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Review

Ecotoxicological methods to evaluate the toxicity of bio-based fertilizer application to agricultural soils – A review

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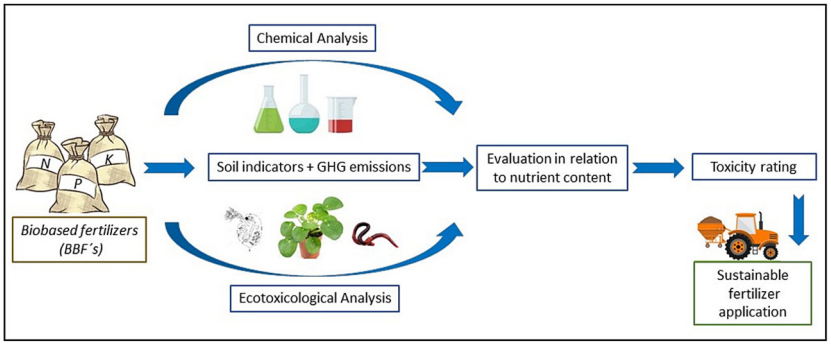
HIGHLIGHTS

- Bio-based fertilizers (BBFs) can contain a multitude of contaminants.
- Test batteries of ecotoxicological tests are suitable for the evaluation of BBFs.
- A combined approach of chemical & ecotoxicological evaluation and soil indicators is recommended.
- Ecotoxicity rating help to improve a sustainable BBF production and to implement circular economy.

GRAPHICAL ABSTRACT



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ABSTRACT

A multitude of possible contaminants can be contained in bio-based fertilizers (BBFs) because of their complex matrix. The chemical characterization of BBFs is a challenging analytical task. Therefore, it is important for sustainable agricultural production to develop standard procedures to assess new bio-based fertilizers for possible hazards related to their application in order to guarantee their safety for soils organisms, plants and the environment.

There is a huge number of ecotoxicological tests for aquatic and terrestrial organisms. They were developed for the evaluation of chemicals, pesticides and industrial wastes on aquatic systems and soil functioning. These tests can be useful for the assessment of BBFs. Ecotoxicological tests in comparison to chemical analysis have the advantage to capture the effects of all possible contaminants and metabolites available in the product. The bioavailability of toxic compounds and their interaction are recorded while the cause-and-effect-chain is not elucidated. Numerous ecotoxicological tests work with liquid media, capturing the effects of pollutants that can be mobilized. Hence, standardized procedures how to produce solvents from BBFs are mandatory. Moreover, tests using the original (solid) material are necessary in order to determine the toxicity of a given BBF in its application form and to cover the potential toxicity of non-soluble compounds. To date there are no rules how to determine the ecotoxicological potential of BBFs. A tiered approach of chemical analytical parameters in combination with a set of ecotoxicological tests and the measurement of sensitive soil indicators seem to be a promising experimental setup for the evaluation of BBFs. A decision tree for such an approach was developed. An extended ecotoxicological test strategy of BBFs is mandatory to identify the most promising raw materials and BBF processing technologies to end up with sustainable fertilizer products showing a high agronomic efficiency.

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Contents

1. Introduction

It is one of the major challenges today to meet the demand of a growing global population for food, energy crops and other agricultural products without depleting natural resources, producing greenhouse gas emissions or contaminating the environment with persistent and toxic pollutants (Nizami et al., 2017). The concept of ´Circular Economy´ is addressing the problem of resource depletion and is aiming to close nutrient cycles. The production of mineral fertilizers is energy consuming in case of nitrogen (N), or finite resources are depleted when elements such as phosphorus (P) or potassium (K) are mined and leaving environmental pollution behind especially at P-mining sites (Basak and Sarkar, 2017; Cordell et al., 2009; Chowdhury et al., 2017; Scholz et al., 2013). The European Commission has added phosphate rock to the list of critical raw materials as the supply security is at risk while the economic importance is high (ESPP, 2022). Europe has only very limited resources in Finland and is therefore depending on import. One way out of this problem is the production and use of recycled fertilizers, which are in most cases bio-based. Bio-based fertilizers (BBFs) can be defined as materials or products derived from biomaterials (plant, animal or microbial origin, often wastes, residues or side-streams from agriculture, industry or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops (Wester-Larsen et al., 2022). Such fertilizers can be produced from very different raw materials originating from animal excretion or residues, human excreta, bio-waste products, residues from landscaping, food processing or producing industries. Bio-based fertilizers are produced from organic materials which nutrients are already in the biological nutrient cycle in opposite to rock materials. Additional other compounds like mineral sources or residues from mining can be used to produce recycled fertilizers but they are not regarded as bio-based raw materials in Fig. 1. Moreover, the technologies to produce BBFs from raw materials are manifold. They range from simple procedures like drying or grinding to complex technologies as thermochemical processing and granulation where additives can enter the product. Many different BBF products were developed in the last years based on different raw materials and production procedures (Fig. 1).

Why should BBFs applied to feed plants undergo an ecotoxicological evaluation? They can contain a broad range of different contaminants due to their origin, complex matrix or processing procedures (Bloem et al., 2017; Kratz et al., 2020). Persistent organic pollutants like drugs or endocrine disruptors were detected in organic materials as well as metallic trace elements and heavy metals that can accumulate in BBFs (Díaz-Cruz et al., 2009; Gong et al., 2019; Hou et al., 2015; Iglesias et al., 2018). For example, most of the over 4000 prescription drugs used in human or animal treatment have been detected in the environment (Scudellari, 2015) and can be ingredients in BBFs based on human or animal excreta. Negative effects can also result from plant-based fertilizer products, which can show phytotoxic effects due to allelopathic interactions (Arowosegbe et al., 2012; Kadioglu et al., 2005; Li et al., 1993; Mersie and Singh, 1988). Selim et al. (2012) showed that a high ammonia-N to nitrate-N ratio in organic amendments such as immature composts can negatively affect crop germination and growth. Regular organic amendments can moreover affect soil organisms such as earthworms (Gunadi and Edwards, 2003; Murchie et al., 2015; Parente et al., 2021) underlining the importance of an ecotoxic evaluation of BBFs (Renaud et al., 2017).

Contaminants can affect the abundant, diverse microbial soil populations that facilitate important ecosystem functions such as decomposition and nutrient cycling (Singh et al., 2014) and interferences due to contaminants can disrupt nutrient cycling and alter soil fertility. Ecotoxicity tests can deliver an important contribution in the selection of suitable raw materials and processing procedures to produce sound and safe fertilizers for sustainable agricultural production.

There is no general recommendation or standard procedure to date how to evaluate BBFs with respect to their ecotoxicological potential when producing agricultural fertilizers. Such information would be valuable for a reasonable life cycle analysis (LCA) or risk assessment of recycled fertilizers in comparison to each other and to mineral fertilizers. It is the aim of the current review to compare and evaluate different methods that can be used for the ecotoxicological evaluation of recycled fertilizers. The review shall give an overview on the diversity of different tests and their scales, on different endpoints and the problems that can occur and impede the interpretation of results when testing BBFs.

In general, there are different possibilities to characterize the toxicity of a product. One approach is assessing the quality by *chemical methods* (i.e. the concentration of selected contaminants). This is a suitable approach to analyze the elemental composition and thus nutrients and heavy metals of rock materials and organic fertilizers. The analysis of bioavailable elements and organic contaminants in unknown composition and concentration is more complex. Several different extraction procedures are necessary to extract and enrich organic contaminants, which are usually present at trace concentrations. The problem becomes worse when complex matrices like sewage sludge or composts need to be characterized (Lehmann and Bloem, 2021). Extensive chemical analyses of BBFs are scarce and available data can result in misinterpretation of the ecotoxicity of a complex product as interactions of compounds and risks for the environment are not recorded (Brasser et al., 1995; Deventer et al., 2004; Ferrari et al., 1999; O'Connor and Paul, 2000). The total content of a compound is rarely related to its toxic effects as the bioavailability is of major importance (Kupper and Fuchs, 2007). Moreover, soil functioning is related to biological processes. Therefore, analytical chemical approaches should be combined with a suitable but economically reasonable set of ecotoxicological studies (Deventer et al., 2004; Kupper and Fuchs, 2007).

Ecotoxicological biotests (also called *bioassays)* are conducted to characterize the toxicity of a single compound or complex mixture. The advantage of ecotoxicological tests is that combinatory effects of different compounds and their bioavailability are captured (Cleuvers, 2003) as well as effects of metabolites or degradation products. This is important as some compounds

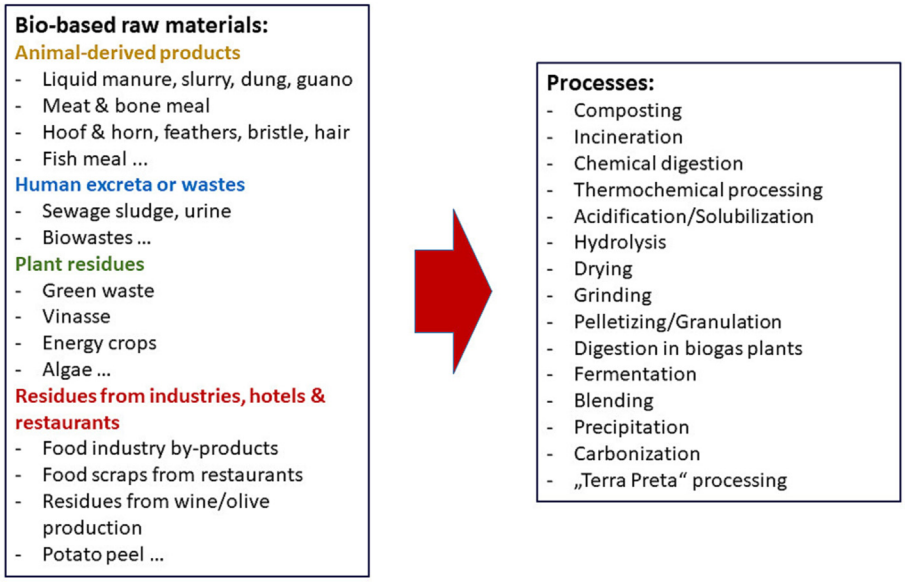

Fig. 1. Examples for bio-based raw materials and processes to produce bio-based fertilizers (BBFs).

become toxic after ingestion and conversion within organisms. Bekaert et al. (2002) suggested a combination of chemical and biological approaches, as for example the toxicity of a leachate can be low whereas its genotoxic effect can be significant. Guidoni et al. (2021) showed that nutrient-rich composts produced from by-products of swine industry and sawdust revealed a high potential for agricultural use based on chemical analysis but impaired germination of different seeds and had a high phytotoxicity together with an electrical conductivity outside the recommended level. Thus, chemical analysis alone could result in wrong recommendations, underlining the importance to combine chemical and ecotoxicological approaches.

Ecotoxicological studies are often conducted under simplified environmental conditions at the organism and population level, where cascading consequences of contaminants can be overlooked if tests are not performed at different trophic levels (Beaumelle et al., 2021; Reineke and Schlömann, 2020). Nevertheless, transfer of results to natural systems is more reliable when using ecotoxicological test systems and derived limit values can be of high relevance. It was concluded by UBA (2013) after laboratory ring tests with solid wastes that a combination of chemical and biological test methods is preferable to characterize the ecotoxicological potential of solid wastes containing unknown contaminants. Results can mirror the effects of all bioavailable contaminants including their potential interactions and pollutants that are not captured by chemical analysis alone.

The scope of ecotoxicological testing can differ from test tubes to pot or field trials and can be conducted over different time scales (acute toxicity versus chronic or long-term toxicity and genotoxicity). An important criterion when looking for suitable test systems for the evaluation of BBFs is the target environmental compartment that is affected by application. In the case of fertilizers, the main compartments are soil, adjacent water bodies and plants, which are directly affected via the rhizosphere. Greenhouse gas (GHG) emissions from organic fertilizers can moreover affect the environment (Amlinger et al., 2008) and should be recorded in the risk assessment of BBFs. The potential of BBFs to release GHGs can be captured by fertilizer incubation studies (Adam and Engels, 2019; Wester-Larsen et al., 2022). Accordingly, ecotoxicological tests for BBFs should address soil organisms, soil functioning, plants and aquatic organisms (Fig. 2).

2. European regulations applying to bio-based fertilizers

BBFs are produced to feed plants and to improve soil life. Nevertheless, they are manufactured from raw materials that are governed by different legal rules. Therefore, BBFs should be investigated for their environmental safety as well.

There are several legal rules that need to be considered with respect to BBFs and their agricultural application. A compilation of the European legal framework governing the use of nutrient rich side streams as BBFs was collected by Kratz and Hermann (2020). The new fertilizing product regulation EC No 2019/1009 that came into force in July 2022 extended its scope from inorganic to organo-mineral and organic fertilizers including recycled materials (European Commission, 2019). While in the old EC No 2003/2003 (European Commission, 2003) fertilizer types were designated, the new regulation EC No 2019/1009 is based on product function categories (PFCs) describing the function of the product, and on component material categories (CMCs) describing the raw materials from which fertilizers are produced. For the first time, limit values for contaminants and pathogens are regulated individually for each PFC. BBFs belong to the category of fertilizers (PFC 1) but can be also organic soil improvers (PFC 3) or even used in blends (PFC 7). The new fertilizing product regulation will open domestic markets of EU countries to several products such as organic or organo-mineral fertilizers, liming materials, soil improvers, growth media, inhibitors, and biostimulants or fertilizing product blends. The new regulation has the intention to harmonize the use of these products EU-wide and to introduce new limit values for toxic compounds (Table 1). Animal by-products as raw materials for BBFs are not directly covered by the new regulation; they are still regulated by EC No 1069/2009 (European Commission, 2009). Generally, fertilizing products according to regulation 2019/1009 have to show an agronomic efficiency and a documentation that they bear no risks for human or animal health, plant growth or the environment. A strategy for ecotoxicity tests is needed to obtain these requirements but is only marginally explained in the new regulation.

In the European REACH regulation (European Commission, 2006), which is the European legislation for the registration of chemicals, some tests are regulated that vary in relation to the tonnage of the chemical that is produced or traded. It would be possible to adapt these methods for the evaluation of BBFs. Ecotoxicity methods have been mainly used to study the toxicity of chemicals and pesticides. Ecotoxicological tests in the REACH regulation focus on aquatic organisms starting with short-term tests on water fleas and algae, and degradation studies, mandatory at low tonnage of 1 ton or more. Additional short-term toxicity testing on fish and sludge respiration and adsorption/desorption studies become necessary when manufacturing reaches 10 tons or more. For higher tonnages above 100 tons elaborate tests such as long-term toxicity studies on *Daphnia* and fish, bioaccumulation studies and short-term effects on terrestrial

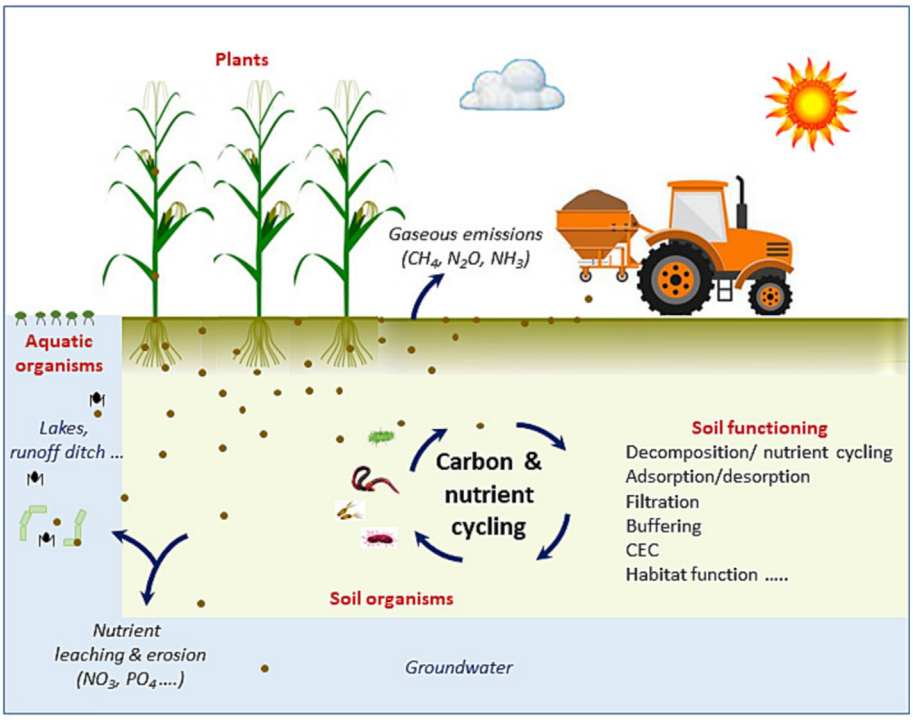

Fig. 2. Compartments that can be the target of ecotoxicity studies with respect to the application of bio-based fertilizers (BBFs).

organisms (invertebrates, soil microorganisms and plants) are requested. Additional long-term toxicity studies on terrestrial organisms need to be carried out when >1000 tons are manufactured or traded. This procedure can be seen as a general guideline for the development of an ecotoxicological test strategy for BBFs but needs to be adapted. BBFs are intentionally used to fertilize plants and to improve soil life. Therefore, they directly interact with the soil - plant interface, and a stronger focus needs to be put on effects on the terrestrial compartment.

The ecotoxicological assessment of wastes is regulated in Europe by 2008/98/EC (European Commission, 2008) where 14 criteria are defined for waste characterization, of which H14 is the ecotoxic characterization. In addition, these rules can be used and adapted for ecotoxic characterization of BBFs.

3. Ecotoxicological tests to evaluate bio-based fertilizers (BBFs)

3.1. General requirements for ecotoxicity tests

Ecotoxicity tests were originally developed for the investigation of toxic effects as a requirement in the registration process of potentially toxic chemicals such as herbicides or pesticides (e.g. ECHA, 2017) that are intentionally released into the environment. Fertilizers are also "intentionally released" into the environment, but with the purpose of feeding plants. Adverse effects are not intended on any organism. For fertilizers no specific ecotoxicity tests are proposed. Nowadays the fertilizer market is expanding and with the numerous BBFs and biostimulants possible ecotoxicological aspects gain in importance.

It is necessary to decide on a selection of ecotoxicity tests from the broad spectrum of possible tests. Generally, two approaches are applied, namely tests performed on water extracts (eluates) using aquatic test protocols, or solid phase tests representing terrestrial tests (Wilke et al., 2008). Preferably, standardized tests (ISO, CEN, DIN) should be used for a better comparability and a higher acceptance of results (Römbke, 2018). Numerous ecotoxicological tests were developed in the past and in the mid-seventies of the 20th century, the OECD initiated a program for the harmonization and standardization of ecotoxicological tests, resulting in tests validated by DIN, ISO, OECD or VDLUFA. For the selection of ecotoxicity tests for testing BBFs rules such as ISO 17616 (2019) or DIN CEN/TR 16110 (2011) can be used and adapted.

Table 1
Limit values for organic, organo-mineral and mineral fertilizers according to the EU Regulation 2019/1009 (European Commission, 2019).

Contaminant		Organic fertilizer	Organo-mineral fertilizer	Mineral fertilizer
Cd [mg/kg DM or mg/kg P_2O_5 if the fertilizer contain ≥5 % P_2O_5]		1.5	3 mg/kg DM if P_2O_5 < 5 % 60 mg/kg P_2O_5 if P_2O_5 ≥ 5 %	
CrVI	[mg/kg DM]	2.0	2.0	2.0
Hg	[mg/kg DM]	1.0	1.0	1.0
Ni	[mg/kg DM]	50	50	100
Pb	[mg/kg DM]	120	120	120
As (inorganic)	[mg/kg DM]	40	40	40
Biuret ($C_2H_5N_3O_2$)	[g/kg DM]	absence	12	12
Perchlorate	[mg/kg DM]			50
Cu	[mg/kg DM]	300	600	600[b]
Zn	[mg/kg DM]	800	1500	1500[b]
Salmonella spp.		Absence	Absence	Absence
Escherichia coli or *Enterococcaceae*		≤1000 CFU/g or ml[a]	≤1000 CFU/g or ml[a]	≤1000 CFU/g or ml[a]

[a] CFU = colony forming units.
[b] These limits shall not apply where copper (Cu) or zinc (Zn) has intentionally been added to an inorganic macronutrient fertilizer for correcting a soil micronutrient deficiency.

The sensitivity of different organisms to potentially toxic compounds varies considerably. Therefore, so-called "test batteries" should be conducted with organisms belonging to different taxonomic groups and trophic levels to build up different physiological and ecological system outputs (Deventer et al., 2004; Mouchet et al., 2005; Reineke and Schlömann, 2020; UBA, 2013). If applicable, the chosen test battery should cover species from the three main trophic groups (microbes, plants and animals - representing decomposers, producers and consumers), and toxicity levels from acute toxicity to chronic toxicity and genotoxicity. Tests should be sensitive, standardized, easy to handle and economically feasible (Römbke et al., 2010). The selection of a test species is driven by ecological, methodological and economical/practical considerations (Versteeg et al., 1997). The choice is always a compromise between the ecological question and practical considerations. A selection of three tests per compartment with three aquatic/eluate and three terrestrial/solid tests is a practical and economic compromise to evaluate waste samples according to Römbke (2018).

A selection of different tests relevant for the soil/water/plant interface is summarized in Table 2 without the intention to offer a complete list but rather to give an indication about different tests that could be relevant for the investigation of BBFs.

Typical test organisms with respect to soil amendments are soil microorganisms, higher soil organisms such as collembola, enchytraeidae or earthworms and plant growth and development (Carbonell et al., 2009; Hilbeck et al., 2008; Höss et al., 2009; Kalsch et al., 2006; Rastetter and Gerhardt, 2017; Römbke and Ketelhut, 2014). In aquatic systems, organisms are in direct contact to compounds, and test systems often react very sensitively to contaminations. Therefore, aquatic organisms are an important part of the ecotoxicological evaluation and usually represent the first test level. Typical aquatic test organisms are *Daphnia magna, Raphidocelis subcapitata, Lemna minor* and *Aliivibrio fisheri* (formerly *Vibrio fischeri*). Most of these tests meet the requirement to be easy and quick to perform: for example the *Daphnia magna* mobility test delivers results after 24 or 48 h; the phytotoxicity tests with plant seeds sown under different compound concentrations can be screened for germination and root development after 120 h (Table 2).

3.2. Preparation of BBFs for ecotoxicological evaluation

BBFs exist in different formulations from liquid fertilizers to powders, pellets and granules. Different test systems can be used with soluble or solid fractions and standardization in eluate preparation is mandatory for comparability of test results. Crucial steps for standardization are already in sample preparation such as drying, grinding and sieving of the material, choice of the eluent, solid to leachate ratio, if there is a shaking step, centrifugation or filtration – all these procedures can affect the composition of the test sample (Deventer et al., 2004). For test systems running with liquid fractions/eluates it is necessary to produce a standardized leachate from a solid fertilizer. A common method is to produce a water eluate because water is considered the "principal carrier of contaminants" (Wilke et al., 2008). This is a reasonable approach for fertilizers, as water-soluble constituents from BBFs have the potential to be leached and transported into adjacent water bodies. Water can mobilize and transport pollutants, especially those with high solubility (Dott et al., 1995). Leaching rates for BBFs can be chosen according to the EN 12457–2 protocol for the preparation of waste eluents from solid wastes where a solid to liquid ratio of 1:10 is proposed (DIN EN 12457-2, 2003) or by EN 14735 "Characterization of waste – Preparation of waste samples for ecotoxicity tests" (DIN EN 14735, 2022). Römbke (2018) recommended a 24 h elution step with distilled water in an end-over-end tumbler, followed by centrifugation for 20 min at 17000 $\times g$ and a final filtration (<0.45 μm) step. For the evaluation of soils, a solid to liquid ratio of 1:2 is proposed to prevent a too high dilution (DIN CEN/TR 16110, 2011; ISO 17616, 2019).

Eluates should be used within 72 h in the test systems without any further additives. The pH needs to be measured as an extreme pH can negatively affect test organisms. The pH optimum is usually in the range of pH 6–9. If the pH is strongly affected by the BBFs the test should be repeated in buffered form to distinguish between a possible pH-effect and that of a toxic compound or mixture of compounds (Becker van Slooten et al., 1999; Timmerer et al., 2020). It is important to consider that changes in toxicity can occur with changing pH, which is why the original eluate should be tested as well. Often soils are well-buffered so that a pH effect observed under test conditions will not necessarily occur under environmental conditions. A second parameter that needs to be assessed in eluates from BBFs is the electrical conductivity. High salt concentration in eluates, especially sodium chloride or sulfate, can disturb aquatic or soil organisms and plants by causing cell plasmolysis (Bhat et al., 2018; Lasaridi et al., 2006; Owojori et al., 2009).

Problems with eluate tests can result from turbidity or coloring of eluates, which can lead to hindered examination or even wrong results due to quenching, which reduces the luminescence in the luminescent bacteria test (Deventer et al., 2004). High contents of dissolved organic matter (DOM) in eluates can stimulate algae growth (Heiden et al., 2000).

For solid phase tests, it is necessary to use standard material as well, since soil characteristics such as pH or organic matter content have an impact on bioassays (Jänsch et al., 2005; Van Gestel et al., 1995). For example, soil type can determine the level of phytotoxicity caused by sewage sludge application (Oleszczuk and Hollert, 2011). The organic matter content is relevant for the mobility and bioavailability of pollutants (Sassman et al., 2007). Therefore, it is recommended to use OECD artificial soils (OECD 207, 1984; ISO 11268-1, 2012) or well-described, available test soils such as one of the reference soils from VDLUFA, from which a soil called St.2.2 is often used in ecotoxicity trials. BBFs should be homogenized and ground but should be used without any further sample preparation to carry out solid phase tests.

Generally, ecotoxicity tests are performed under controlled conditions in a "dose-response-design" and a dilution series consists of at least 5 dilutions, often chosen on the basis of the number 2 (or 3) such as the dilution series 1:2, 1:4, 1:8, 1:16 and so on (UBA, 2013). For a reliable test evaluation, at least two of the test concentrations need to be between 0 and 100 % of inhibition (Becker van Slooten et al., 1999; EFSA, 2017). While dilution steps with eluates are performed with water, solid phase tests can be conducted by diluting the solid material with an appropriate amount of control medium such as sand, standardized soil or OECD artificial soil (Deventer et al., 2004). Adaptation of terrestrial tests for BBF testing is a challenging task, as the test design should be adapted in a way to separate between the positive fertilizer effect that is usually observed with BBFs (Jamil et al., 2006; Zuo et al., 2019) and a potential negative effect due to contamination. Therefore, it is necessary to work with more than one control to cover the different fertilizer levels. The nutrient concentration of the BBFs should be included in the ecotoxicological evaluation to allow for a better comparability of the fertilizers in relation to field application amounts.

3.3. Test strategy for ecotoxicological evaluation of BBFs

For the evaluation of pesticides or waste materials, "test batteries" of ecotoxicity methods have been proposed which can be used as a starting point for the selection of tests for BBFs as well (Hilbeck et al., 2008; Römbke et al., 2009; UBA, 2013).

For pesticide registration, a more extensive test battery of ecotoxicity tests is recommended, including tests with higher organisms such as fish species and birds (Hilbeck et al., 2008). BBFs have the intention to feed plants and to improve soil organic carbon but they can contain harmful substances as well. BBFs are more like wastes than pesticides also in their complex matrix and high variability, and an ecotoxicological investigation following the rules for wastes seems to be a reasonable approach.

The evaluation of wastes is starting with the utilization of accessible information such as classification, labeling and packaging followed by tests with eluates and tests with solid materials (Römbke, 2018; UBA, 2013). Effect testing is always following a hierarchical order starting with short-term, low-cost tests under worst-case assumptions. The elected tests should comply with the selection criteria mentioned in chapter 3.1: tests are

Table 2
Selection of ecotoxicity tests suitable for the evaluation of BBFs: Aquatic and terrestrial tests on different trophic levels and with different endpoints are summarized.

Test organism	Test	Duration	Typical Endpoint	Guideline
Aquatic tests for (waste) eluates				
Bacteria *(Aliivibrio fischeri)*	Acute luminescent bacteria test - inhibitory effect on light emission	0.5 h; pH 6.0–8.5	Inhibition of luminescence, EC_{20}, EC_{50}	ISO 11348-1, 2007 ISO 11348-2, 2007 ISO 11348-3 (2007)
Bacteria *(Pseudomonas putida)*	Growth inhibition test; Inhibition of respiration	48 h	Growth inhibition of EC_{50}	Vaajasaari et al., 2000; ISO 10712 (1995)
Protozoa *(Tetrahymena thermophile)*	Inhibitory effect on reproduction, Chronic toxicity	24-28 h pH 6.0–9.0	Reproduction EC_x	Pauli and Berger (2000)
Freshwater algae and Cyanobacteria (e.g. *Desmodesmus subspicatus, Raphidocelis subcapitata)*	Chronic toxicity Growth inhibition test	72 h; pH 8.3 ± 0.2	Biomass, NOEC, EC_x	ISO 8692 (2012) OECD 201 (2011)
Marine algae *(Skeletonema costatum or Phaeodactylum tricornutum)*	Chronic toxicity Growth inhibition test	72 h; pH 8.0 ± 0.2	Growth, EC_{10}, $EC_{50;}$ NOEC	ISO 10253 (2016)
Water fleas (*Daphnia magna*)	Acute toxicity (immobilization)	24 h + 48 h; pH 7.8 ± 0.2	Mobility, EC_{50}	ISO 6341 (2012) OECD 202 (2004)
Water fleas (*Daphnia* spp.)	Chronic toxicity (reproduction)	21 d; pH 7.5 ± 1.5	Reproduction NOEC, LOEC	ISO 10706 (2000) OECD 211 (2012)
Water fleas (*Ceriodaphnia dubia*)	Chronic toxicity (reproduction)	7 d; pH 8 ± 0.3	Reproduction NOEC, EC_x	ISO 20665 (2008)
Rotifer (*Brachionus calyciflorus*)	Acute and chronic toxicity (population growth inhibition)	24, 48 h; pH 7.6 ± 0.3	Mortality, NOEC, EC_x LC_{50}	ISO 20666 (2008)
Midge larvae (sediment) (*Chironomus riparius*)	Emergence of larvae	28 d	Emergence, EC_x	OECD 218 (2018) OECD 219 (2004)
Duckweed (*Lemna minor*)	Growth inhibition test	7 d; pH 5.5 ± 0.2	Frond numbers, dry mass, EC_x, LID	ISO 20079 (2005)
Higher plants (*Lemna gibba*)	Chronic test (growth, biomass)	7 d	Biomass, EC_{50}	OECD 221 (2006)
Higher Plants (*Sorghum saccharatum, Lepidium sativum, Sinapis alba*)	Toxic effects on germination and early growth	120 h	Germination and root length, EC_x	ISO 18763 (2016)
Umu test *(Salmonella typhimurium TA 1535/psK1002)*	Gene activation test	4 h; pH 7 ± 0.2	Gene activation	ISO 13829 (2000)
Bacteria (Ames fluctuation test) *(Salmonella typhimurium TA 100, TA98)*	Genotoxicity test	48 + 72 h, pH 7 ± 0.2	Mutation	ISO 11350 (2012)
Tests for solid wastes and for the terrestrial compartment				
Arthrobacter globiformis	Contact toxicity test using the dehydrogenase activity	6 h	Activity; EC_x	ISO 18187 (2016)
Soil microorganisms	Soil respiration	28 d	Respiration rate, EC_x	ISO 16072, 2011
Nematodes (*Caenorhabditis elegans* var. Bristol)	Acute toxicity test with soil	3–4 d	Mortality, LC_{50}	ISO 10872 (2020)
Nematodes (*Caenorhabditis elegans* var. Bristol)	Chronic test (growth, fertility, reproduction)	96 h	Growth	Höss et al., 2009
Predatory nematodes *(Seinura tenuicaudata, Monobutlerius degrissei, Panagrellus redivivus)*	Acute test (water) or chronic test (soil) (mortality, growth)	24 h (acute test); 28 d (chronic test)	Mortality, Growth, $EC_{X,}$ LC_X	Wilms (1992)
Non-target arthropod: Predatory mite (*Aphidus rhopalosiphi*)	Acute toxicity (mortality, reproduction of surviving individuums)	2, 24, 48 h +24 h for fecundity	Mortality (48 h) Fecundity (+24 h), $LC_{50,}$ $EC_{X,}$	(Candolfi et al., 2000)
Non-target arthropod: Predatory mite (*Typhlodromus pyri*)	Chronic toxicity (mortality, reproduction)	7 d, 7–14 d	Mortality (after 7 d) + reproduction (number of eggs after 14 d), $LC_{50,}$ $EC_{X,}$	(Candolfi et al., 2000)
Non-target arthropod: Predatory mite (*Typhlodromus pyri*)	Field test	28 d	Abundance EC_X	(Candolfi et al., 2000)
Predatory mite (*Hypoaspis aculeifer*)	Chronic toxicity	16 d	Reproduction EC_{50}, EC_X	OECD 226 (2008)
Collembola (*Folsomia candida*)	Acute test	24 h, 72 h, 7 d	Mortality of adults, number of juveniles, LC_{50}	Kiss and Bakonyi (1992)
Collembola (*Folsomia candida*)	Reproduction (14 d), chronic toxicity (28 d)	14/28 d; pH 6.0 ± 0.5	Reproduction EC_{50}, EC_X, NOEC	ISO 11267 (2014)
Isopods *(Trichoniscus pusillus, Porcelio scaber)* and Oribatiden *(Platynothrus peltifer)*	Chronic toxicity (mortality, growth, reproduction)	28 d + 28 d (long life cycle of 1 year – not practicable)	Reproduction NOEC, (EC_{50}), LC_{50}	Deventer et al., 2004
Coleoptera *(Oxythyrea funestra)*	Acute test	10 d; pH 6.0 ± 0.5	Mortality LC_{50}	ISO 20963 (2005)
Earthworms (*Eisenia fetida/andrei*)	Acute toxicity (mortality)	14 d; pH 6.0 ± 0.5	Mortality of adults, growth; LC_{50}	ISO 11268-1 (2012) OECD 207 (1984)
Earthworms (*Eisenia fetida/andrei*)	Chronic toxicity	56 d; pH 6.0 ± 0.5	Reproduction EC_{50}, NOEC	ISO 11268-2 (2012) OECD 222 (2016)
Earthworms (*Eisenia fetida/andrei*)	Avoidance test	48 h	Distribution in the sample	ISO 17512-1 (2008)

Table 2 (*continued*)

Test organism	Test	Duration	Typical Endpoint	Guideline
Earthworms (whole community)	Field test (abundance, biomass, species diversity)	180–365 d	Abundance, biomass, diversity	ISO 11268-3 (2014)
Pot worms (*Enchytraeus albidus*)	Behavior test	14–28 d	Distribution in the sample	Achazi et al., 1996
Pot worms (*Enchytraeus albidus*)	Acute toxicity (mortality) + Chronic toxicity (reproduction)	7, 21, 42 d; pH 6.0 ± 0.5	Mortality and reproduction LC_{50}, EC_X	ISO 16387 (2014) OECD 220 (2016)
Earthworms or Pot worms	Bioaccumulation & Elimination	21 d	Concentration	Bruns et al., 2002
Micro-organisms (whole community)	Acute toxicity (carbon and nitrogen mineralization)	7, 14, 28 d	Nitrate formation or carbon dioxide release EC_X	OECD 216 (2000) OECD 217 (2000)
Micro-organisms (whole community)	Respiration	3–5 d	Carbon dioxide release EC_X	ISO 16072 (2011)
Soil Micro-organisms	Potential ammonium oxidation test (PAO), nitrification test	6 h	Rate of nitrate formation EC_X	ISO 15685 (2012) Campbell et al., 2003
Soil Micro-organisms	Exoenzyme test	30 min – 4 h	Hydrolase activity, EC_X	ISO 20130 (2018)
Soil Micro-organisms	Respiration curves	Up to 7 d	Maximum substrate-induced respiration	ISO 17155 (2012)
Bacterial community (sewage sludge)	Acute toxicity (respiration rate)	3 h	Oxygen uptake inhibition, EC_X	OECD 209 (2010)
Plant tests				
Plants (6–10 species) (e.g. *Brassica oleracea, Lycopersicum esculentum, Avena sativa*)	Seedling emergence, biomass dry weight and shoot length	14–21 d	Emergence and biomass, EC_{10}, EC_{50}	OECD 208 (2006)
Avena sativa or *Brassica rapa*	Acute toxicity (Growth and bioavailability of the test substance) Test in "Neubauer" pots	10 d (*B. rapa*); 14 d (*A. sativa*)	Biomass, EC_x	Günther and Pestemer (1990)
Lettuce (*Lactuca sativa* L.)	Acute toxicity	5 d	Seedling emergence EC_{10}, EC_{50}	ISO 17126 (2005)
Hordeum vulgare L. (Variety: CV Triumph)	Acute toxicity	2 d + 5 d	Root elongation LOEC, NOEC	ISO 11269-1 (2012)
Arabidopsis thaliana	Life cycle test	Up to 8 weeks	Number & length of leaves, duration till flowering	Ratsch et al. (1986)
Avena sativa or *Brassica rapa*	Chronic toxicity (Emergence, Growth, Biomass)	7–14 - 21d pH 5.5–7.5	Seedling emergence; 7d biomass production; LOEC, NOEC	ISO 11269-2 (2012) Heiden et al., 2000
Avena sativa or *Brassica rapa*	Life cycle test	6 weeks	germination, growth, seed development; EC_X	ISO 22030 (2005) Heiden et al., 2000
32 test crop species	Vegetative vigor test - spraying of test substance on plant/leaf surface at the 2–4 true leaf stage	21–28 d after treatment	Mortality, biomass, EC_X	OECD 227 (2006)
Rhizobia formation with *Medicago sativa* and *Rhizobium melioti*	Nodulation of roots and plant growth	14 d	Number of root tubercle, biomass of roots and shoots; EC_X	Wetzel et al., 1991
Soil functioning tests				
Organic matter degradation (litter bags)	Field test (organic matter mass loss)	180–365 d	decomposition	(Römbke et al., 2003)
Bait lamina test – feeding activity of soil-living animals	Field test on biological activity	10 d	Feeding activity	ISO 18311 (2016) (von Törne, 1990)
"Microcosm" experiment with undisturbed soil columns	Chemical residues in different soil layers, in leachate and plants	Sampling after 1, 8, 16 weeks	Leachate and plant analysis for radiolabeled compounds	ASTM-E-1197-87 (1998)
Soil microorganisms	Dehydrogenase activity in soils - Method using TTC or INT*	16–18 h	Dehydrogenase activity; EC_x	ISO 23753-1 (2019) ISO 23753-2 (2019)

*TTC = Triphenyltetrazolium chloride; *INT = Iodotetrazolium chloride; h = hours, d = days, EC = effect concentration, LC = lethal concentration, NOEC = no observed effect concentration, LOEC = low observed effect concentration, LID = lowest inhibition dilution.

standardized (ISO, CEN, OECD) guaranteeing for sufficient experience, different exposure pathways are covered (tests for eluates and solid phases), and sufficiently sensitive endpoints and different trophic levels and taxonomic groups are chosen according to their ecological relevance. High practicability, reproducibility and replicability of the tests and low efforts with respect to short test durations and low demand of equipment are important selection criteria so that a high number of laboratories is able to perform the tests (Deventer et al., 2004; ECHA, 2017; Hilbeck et al., 2008; Römbke, 2018; UBA, 2013).

The test battery proposed for wastes (Table 3) is the minimum set of methods according to a European inter-laboratory comparison to identify potential biological effects of environmentally dangerous waste constituents (Moser and Römbke, 2009; Moser et al., 2011; Pandard et al., 2006; Römbke et al., 2009, 2010). It includes three tests for waste eluates (aquatic tests) and three for solid wastes (terrestrial tests).

For practical reasons, three tests per compartment were chosen for waste evaluation. For a more detailed ecotoxicological characterization, additional tests are recommended (UBA, 2013), especially if a substance is already classified as a hazardous material but ecotoxicological risks are not clear. As additional tests the duckweed growth inhibition test (ISO 20079, 2005), the water flea long-term toxicity test (ISO 10706, 2000), the earthworm acute toxicity test (ISO 11268-1, 2012) or the inhibition test on reproduction of collembola (ISO 11267, 2014) are proposed by UBA (2013).

If substances can be present that are highly persistent, more complex, longer lasting tests should be chosen like laboratory, semi-field or field

Table 3
Minimum "test battery" for the ecotoxicological classification of waste materials (Pandard and Römbke, 2013; UBA, 2013).

Test organism	Test and endpoint	Duration	Threshold values	Guideline
Tests for waste eluates				
Bacteria (*Aliivibrio fisheri* formerly *Vibrio fischeri*)	Luminescent bacteria test - inhibitory effect on light emission	0.5 h	<20 % of inhibition	ISO 11348-1 (2007) ISO 11348-2 (2007) ISO 11348-3 (2007)
Algae (unicellular algae e.g. *Desmodesmus subspicatus)*	Chronic toxicity Growth rate	72 h	<25 % growth reduction	ISO 8692 (2012) or OECD 221 (2006)
Water fleas (*Daphnia magna*)	Acute toxicity (immobilization)	48 h	≤1 of 10 daphnids immobilized	ISO 6341 (2012) or OECD 202 (2004)
Tests for solid wastes				
Bacteria *(Arthrobacter globiformis)*	Contact toxicity test using the dehydrogenase activity	6 h	30 % of toxicity	ISO 18187 (2016)
Plants *(Brassica rapa)*	Emergence and growth	14 d	<30 % yield reduction	ISO 11269-1 (2012) ISO 11269-2, 2012
Earthworms (*Eisenia fetida, E. andrei*)	Avoidance test	48 h	80 %	ISO 17512-1 (2008)

experiments, which have a higher demand for resources (Bradbury et al., 2004). Terrestrial micro − / mesocosms can be used to analyze the effect and fate of compounds under more realistic conditions and thus represent a link to field conditions (Knacker et al., 2004; Paulus et al., 1999). Compounds with a high tendency to sorb to soil or sludge matrix can show a higher toxicity in water than in soil, as sorption of toxicants is one of the main mechanisms controlling toxicity via reduction of bioavailability (Girardi et al., 2011; Welp and Brümmer, 1999).

Genotoxicity seems to be a very special endpoint and to be less dose-dependent than other endpoints. Römbke (2018) detected no genotoxicity in 23 different waste samples indicating that these tests are only necessary if genotoxicity is expected in a product. If a genotoxic potential cannot be excluded the "umu test" (ISO 13829, 2000) allows a screening of the genotoxic potential.

Probably the test battery for BBFs can be reduced when enough data exist. Deventer and Zipperle (2004) identified the following three ecotoxicity tests as suitable to characterize the toxicity of 24 waste types: the algae test (DIN 38412-33, 1991) as aquatic test and the higher plant test (OECD 208, 2006) together with the bacterial contact test (ISO 18187, 2016) for solid phase examination. Pandard et al. (2006) evaluated a test battery of six standardized ecotoxicity tests in waste evaluation and was able to optimize the test battery to three tests without loss of information: one plant growth test for solid materials with lettuce (*Lactuca sativa)* and two tests for water extracts (*Aliivibrio fischeri* and *Ceriodaphnia dubia*). Wilke et al. (2008) recommended the use of at least two test systems for the terrestrial and for the aquatic environment each representing a producer and a consumer such as the higher plant test and the collembolan reproduction test for the terrestrial and the duckweed test and the daphnia reproduction test for the aquatic environment. They observed that chronic endpoints such as growth and reproduction are more sensitive than acute endpoints like mortality. Similarly, Ferrari and coworkers reported higher toxicity in chronic compared to acute tests for six pharmaceuticals (Ferrari et al., 2004). For example, biochar produced from sewage sludge with addition of plant material was tested with a test battery consisting of three tests (*Lepidium sativum*, *Folsomia candida* and *Aliivibrio fischeri*): Short-term tests revealed a low toxicity (Gondek et al., 2014) while under long-term conditions the results were more diverse, presumably due to aging of biochar products (Godlewska et al., 2022).

The selection of suitable test organisms is of high relevance as they differ considerably in their sensitivity to different contaminants. The algae growth test is reported to belong to the most sensitive test systems. This test can be a valuable part of the test battery for BBFs, because of the fact that green algae comprise a key-function in aquatic systems as biomass producer and food for other organisms (Deventer et al., 2004). Moreover, algae are also part of the soil microbiome and can be found on plant tissues, highlighting their ubiquitous abundance (Lee and Ryu, 2021). It is advantageous to have very sensitive tests included in the test battery as they can give a first hint to a possible toxicity. Rooker (2000) determined the Microtox™ test with *Aliivibrio fischeri* and the chronical growth test with algae to be more sensitive than tests with *Daphnia*. Clement et al. (1997) determined the sensitivity of test organisms in the following order of sensitivity: *Aliivibrio* > *Scenedesmus* > *Lemna* > *Ceriodaphnia* > *Thamnocephalus* > *Daphnia* > *Spirostomum* > *Brachionus*. However, the sensitivity of test organisms changes in relation to the toxic compound under evaluation. The nematode *Caenorhabditis elegans* responded very sensitively to toxic organic pollutants but was less sensitive to heavy metals when compared with other test organisms like oligochaetes, collembolans or plants (Höss et al., 2009) while daphnids and plants like *Lepidium sativum* react stronger to heavy metals (Gondek et al., 2014; Seco et al., 2003).

Even within a given test, the choice of species is of relevance. Tests with earthworms are often conducted with *Eisenia fetida*, a compost worm that is easy to obtain and can use fresh organic matter as nutrient source. Roß (2017) found a different avoidance behavior of *Eisenia fetida* and the endogeic worm *Aporrectodea caliginosa* in response to different organic fertilizers: while *E. fetida* preferred soil with organic fertilizer amendment, the opposite was observed for *A. caliginosa*. Therefore, it is important which earthworm species is used, and the eco-morphological classification (epigeic, endogeic, anecic) should be considered in the experimental design as there are species-specific differences in behavior (Fründ et al., 2010); preferably species should be chosen that naturally occur in the agricultural soil system and have an important ecological function.

Different plant species are recommended in the plant growth tests and show different sensitivity to toxic compounds, underlining the necessity for parallel testing with different plant types (Deventer et al., 2004). From the dicotyledonous species, *Brassica rapa* and *Brassica napus* revealed a good sensitivity (Kalsch et al., 2006) and often showed a higher sensitivity than the monocotyledonous species *Avena sativa* (Roß, 2017).

Römbke (2018) determined the toxicity of 23 wastes by using three eluate tests (genotoxicity, algae and daphnid tests) and three solid phase tests (*Arthrobacter globiformis* test, plant growth and earthworm avoidance tests). He observed that algae reacted much more sensitive and showing a graded response compared to daphnids that revealed a yes/no effect pattern. From the terrestrial tests, the contact test using *Arthrobacter globiformis* and the plant growth test were more sensitive than the earthworm avoidance test. *Arthrobacter globiformis* is a commonly found soil microorganism. Therefore, the test is of high ecological relevance.

Results from aquatic and terrestrial tests differ because each test has its own "effect profile" and results from different tests did not necessarily correlate (Römbke, 2018). The author concluded that a combination of the two most sensitive tests (the algae and the *Arthrobacter* test) was able to detect the same ecotoxicity like the whole test battery consisting of six tests.

A comparative evaluation of different genotoxicity tests (Comet assay versus micronucleus test) revealed that tests performed with larvae of the frog *Xenopus laevis* showed a higher sensitivity than bacterial assays (Microtox or Ames test) because of the high sensitivity of amphibians to genotoxic pollutants in aqueous solutions (Mouchet et al., 2005; Jaylet et al., 1990; Gauthier et al., 1993).

Overall, the selection of suitable test organisms is of high relevance and if the character of possible contaminants is unknown this uncertainty can be only overcome by a higher number of different tests so that the likelihood is higher that at least one sensitive organism is part of the test battery.

The test battery shown in Table 3 seems to be suitable for the evaluation of BBFs. The test battery contains very sensitive test organisms like algae and bacteria and covers the three trophic levels of producers (e.g. algae), consumers (e.g. crustacean like *Daphnia*) and decomposers (bacteria like *Aliivibrio*) tested under acute and chronic test conditions. According to Clement et al. (1997), such test batteries are suitable to detect 90 % of all toxic compounds.

Other tests can be chosen for a test battery for testing BBFs: effects on soil dwelling organisms such as earthworms, nematodes, collembolan, mites, isopods or soil bacteria (see Table 2) are of special relevance as breakdown and transformation of soil organic matter determine soil fertility and ecosystem functioning. The European Food Safety Authority (EFSA) identified community-based microbial test systems, which cover a broad range of metabolic processes as important ecotoxicity tests (EFSA, 2017; Hund-Rinke et al., 2019). The carbon and nitrogen transformation tests (OECD 216, 2000; OECD 217, 2000) designed to detect long-term adverse effects of compounds on aerobic soils over 28 days are regarded as important microbial tests, and they are commonly used since OECD guidelines exist (ECHA, 2017).

Earthworms are excellent bio-indicators for toxic compounds entering the soil as they are covered by a water film and come into direct interaction with soil water and possible contaminants via their whole surface (Fründ et al., 2010). Moreover, they represent 90 % of the invertebrate's biomass and can reach population densities of up to 500 individuals per square meter, underlining their immense meaning for mineralization of organic matter, soil structure and soil fertility (Edwards and Bohlen, 1996). BBF application can directly affect soil dwelling organisms. Nematodes belong to the most abundant metazoans in soils, having a key position in terrestrial food webs and thereby agricultural production (Andrassy, 1992). For strongly adsorbing or binding substances, soil-dwelling organisms that feed on soil particles are most relevant. From the different earthworm tests the avoidance test was shown to be more sensitive than the earthworm acute test (ISO 11268-1, 2012) (Hund-Rinke and Wiechering, 2001).

In addition, various soil functioning tests such as the Litterbag or the Bait-Lamina-test can be performed to show the effect of a soil amendment on organic matter decomposition and biological activity, which are central constituents in nutrient cycling and soil fertility (Kratz, 1998; Kula and Römbke, 1998). Bags filled with organic matter that were placed inside the soil and were evaluated after half a year represent very well the activity of soil organisms (Paulus et al., 1999).

Tests on soil organisms can show a high variability in relation to soil properties that is why standardization of such tests is mandatory (Höss et al., 2009) to separate between natural variability and toxic effects.

3.4. Evaluation of the ecotoxicological test results

Ecotoxicological endpoints characterize the toxicity of a compound or mixture in comparison to a control. Typical endpoints measured in aquatic tests are growth in case of algae, and immobilization (or mortality) in case of crustacea like Daphnia (Clement et al., 1996; Ferrari et al., 1999; Moser and Römbke, 2009). Reproduction as an endpoint was shown to be more sensitive and ecologically relevant for crustacea but also more demanding in terms of time and efforts (Ferrari et al., 1999). In terrestrial plant tests germination rate was a less sensitive endpoint compared to biomass development combined with shoot length (Latif and Zach, 2000; Lors et al., 2010). Mortality is the oldest but least sensitive endpoint in case of terrestrial animals like earthworms and enchytraeids, while biomass loss, avoidance reactions, feeding inhibition or reproduction parameters are more relevant (Römbke and Moser, 2007). Results are also affected by other parameters such as soil type or selected species. Therefore, the test design and execution are crucial points in ecotoxicological studies (Isidori et al., 2003; Lors et al., 2010).

Römbke (2018) gives an overview about the effect criteria for different tests that can be used for waste materials. For each ecotoxicological test a specific threshold level is fixed beyond which an effect is considered toxic (see Table 3, Deventer et al., 2004).

The most important values, terms and abbreviations are summarized in Table 4. Specific values facilitate the direct comparability of the toxicity of different compounds and can be used to determine limit values for compounds in environmental systems (UBA, 2013; Pandard et al., 2006). Commonly used indicator values include the LC_{50} (lethal concentration of a chemical that kills 50 % of the test population), the EC_{50} (concentration that affects 50 % of the population) or the IC_{50} (concentration that inhibits 50 % of the biological activity) (ISO 11269-2, 2012; Walton et al., 2021). Accordingly, the result of an ecotoxicological evaluation is often expressed as EC_x value (CEN/TR 17105, 2017). Another often-used value, especially in Germany, is the LID-value, which is the lowest inhibition dilution or in other words the highest test concentration at which no inhibition or mortality higher than the test specifications was observed (Römbke, 2018).

It needs to be discussed how to define reasonable ecotoxicological limit values for the application of BBFs. Typical evaluations of test results for waste material follow the rule if the EC_{50} is ≤ the concentration in a 10 % dilution this is indicating to a distinct negative effect on test organisms. Deventer et al. (2004) regarded a compound as toxic if its water extract with a concentration of 1 % inhibits reproduction of *Ceriodaphnia dubia* by >20 % or if the compound in its solid form applied at a concentration of 10 % to a test substrate inhibits emergence and growth of a test plant by >50 %.

Ferrari et al., 1999 used toxic units ($TU = 100/LC_{50}$) for classification. An observed $TU \leq 1$ indicate no toxicity, $1 > TU \leq 10$ low toxicity, $10 > TU \leq 30$ moderate toxicity, $30 > TU \leq 100$ a toxic compound, $100 > TU \leq 1000$ a high toxicity and $TU > 1000$ a very high toxicity of the test compound. For aggregation of different test results, Lapa et al. (2002) assigned scores in relation to the TU classes (Score 1 if $TU \leq 1$, Score 2 if $1 > TU \leq 10$, Score 3 if $10 > TU \leq 100$ and Score 4 if $TU > 100$). The sum of all test scores divided by the test number delivers the class weight score.

According to Hilbeck et al. (2008) no further tests are required if no effect was observed at the highest test concentrations (usually 1000 mg/kg or 1000 mL/L).

Such approaches can be adapted for BBFs but results should be evaluated in relation to the nutrient content because the environmental application is determined by the nutrient composition. Especially if the ecotoxicological evaluation of different BBFs is performed for a risk assessment, the toxicity should be evaluated in relation to the nutrient content

Table 4
Ecotoxicological endpoints characterizing the toxicity of a test compound.

Specific toxicity value	Explanation
LC_{50}	Lethal concentration where 50 % of the test organisms are dead
EC_{50}	Effect concentration where 50 % of the test organisms show an effect such as growth reduction or on mobility
IC_{50}	Inhibition concentration - concentration giving half inhibition
LC_x, EC_x	Lethal/effect concentration where x% of the test organisms are dead or affected
LID values	Lowest Inhibition Dilution: the lowest dilution concentration where the fixed effect criterion is not exceeded, e.g. if this is the case in a 1:4 dilution the LID value is 4
TU	Toxic units: $TU = 100/LC_{50}$
NOEC	No observed effect concentration – concentration with no observed effect
LOEC	Lowest observed effect concentration - lowest concentration where an effect was found after long-term exposure
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
NEC	No effect concentration
TER	Toxicity exposure ratios (TER = toxicity [EC_{50}, LC_{50} or NOEC]/exposition [PEC])
HQ	Hazard quotient (NEC/PNEC)
RQ	Risk quotient (PEC/PNEC) >1 indicate a risk for the environment

and the potential application amount. For this purpose the toxicity exposure ratio (TER) could be an approach:

$$TER = \frac{Toxicity\ [EC50, LC50\ or\ NOEC]}{Exposure\ [PEC]} \quad (1)$$

It would be possible to change the factor in formula (1) to a specific nutrient amount of nitrogen (N) or phosphorus (P) to allow for a direct comparison of different BBFs regarding their ecotoxicity when applied to the field based on the nutrient content.

According to the European Commission (2011) a compound can be only authorized if for example the earthworm acute toxicity/exposure ratio of a compound is below 10 and in case of long-term exposure below 5; microbial nitrogen and carbon mineralization should be less affected than 25 % after 100 days.

Environmental Risk Assessment (ERA) for chemicals is done by dividing the predicted environmental concentration (PEC) of a compound by the predicted no effect concentration (PNEC). If the resulting risk quotient (RQ) is ≤ 1 this indicates that no concern for the environment has to be expected after field application. A value >1 is indicating a potential environmental risk, making measures necessary for risk reduction (Kreuzig et al., 2021). PEC can be either determined by chemical methods or via the intended use pattern of the BBF and preferably by a combination of both approaches. It is suggested by DIN CEN/TR 16110 (2011) to calculate the concentration of compost in ecotoxicity tests via its nutrient content and application amount but to also test the threefold and tenfold concentration as erroneous application or storage in the field can cause these higher concentrations. This approach can be used for BBFs as well to come up with safe recommendations.

In German studies, the Lowest Inhibition Dilution (LID value) is a commonly used approach to evaluate wastewater and contaminated soils (Römbke, 2018). It is necessary to define a limit concentration, where the effect criteria of a particular test is reached. In case of the aquatic algae growth test the effect criterion is 25 % of growth reduction. The test result is rated as non-ecotoxic if in a 1:4 dilution (LID = 4) the growth reduction is lower than 25 %. Based on results of an "European Ringtest" on wastes a LID ≤ 4 for aquatic tests and a LID ≤ 8 for terrestrial tests is regarded as being acceptable (Moser and Römbke, 2009; Römbke, 2018). This approach is quite easy to use also for test batteries. Pandard and Römbke (2013) proposed a "tiered approach" where aquatic tests were regarded before terrestrial ones. Materials that failed already the aquatic tests are regarded as toxic. The materials that were evaluated as non-ecotoxic in the aquatic tests were assessed in a second step with terrestrial tests. Only materials that show no ecotoxicity in any test were considered as non-ecotoxic materials. The LID approach is also appropriate for eluates with low toxicity where no dose response curve can be observed and where the LID approach delivers fast results (CEN/TR 17105, 2017).

BBFs should not show negative effects on any organism in the amounts that are usually applied to the field including a buffer (the tenfold amount) like proposed by DIN CEN/TR 16110 (2011). The toxicity values should be carefully evaluated with respect to possible environmental concentrations and the hazard to be leached to adjacent water bodies. Unlike waste materials, BBFs were applied to the field in relation to their nutrient content, especially N or P. Accordingly, EC_{50} values calculated for fertilizers should be evaluated in relation to their nutrient content. Following the recommendation given in ISO 17616 (2019) a material is classified as "ecotoxic" in case the threshold values have been breached in one out of six tests (Römbke, 2018). Römbke and Ketelhut (2014) recommended the use of statistical approaches for the evaluation of ecotoxicity studies as a more reliable method in comparison to threshold values to detect significant effects of test compounds.

3.5. Combination of ecotoxicological tests, chemical analysis and soil indicators for the valid evaluation of BBFs

Ecotoxicity tests conducted under simplified experimental conditions as summarized in chapter 3.3 will deliver information on direct toxicity of the tested fertilizer to single organisms. Such tests are valuable to derive standard toxicity values like the EC_{50}. However, under real-world scenarios species are not alone but interact with each other and with their abiotic environment and adverse effects can emerge through indirect effects mediated by species interactions (Rohr et al., 2006; Yamamuro et al., 2019). Effects on soil microbial communities are such an example, as diversity rather than the absolute quantity of soil life determines soil functioning and maintenance of biogeochemical nutrient flows (Wagg et al., 2014; Bender et al., 2016). Beaumelle et al. (2021) could show that indirect effects mediated by species interaction, ecosystem functioning and interaction between stressors and climate change are not sufficiently addressed in literature, and that there is an urgent need to improve this integrative knowledge as soil biodiversity is facing multiple threats such as climate change, soil pollution and soil degradation.

The additional assessment of selected soil indicators can help to overcome the problem that ecotoxicity tests and chemical analysis do not cover all possible environmental effects. Bünemann et al. (2018) reviewed literature for sensitive soil quality indicators: they identified a set of 65 soil indicators from 62 reviewed papers from which soil respiration as biological, total organic matter content as chemical and water storage capacity as physical parameter were most often mentioned as soil quality indicators. Kumpiene et al. (2014) identified a phytotoxicity test in combination with analyzing plant enzyme activities related to stress response as most responsive indicators for risk assessment of trace-element-contaminated soils. Chaudhary et al. (2022) suggested to use a set of enzymatic activity tests in combination with quantifying the bacterial population using quantitative PCR as the microbial population with its enzyme activities is the main driver of micro−/macronutrient availability, soil health and fertility (Jacoby et al., 2017; Tahat et al., 2020). Special requirements for the selection of additional soil indicators would be an easy sampling and measurement of the parameter as well as a high reliability and interpretation scheme and low costs (Bünemann et al., 2018).

BBFs can be a source of chemical stressors such as heavy metals that need to be analyzed in addition to the ecotoxicological test battery. Rodríguez-Eugenio et al. (2018) identified the chemical soil pollution as a main factor affecting soil functioning worldwide, which is why it is important to avoid or minimize soil pollution by fertilizer application. Assessment of soil health indicators can help to identify changes in response to fertilizer application.

In conclusion, a reasonable approach to investigate BBFs for their environmental safety would be a combination of chemical analysis and a battery of ecotoxicity tests. Additionally parameters like the assessment of soil indicators such as soil respiration, total organic matter content or the effect on water holding capacity after fertilizer application in comparison to a control would allow an integrated assessment of BBF application. If a scoring for each test level is defined (Fig. 3), this scoring can be used together with other data such as the emission potential for GHGs of such bio-based fertilizers (Wester-Larsen et al., 2022) to conduct an integrated life cycle assessment comparing the different products for their environmental relevance (Fig. 3).

4. Conclusions und future research

Combined biological-chemical approaches have the potential to describe the toxicity of complex BBFs more accurate than chemical evaluation alone could do. Future studies need to unravel the most suitable tests to characterize materials showing conflictive characteristics such as organic fertilizers with the potential to improve soil life and plant growth by delivering organic matter and nutrients but on the other side showing the risk to contain varying concentrations of harmful substances. Future progress in the development of ecotoxicological tests such as "higher tier tests" and the establishment of molecular biological methods have the potential to detect also synergistic effects of complex mixtures and can help to determine more sensitive endpoints (Kreuzig et al., 2021). This could further improve the risk assessment for BBFs.

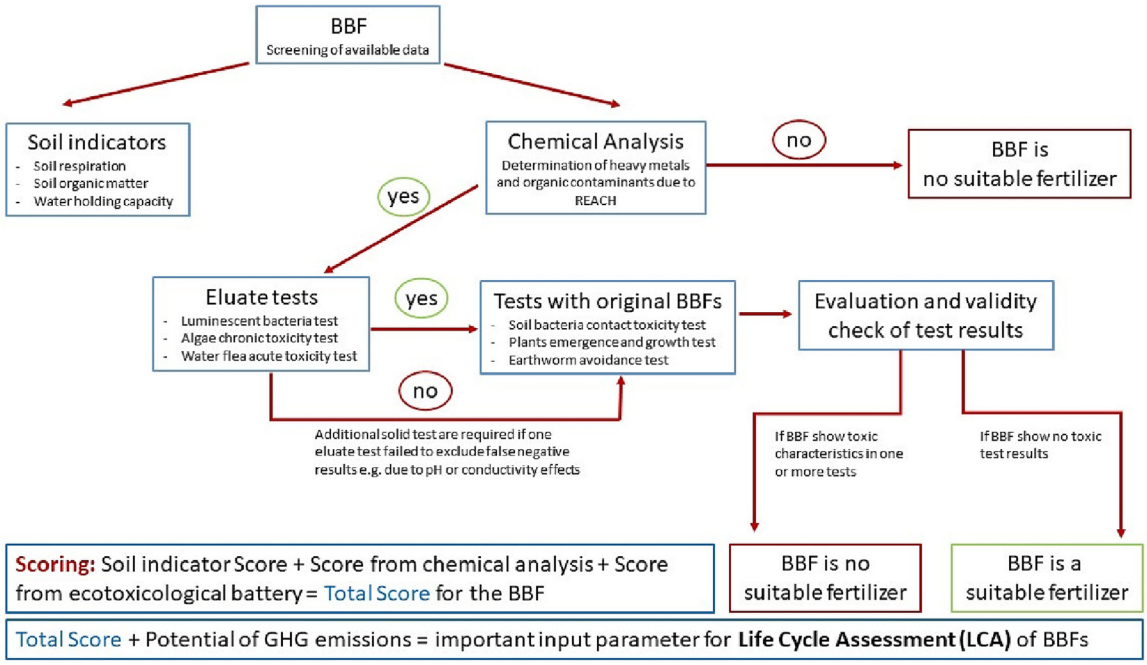

Fig. 3. Decision tree for the evaluation of BBFs by ecotoxicological studies in combination with chemical characterization and soil indicator evaluation."No" on the arrows means that limit values were exceeded or tests failed, while "yes" denotes that guidelines were met. The overall scoring from chemical analysis, soil indicators and ecotoxicological assessment can be used together with the potential of GHG emissions from these fertilizers as toxicological input parameters for a life cycle assessment (LCA) of the products.

In future research aspects related to climate change should be considered and how these changes can affect ecotoxicity tests and the toxicity of BBFs. Different environmental conditions such as climatic differences or soil features can affect the informative value of ecotoxicological tests, which needs to be considered when selecting tests for a test battery.

As BBFs have the intention to be used in agriculture to feed plants or even to improve soil life, it is possible that future research will show that a simplification of the ecotoxicological test battery is possible. However, following the precautionary principle but also being open for potentially critical raw materials such as sewage sludge based products, an evaluation in accordance to that for waste materials is recommended even if BBFs are valuable nutrient-rich products that are urgently needed to close nutrient cycles and to improve circular economy.

CRediT authorship contribution statement

Sophia Albert (SA): Writing- Original Draft Preparation.

Elke Bloem (EB): Conceptualization, Visualization, Supervision, Writing – Review and Editing.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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