ g	weight of excrement collected after 120 hrs	_____ Date / Signature
__ __ __ g	total weight of excrement collected	_____ Date / Signature
__ __ __	number of earthworms without intestinal content	_____ Date / Signature
__ __ __ __ g	weight of earthworms without intestinal content	_____ Date / Signature

Storage

number of stainless steel containers	weight empty [g]	
		excrement collected
		worms without intestinal content

German Environmental Specimen Bank

Sampling Protocol

Earthworm (*Lumbriucs terrestris* / *Aporrectodea longa*)

Sampling Area: _____ Identification: __ __ __ __ __

Underlying version of the sampling guideline __ __ . __ __ . __ __

Underlying version of the sampling scheme __ __ . __ __ . __ __

1. Objective of the Sampling: _____

_____

2. Actual Timeframe of the Sampling:

Start		End		Sampling Leader	Remarks
date	time	date	time		

3. Participants internal _____

_____

external _____

4. Checklist Referring to Sampling Scheme and Sampling Guideline: ☒ as prescribed

- ☐ 4.1 Sampling period
- ☐ 4.2 Sampling site and sampling point (selection/definition)
- ☐ 4.3 Selection of the individual specimens
- ☐ 4.4 Technical preparations
- ☐ 4.5 Cleaning procedures for the packages
- ☐ 4.6 Sampling technique / method of capture
- ☐ 4.7 Sampling amount
- ☐ 4.8 Data collection
- ☐ 4.9. Transport and interim storage

Number, kind and reason for deviation (clear text):

_____

_____

_____

Remarks: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature