Ecological risk assessment of agricultural soils for the definition of soil screening values: A comparison between substance-based and matrix-based approaches

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Abstract

The Italian legislation on contaminated soils does not include the Ecological Risk Assessment (ERA) and this deficiency has important consequences for the sustainable management of agricultural soils. The present research compares the results of two ERA procedures applied to agriculture (i) one based on the “substance-based” approach and (ii) a second based on the “matrix-based” approach. In the former the soil screening values (SVs) for individual substances were derived according to institutional foreign guidelines. In the latter, the SVs characterizing the whole-matrix were derived originally by the authors by means of experimental activity. The results indicate that the “matrix-based” approach can be efficiently implemented in the Italian legislation for the ERA of agricultural soils.
method, if compared to the institutionalized “substance based” approach is (i) comparable in economic terms and in testing time, (ii) is site specific and assesses the real effect of the investigated soil on a battery of bioassays, (iii) accounts for phenomena that may radically modify the exposure of the organisms to the totality of contaminants and (iv) can be considered sufficiently conservative.

Keyword: Environmental science

1. Introduction

In 2013, almost 150 Italian public and private stakeholders (researchers, decision and policy makers, entrepreneurs), working on contaminated sites, established a committee called CTTC (Italian technical committee on contaminated sites) [1]. Its aim was to develop a technical and scientific document that would be instrumental to policy-makers engaged in renewing the legal-framework regarding the remediation of contaminated sites. Among several issues concerning the application of Ecological Risk Assessment (ERA) to agricultural soil, the CTTC focused on inconsistency between the role of Risk Assessment (RA) procedures assigned by the current law-framework [2] and its use to define soil Screening Values (SVs).

Nowadays, SVs are used in screening ERAs to define a Level 2 risk. They identify contaminants of potential concern and these are defined as the substance-specific concentration values that if exceeded, trigger a further site-specific risk assessment (trigger values). Therefore, they are not meant to serve as site-specific remediation levels (target values) nor do they represent a sufficient condition to start remediation activities (cut off values) (Fig. 1) [3]. Italian law [2] considers SVs only for residential and industrial land-use and not for agricultural soil.

In recent years, several authors [4, 5] have discussed the similarities within the guidelines for developing ecological soil SVs [6, 7, 8, 9]. The comparison is reported in Table 1 and the main differences are related to:

![Fig. 1. Screening values uses. Level 1. This level identifies long term negligible risk; Level 2. This level indicates an intermediate (warning) risk, with the need of further assessments; Level 3. This level reports a potentially unacceptable risk, which requires an action to decrease the intensity of the stress factor(s) causing the unacceptable risk.](http://dx.doi.org/10.1016/j.heliyon.2017.e00284)
Table 1. Comparison between national and international methodologies for the derivation of ecological soil Screening Values.

| Derived soil screening value (SV) name | Soil screening value extrapolation method | Land Use specific SV | Screening value protected receptors | Soil specific normalization method | Background Concentration accounting method |
|---------------------------------------|------------------------------------------|---------------------|-------------------------------------|----------------------------------|---------------------------------------------|
| **NL**                                | Maximum Permitted Concentration for soil compartment (MPCsoil) | Where terrestrial toxicity data are available, 5% SSD is used, if 4 or more NOECs from at least 4 taxa are present. If fewer terrestrial toxicity data are available, the Assessment Factors method is used, based on the lowest available value. In the case that no terrestrial toxicity data are available, the Equilibrium Partitioning method is used based on aquatic toxicity data. | NO | Where terrestrial toxicity data are available, Primary producers (plants), primary consumers (soil invertebrates) and decomposers (microorganisms) are considered. An MPC is calculated for the soil microbial community and for the other considered species separately. The MPC is set equal to the lower value obtained. | The standardized MPC (MPCsoil,std) is equivalent to the experimentally derived MPC (MPCsoil,exp) multiplied by the ratio of a soil standardized organic matter content (foc,std) and the soil experimental organic matter content (foc,exp). MPCsoil,std = MPCsoil,exp*(foc,std/foc,exp) | Risk addition method |
| **TGD-EU**                             | Predicted No Effect Concentration for soil compartment (PNECsoil) | Where terrestrial toxicity data are available, the Assessment Factors method is used based on the lowest available value. If 10 NOECs covering at least 8 taxa are available, the 5% SSD is used divided by an assessment factor. In the case that no terrestrial toxicity data are available, the Equilibrium Partitioning method is used based on aquatic toxicity data. | NO | Primary producers (plants), primary consumers (soil invertebrates) and decomposers (microorganisms) are considered together. | The standardized PNEC (PNECsoil,std) is equivalent to the experimentally derived MPC (PNECsoil,exp) multiplied by the ratio of a soil standardized organic matter content (foc,std) and the soil experimental organic matter content (foc,exp). PNECsoil,std = PNECsoil,exp*(foc,std/foc,exp) | Demanded to further site specific ERA |
| **CANADA**                             | Soil Quality Guideline (SQG) | Where at least ten chronic terrestrial toxicity data from at least three studies are available, a SQGi is derived from the 25% SSD divided by an assessment factor. If less chronic terrestrial toxicity data are available, the Assessment Factor method is used based on the lowest available NOEC. When only acute terrestrial toxicity data are available, the Assessment Factor method is used based on the lowest available data. | Agricultural, Residential, Parkland, Commercial and Industrial | Soil dependent organisms (microorganisms, plants and invertebrates) and consumers (both livestock and wildlife). | Demanded to further site specific ERA | Demanded to further site specific ERA |

(Continued)
Table 1. (*Continued*)  

| Derived soil screening value (SV) name | Soil screening value extrapolation method | Land Use specific SV | Screening value protected receptors | Soil specific normalization method | Background Concentration accounting method |
|---------------------------------------|-----------------------------------------|---------------------|-------------------------------------|----------------------------------|---------------------------------------------|
| USEPA Ecological Soil Screening Level (ECO-SSL) | First literature toxicological data are selected based on a given score assigned to assess the quality (e.g. chronic data are ranked higher than acute data) and conservative conditions of bioavailability. Then EC20, EC10 and GMTA (which is the geometric mean of NOAEC and LOAEC) are listed from each high quality selected study. Finally the geometric mean of the selected values is finally used. | NO | Plants, invertebrates, birds and mammals. | NO | Demanded to further site specific ERA |

Moreover, a modeled SQGi is derived for each land-use identified exposure pathway:  
- soil contact (1 pathway);  
- microbial nutrient and energy cycle (1 pathway);  
- food and soil ingestion (3 pathways);  
- freshwater and groundwater ingestion and contact (3 pathways)  
The minimum SQGi is used as definitive land-use specific SQG.
• the extrapolation methods (i.e. Species Sensitivity Distribution, Assessment Factors, Equilibrium Partitioning Method or tailored modeling procedures as in the case of Canadian guidelines).

• the receptors to be protected (soil organisms and/or wildlife).

• the specific soil features (as organic matter content) to be considered.

• the methods to account for the bioavailability of contaminants and the background concentrations.

• the specific protections goals.

Guidelines differ on the assumptions made to understand and model the complexity of soil medium and the behavior of single substances in the environment. In addition, despite the wide range of magnitude characterizing the derived SV [4], these differences lead to a variable level of conservatism, transparency and accuracy. The totality of SVs derived by the guidelines can only be used within screening ERA procedures using the so-called “substance-based” approach. This approach is the simplest and it relies on well-established laboratory physicochemical analyses of single chemical concentrations and on institutionally recognized ecotoxicological databases. Thus, it characterizes the risk of the single compound independently. This approach does not take in account the bioavailability of the assessed chemicals and implements a modeling approach (i.e. Concentration addiction or Independent Action models) to predict the combined effects of a mixture of pollutants [10, 11, 12, 13, 14, 15]. Also the effect of the specific soil matrix in assessing the effective exposure of organisms to the chemicals are not considered. Therefore, the results cannot be considered as being representative of site-specific conditions leading to an unrealistic estimation of risk to the considered ecosystems [16].

Conversely, the “matrix-based” approach may provide a methodology that is able to overcome the defined drawbacks of the “substance-based” approach. In fact, it assesses the effects to target receptors from the chemicals in mixture and within their real matrix thereby considering the entire source of the chemicals as a whole. It relies on conducting a battery of both mono-specific or multi-specific (mesocosm, microcosm, etc.) bioassays testing different concentrations of the matrix imposing defined biotic (i.e. feeding periods, previous breeding conditions, etc.) and abiotic conditions (i.e. temperature, soil pH, light-dark cycles, etc.), which are often standardized to allow easier comparison of results. Such tests determine the concentration of the matrix causing an effect to representative target organisms that are chosen to pertinently relate the results to the land-use specific risk assessment. This approach is more complex but more realistic since it accounts for phenomena that may radically modify the exposure of the organisms to the totality of contaminants (e.g. exposure to minor pollutants not chosen to be assessed when
formulating the problem, chemicals partially adsorbed on soil particles, interactions between substances, etc.) [10, 17]. Thus, this approach avoid the necessity of preliminarily identifying chemicals of concern.

Whilst risk thresholds for individual chemicals are set by tabular values, defined *a priori*, in a substance-based approach, defining thresholds of environmental acceptability for a matrix-based approach is still an incomplete task. In this respect, the scientific community has not yet reached a consensus on which effect values (and species) can be considered viable for being representative of the ecosystems [18]. Similarly, the definition of an ecosystem “in danger” is still under debate [10, 19].

An agroecosystem is considered “healthy” when it can be perceived as being sustainable, which means both stable and resilient [20]. It implies that a healthy agroecosystem must be capable of maintaining constant levels of crop-productivity indefinitely in spite of environmental stresses [21] thereby not needing increasing anthropogenic inputs (such as pesticides, fertilizers, etc.). These system properties refer not only to ecological aspects but also to socio-economic issues. They rely on different parameters such as indexes of diversity or ecosystem services [20, 22, 23, 24, 25]. Instead, laboratory scale mono-specific bioassays with the assessment of several common endpoints (such as growth, reproduction and mortality) are faster, cheaper and more easily executable tests in order to obtain effects data. These bioassays represent a more viable tool for risk characterization in an ERA screening phase. However, the intrinsic simplicity of these tests leads to an unknown degree of realistic ecological representativeness. These observations should be taken into account (or “considered as suggestions”) when proposing assumptions for the derivation of ecological soil SVs.

Italian law [2] provides an example of this kind of value when establishing the maximum concentrations of an effluent discharged by a municipal wastewater treatment plant. In detail, treated wastewater can be legally discharged into a surface water stream if the results of an acute 24 hour-bioassay on crustaceans (e.g. *Daphnia magna*) does not result in the immobilization of 50% of the individuals tested. It is a concrete case of application of the principle known as “whole effluent toxicity”, used in the field of risk assessment of wastewaters.

The main aim of this paper is to apply and discuss a scientific methodology based on the Ecological Risk Assessment (ERA) for the derivation of agricultural soil screening values (SVs). A case study is presented where a comparison was performed between the results of two ERA procedures: the first, using a “substance-based” approach to exploit soil SVs as proposed by institutional foreign guidelines, and the second based on a “matrix-based” approach with the SVs defined in the current paper.
The proposed “matrix-based” approach provides suggestions for possible solutions for the limitations of the “substance-based” approach. The intention is not to define a fully-comprehensive procedure to determine agricultural SVs, but rather to offer a preliminary screening on this topic. The paper presents a scientific-based discussion aimed at refining a complete methodology that will impact upon policy and decision making in Italy and possibly other countries.

2. Materials and methods

2.1. General scheme of the procedure

The comparison between the results obtained by the ERAs following the substance-based and the matrix-based approach was made possible by a stepwise procedure, described schematically in Fig. 2. Both methodologies provided a risk value expressed as the ratio between an exposure value and an effect value, which was obtained differently according to the selected approaches.

![Figure 2](http://dx.doi.org/10.1016/j.heliyon.2017.e00284)

**Fig. 2.** Scheme of the general procedure implemented for the comparison of the results obtained by the substance-based and the matrix-based screening ERAs.
2.2. Sampling campaign

The selected area for the application of ERA is agricultural soil located in the province of Venice (Italy). The specific site is characterized by being a reclamation land since the first decades of the 20th century. The sampling area measures 4.2 ha and it is part of a larger farmland of about 50 ha, all cultivated conventionally as vineyards.

According to the USDA geologic characterization, the site is constituted by a silty-clayey soil specifically made by an Aquertic Eutrudepts fine, carbonatic and mesic soil type. Small water ponds are visible and distributed throughout the whole farmland [26].

The soil samples came from a farmland exploiting conventional methodologies for vineyard cultivation, thus making use of a wide range of plant protection products (PPPs). PPPs were applied by spraying machines exploiting anti-drift and recovery nozzles in order to decrease the fraction of active substance reaching the soil thus improving cost-effectiveness of the process. Table 2 describes the type and mass load of pesticides applied on the sampling area (4.2 ha) during the year before the soil sampling campaign was conducted. The load refers to the masses of active ingredients only and does not refer to the amounts of coformulants used.

The sampling procedure followed the guidelines on contaminated soil proposed by APAT (Italian Environmental Protection Agency) [27]. Soil samples were obtained from a depth of 30 cm, after removal of the upper sward. The total mass (about 40 kg) of sampled soil was then mixed together in order to reduce possible physical-

| Active Substance    | Yearly load on sampling area (kg/(y*4.2 ha)) |
|---------------------|---------------------------------------------|
| Mancozeb           | 15.8                                        |
| Sulphur            | 188.2                                       |
| Copper (Total)     | 34.7                                        |
| Dimetomorf         | 21.0                                        |
| Spiroxamine        | 32.8                                        |
| Fluopicolide       | 1.1                                         |
| Fosetyl-Aluminum   | 37.0                                        |
| Metrafenone        | 0.5                                         |
| Ciprodinil         | 1.3                                         |
| Fludioxonil        | 0.8                                         |
| Cyazofamid         | 0.5                                         |
chemical and spatial heterogeneities. Once mixed, soil samples were transferred into 20 liter lockable plastic containers and stored at 4 °C before any test.

One sampling campaign was performed, about 30 days after the last pesticides application. No temporal distributions were considered by this study, but rather an instantaneous characterization of sampled soil during a period of absence of pesticide application. Therefore, the toxicity of the sampled soil is assumed to be representative of the residual soil toxicity of the sampling area.

2.3. Physical and chemical characterization the sampled agricultural soil

The physical and chemical parameters of the sampled soil are presented in Table 3. The list of chemicals is defined as “contaminants of concern” and it is integrated with the PPPs specifically applied in the selected vineyard together with other common chemical products used in vineyards in North-East Italy.

2.4. Ecotoxicological testing

APAT guidelines [27] suggest a set of ecotoxicological bioassays in order to characterize a sampled matrix. Moreover, other guidelines [28, 29] were consulted for other available tests. In general, the choice about which ecotoxicological bioassay to be performed, and the endpoint to be assessed, was based on different factors such as sensitivity and response time, cost-effectiveness, ease of measurement and diagnostic ability.

Based on the above considerations, four different inhabiting soil invertebrates were selected and analyzed: Eisenia fetida (Savigny 1826) (Annelida: Lumbricidae), Folsomia candida Willem, 1902 (Collembola: Isotomidae), Caenorhabditis elegans (Maupas, 1900) (Nematoda: Rhabditidae) and Steinernema carpocapsae (Weiser, 1955) (Nematoda: Steinernematidae). The main features of the ecotoxicological tests performed are presented in Table 4.

As suggested by USEPA [30], test concentrations were chosen following a geometrical series having a ratio of 1.5. Therefore, eleven different concentrations were defined for ecotoxicological tests: 0% (control), 2%, 3%, 5%, 7%, 10%, 20%, 30%, 50%, 70% and 100%. Both direct (i.e. direct exposure of test organism to different concentrations of sampled soil) and indirect tests (i.e. exposure of organism using an eluate) were performed on these eleven concentrations. In direct tests, concentrations are expressed on a weight base (w/w referring to the dry matter) while in indirect tests they are expressed on a volume base (v/v).

Direct ecotoxicological tests were performed using different mixtures of the sampled agricultural soil and a standardized soil. This soil was artificially
composed according to the specific guidelines [27, 28, 31]. The standardized soil only was used as the control concentration.

Indirect ecotoxicological tests were performed using an eluate of the sampled agricultural soil. The eluate was obtained according to the specific guidelines [32], using a solid-to-liquid ratio of 1:10 (referring to the dry matter). Therefore, specific

Table 3. Physical and chemical properties of the sampled agricultural soil.

| Substance                          | Units     | Value | RL   | Method                                      | Background Concentration |
|------------------------------------|-----------|-------|------|---------------------------------------------|--------------------------|
| Humidity                           | %         | 19    | -    | IRSA-CNR Q64/84 vol. 2 n. 2                 |                          |
| Dry matter                         | %         | 81    | -    | IRSA-CNR Q64/84 vol. 2 n. 2                 |                          |
| pH                                 | -         | 6.99  | -    | IRSA-CNR Q64/84 vol. 3 n. 1                 |                          |
| Water Holding Capacity (WHC)       | %         | 113   | -    | OECD 222/2004 Annex 2                       |                          |
| TOC                                | % dm      | 2.2   | -    | UNI EN 13137                                |                          |
| Aluminum                           | mg/kg dm  | 24100 | 0.8  | EPA 6010 D 2014                             |                          |
| Antimony                           | mg/kg dm  | <RL   | 0.4  | EPA 6010 D 2015                             | 1.06                     |
| Arsenic                            | mg/kg dm  | 12.3  | 0.8  | EPA 6010 D 2016                             | 15.1                     |
| Barium                             | mg/kg dm  | 103   | 0.8  | EPA 6010 D 2017                             |                          |
| Berillium                          | mg/kg dm  | 1.41  | 0.4  | EPA 6010 D 2018                             | 1.07                     |
| Cadmium                            | mg/kg dm  | <RL   | 0.4  | EPA 6010 D 2019                             | 0.47                     |
| Chrome (Total)                     | mg/kg dm  | 39.9  | 0.8  | EPA 6010 D 2020                             | 49.9                     |
| Cobalt                             | mg/kg dm  | 14.4  | 0.8  | EPA 6010 D 2021                             | 12.4                     |
| Copper (Total)                     | mg/kg dm  | 80.4  | 0.8  | EPA 6010 D 2022                             |                          |
| Iron                               | mg/kg dm  | 17.4  | 1.7  | EPA 6010 D 2023                             |                          |
| Sulfur                             | mg/kg dm  | 290   | 20   | UNI EN 15309:2007                           |                          |
| Dieldrin                           | mg/kg dm  | <RL   | 0.01 | MP 1555 rev 1 2011                          |                          |
| Dimetomorf                         | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2011                          |                          |
| Spiroxamine                        | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2012                          |                          |
| Fluopicolide                       | mg/kg dm  | 0.041 | 0.01 | MP 1503 rev 1 2013                          |                          |
| Fosetyl-Aluminum (as etylfosfonic acid) | mg/kg dm | <RL   | 0.1  | MP 0940 rev 10 2015                         |                          |
| Metrafenone                        | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2013                          |                          |
| Ciprodinil                         | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2014                          |                          |
| Quinoxyfen                         | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2015                          |                          |
| Cyazofamid                         | mg/kg dm  | <RL   | 0.01 | MP 1503 rev 1 2014                          |                          |
| Pentachlorphenol                   | mg/kg dm  | <RL   | 0.01 | EPA 3550C 2007 + EPA 8082 A 2007            |                          |
| Sum of DDT/DDE/DDD                 | mg/kg dm  | <RL   | 0.01 | EPA 3550C 2007 + EPA 8270 D 2014            |                          |
| Sum of PAHs                        | mg/kg dm  | <RL   | 0.025| MP 1555 rev 1 2012                          |                          |
| Sum of PCBs                        | mg/kg dm  | <RL   | 0.003| EPA 3550C 2007 + EPA 8082 A 2007           |                          |

RL = detection limit.

*From Veneto Environmental Agency (ARPAV) [42].
**Table 4. Summary of ecotoxicological test performed.**

| Test                              | Organism               | Number per concentration | Replicates | Media                        | Dose       | Quantity (dm) per replicate | Duration | Temperature | L:D | L(lux) | WHC (%) | Endpoint | Method     |
|-----------------------------------|------------------------|--------------------------|------------|------------------------------|------------|------------------------------|----------|-------------|-----|--------|---------|----------|------------|
| **Plant bioassays**               |                        |                          |            |                              |            |                              |          |             |     |        |         |          |            |
| Seed Germination Bioassay (SGB)   | *Lepidium sativum*     | 10                       | 4          | Soil (sand)                  | Sampled    | 10 g                         | 72 h     | 25 ± 2      | 0.02| -      | 100     | Germination| APAT, 2004 |
| Seed Germination Eluate Bioassay  | *Lepidium sativum*     | 10                       | 4          | Solution (distilled water)   | Eluate (1:10) | 5 ml                         | 72 h     | 25 ± 2      | 0.02| -      | -       | Germination| APAT, 2004 |
| **Earthworm bioassays**           |                        |                          |            |                              |            |                              |          |             |     |        |         |          |            |
| Earthworm Chronic Bioassay (ECB_a)| *Eisenia fetida*       | 10                       | 4          | Soil (OECD soil)             | Sampled    | 500 g                        | 28 d     | 20 ± 2      | 0.67| 400–800 | 40      | Survivor | OECD 222/2004 |
| Earthworm Chronic Bioassay (ECB_b)| *Eisenia fetida*       | 10                       | 4          | Soil (OECD soil)             | Sampled    | 500 g                        | 28 d     | 20 ± 2      | 0.67| 400–800 | 40      | Growth    | OECD 222/2004 |
| **Collembola bioassay**           |                        |                          |            |                              |            |                              |          |             |     |        |         |          |            |
| Collembola Chronic Bioassay (CCB) | *Folsomia candida*     | 10                       | 4          | Soil (OECD soil)+ yeast      | Sampled    | 10 g                         | 28 d     | 20 ± 2      | 0.67| 400–800 | -       | Survivor | ISO 17512 guideline |
| **Nematode bioassays 1**          |                        |                          |            |                              |            |                              |          |             |     |        |         |          |            |
| Nematode Bioassay 1 (NB1)         | *Caenorhabditis elegans* | 10                      | 4          | Soil (OECD soil) + agar and *E.coli* | Sampled | 5 g                          | 24 h     | 20 ± 2      | Dark| 0        | 85      | Survivor | ASTM E2172-01, 2014 |
| **Nematode bioassays 2**          |                        |                          |            |                              |            |                              |          |             |     |        |         |          |            |
| Nematode Bioassay 2 (NB2)         | *Steinernema carpocapsae* | 10                      | 4          | Soil (OECD soil)             | Sampled    | 5 g                          | 48 h     | 20 ± 2      | Dark| 0        | -       | Survivor | - |
test concentrations (v/v) were obtained by different dilutions of the eluate with distilled water. The distilled water was used as the control concentration.

Plant bioassays involved phytotoxicity testing of the garden cress, *Lepidium sativum*, through two different methodologies, following the guidelines proposed by the Italian Environment Protection Agency (APAT) [27, 28].

The *Seed Germination Bioassay* (SGB) is a direct test. It was conducted by measuring the elongation of the emerged roots of seeds previously placed on a 10 g mixture of sampled soil and quartz sand.

The *Seed Germination Eluate Bioassay* (SGB_E) is an indirect test. It was conducted by measuring the elongation of the emerged roots of seeds previously placed on a dilution of distilled water and eluate of the sampled soil.

Both for SGB and for SGB_E, each concentration was tested in four replicates resulting in 44 tested samples and 440 cress seeds assessed. Sample preparations and test conditions for both the tests fully followed guideline prescriptions. The ecotoxicological parameter for both tests is expressed in terms of the Germination Index (GI in percentage respect the control) [27, 28].

Invertebrate bioassays explore the effect of direct exposure to the sampled agriculture soil to different soil-inhabiting taxa (Table 4).

The *Earthworm Chronic Bioassays* consisted of monitoring survival (ECB_a) and growth (ECB_b) of ten mature earthworms placed in 1.2L plastic containers filled with a 500 ± 5 g mixture of agricultural soil and standardized soil. Each concentration was tested in four replicates, thus resulting in 44 samples and 440 earthworms tested. The tests were performed according to the OECD 222/2004 [31] and the results were expressed as survival percentage (Su, %) and growth (Gr, %), which is defined as the percentage of change in weight from the initially inoculated earthworm weight.

*Collembola Chronic bioassay* (CCB) used the common springtail (*F. candida*) according to the ISO 17512 guideline [33]; survival percentage (Su, %) was considered as endpoint.

*Nematode Bioassay 1* (NB1) investigated the exposure effect to the bacterial feeder *C. elegans*. The test was carried out according to the ASTM guidelines E2172-01 [34] and to the principles of ISO 10872 [35]. Survival percentage (Su, %) was considered as the endpoint.

*Nematode Bioassay 2* (NB2) analyzed the direct exposure to the most used entomopathogenic nematode (EPN) in biological control and one of the most common species living in agricultural soil, *S. carpocapsae*. Monoxenic infective juveniles (IJ) of *S. carpocapsae* culture (Becker Underwood, Ltd) were used for the
bioassay. The toxicity test was carried out according to ASTM guidelines E2172-01 [34] and to ISO 10872 [35]. The results were expressed as survival percentage (Su, %).

2.5. Methods applied for the definition of ecological soil screening values

Two methods were performed for the definition of ecological soil Screening Values: the substance-based approach and the matrix-based approach.

When the first method was applied, Ecological soil SVs were individually assumed equal to the Predicted Non-Effect Concentrations (PNECs), determined for each individual chemical according to the “Technical Guidance Document on Risk Assessment” (TGD_EU) [8]. The assessment of the effects of tested chemicals relied upon literature data coming from four different institutionally acknowledged databases [36, 37, 38, 39]. Collected data referred to the following endpoints: growth, mortality and reproduction. Decomposers (microorganisms and earthworms), primary producers (plants), primary consumers (invertebrates) and crop-pest antagonists (entomopathogenic nematodes) were at the trophic levels considered within terrestrial toxicity data collected. Primary producers (algae), primary consumers (i.e. crustaceans) and fish were instead considered for aquatic toxicity, when no terrestrial data was available for the specific chemical.

Where terrestrial toxicity data was available, the PNECs for soil compartment were derived by applying the assessment factor method to the lowest ecotoxicological parameter collected, according to the following equation:

\[ PNEC_{SOIL} = \frac{\text{Chosen Ecotoxicological Parameter}}{\text{Assessment Factor}} \]

The use of the Assessment Factor (dimensionless) highlights the uncertainty of the defined extrapolation method. The chosen value for the chemical-specific AF was set according to the TGD_EU [8].

Where terrestrial toxicity data were not available (as for many metals) the TGD_EU suggests exploiting the Equilibrium Partitioning Method based on aquatic toxicity. In details, the PNEC relies on the following equation:

\[ PNEC_{SOIL} = \frac{K_{SOIL-WATER}}{\text{RHO}_{SOIL}} \times PNEC_{WATER} \times 1000 \left( \frac{mg}{kg \text{ dm}} \right) \]

Where:

- \( \text{RHO}_{SOIL} \) is the bulk density of a specific soil constituting the standardized environment as proposed by the TGD_EU [8];


- \( PNEC_{\text{WATER}} \) is obtained by applying the assessment factor method to the lowest ecotoxicological parameter between the aquatic toxicity data collected. The chosen value for the chemical-specific AF was set according to the TGD_EU [8].

- \( K_{\text{SOIL-WATER}} \) is the substance specific total soil-water partition coefficient, calculated according to the same TGD_EU guideline [8].

Table 5 shows the derived \( PNEC_{\text{soil}} \) values for the chemicals assumed as representatives of the spraying activity of plant production products.

The values used to define SVs for the sum of Poly Aromatic Hydrocarbons (PAHs) and sum of Polychloro-bifenils (PCBs) came from the soil screening values characterizing the human health risk assessment for residential use as established by the Italian Regulation about contaminated sites [2].

When the matrix-based method was applied, SVs for matrix-based approach were expressed as effect values (i.e. percentages), corresponding to different endpoints, considered as protective for the ecological functions under assessment (i.e. soil functions). One of the priorities of this study was the definition of this threshold value to be used within a screening ERA correspondent to a specific percentage of sampled agricultural soil, on a dry weight base. Therefore, the proposed approach was considered to be similar to the “whole effluent toxicity” as established by the Italian regulation [2] about toxicity testing of effluents from wastewater treatment plants, but applied to a soil screening ERA.

This study assumed that a soil matrix could be screened out from a more specific ERA (i.e. the related risk can be considered “acceptable”) if a sample consisting of 100% on a weight base does not cause a negative effect higher than 50% compared with the control. In this context, the control is defined as a media where no perturbations occur to the tested organisms. For example, if the Germination Index (GI, %) derived from plant bioassays coming from the samples representing the 100% concentration of tested soil resulted in equal or lower than 50% of the control, the assessed soil should be assumed to be “risky” at the screening level. However, each ecotoxicological bioassay tested 11 concentrations, thus leading to a series of eleven effect values. Efforts should be made to justify the reason why values derived from increasing soil concentrations do not show monotony. Equally the mode and the mechanism of action for each active substance in the mixture should be better investigated. This acceptable threshold had to be assessed for general endpoints of growth and survival, specifically for the three trophic levels considered by this study.

### 2.6. Risk characterization

Risk for the substance-based approach was characterized by calculating the Hazard Quotient (HQ) for single substances as the ratio between the Predicted
Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC) for the soil compartment. The PEC was assessed by physical-chemical laboratory analysis while the PNEC, defined equivalently as SV, was estimated according to the TGD_EU [8]. Where the risk characterization of a single chemical determines an HQ > 1, it entails the need of a further site-specific risk assessment to better investigate the specific risk posed by the responsible compounds.

Risk for a matrix-based approach was assessed by the comparison of the effects values, related to 100% (w/w dm) of the sampled soil and derived from the performed ecotoxicological bioassays (see Section 2.4), with the SVs for matrix-based approach as established in previous Section 2.5.

### Table 5. Calculated Hazard Quotients (HQi) for the soil compartment.

| Substance                  | CAS Number | PEC (mg/kg dm) | PNECsoil = SV (mg/kg dm) | HQ = PEC/SV |
|----------------------------|------------|----------------|--------------------------|-------------|
| Aluminum                   | 7429905    | 24,100         | 3.176                    | 7,588.161   |
| Antimony                   | 7440360    | 0.400<sup>*</sup> | 0.051                   | 7.843       |
| Arsenic                    | 7440382    | 12.300         | 7.535                    | 1.632       |
| Barium                     | 7440393    | 103.000        | 15.720                   | 6.552       |
| Berillium                  | 7440417    | 1.410          | 0.530                    | 2.660       |
| Cadmium                    | 7440439    | 0.400<sup>*</sup> | 0.030                   | 13.333      |
| Chrome (Total)             | 7440473    | 39.900         | 0.005                    | 7,980.000   |
| Cobalt                     | 7440484    | 14.400         | 6.000                    | 2.400       |
| Copper (Total)             | 7440508    | 80.400         | 0.105                    | 765.714     |
| Iron                       | 7439896    | 17.400         | 0.002                    | 8,700.000   |
| Sulfur                     | 7704349    | 290.000        | 0.976                    | 297.131     |
| Dieldrin                   | 2004845    | 0.010<sup>*</sup> | 0.003                   | 3.333       |
| Mancozeb                   | 8018017    | 0.010          | 0.020                    | 0.500       |
| Dimetomorf                 | 11048705   | 0.010<sup>*</sup> | 0.600                   | 0.016       |
| Spiroxamine                | 11813408   | 0.010<sup>*</sup> | 1.000                   | 0.010       |
| Fluopicolide               | 239110157  | 0.041          | 0.036                    | 1.138       |
| Fosetyl-Aluminum (as etylfosfonic acid) | 39148284 | 0.100<sup>*</sup> | 1.000                   | 0.100       |
| Metrafenone                | 220899036  | 0.010<sup>*</sup> | 0.040                   | 0.250       |
| Ciprodinil                 | 121552612  | 0.010<sup>*</sup> | 0.267                   | 0.037       |
| Quinoxyfen                 | 124495187  | 0.010<sup>*</sup> | 0.106                   | 0.094       |
| Fludioxonil                | 13141861   | 0.010<sup>*</sup> | 0.033                   | 0.303       |
| Cyazofamid                 | 124495187  | 0.010<sup>*</sup> | 0.106                   | 0.094       |
| Pentachlorphenol           | 87865      | 0.025<sup>*</sup> | 0.064                   | 0.390       |
| Sum of DDT/DDE/DDD         | 50293/72559/72548 | 0.010<sup>*</sup> | 0.061                   | 0.163       |
| Sum of PAHs                | –          | 0.010<sup>*</sup> | 10.000                  | 0.001       |
| Sum of PCBs                | –          | 0.003<sup>*</sup> | 0.060                    | 0.050       |

<sup>*</sup> PEC assumed equal to the Detection Limit (RL).
3. Results and discussion

3.1. Substance-based approach risk characterization

A HQ has been calculated for each tested chemical. Table 5 shows the estimated substance specific HQs.

Exposure values for each chemical are set equal to the concentrations obtained with the performed chemical analysis (PEC\(_i\)=C\(_i\)) (Table 3), while effect concentrations are assumed equal to the derived substance-based screening values (PNEC\(_{soil,i}\)=SV\(_i\)) (Table 5). Where concentration values have been detected under the Quantification Limit (RL), the exposure values are assumed equal to the specific Quantification Limits (if Ci < RLi, PECi=RLi), under a conservative assumption.

As it can be seen, no PECs for applied PPPs can be derived, since the specific concentrations are under the detection limits (<RL). This fact is probably a consequence of the timing of the sampling campaign which occurred a month after the last pesticide application. During the time that elapsed from the last application, pesticides may be transformed, transported or transferred by environmental dissipation processes such as degradation (photolysis, oxidation, biotransformation, etc.) or transport processes (rain-out, volatilization, transport with the matrix, bioconcentration, etc.) [40]. Furthermore, not all tested products were applied during the last pesticide application. Fluopicolide represents the unique exception, since a concentration of this substance could be detected. In addition, the use of high efficiency spraying machines exploiting anti-drift and recovery nozzles minimizes the active substances reaching the soil during application.

In Table 5, the results show that the totality of derived HQs for inorganic compounds is considered “risky” and in some cases (Aluminum, Chrome, Copper, Iron and Sulfur) “very risky” since the derived HQs resulted in various orders of magnitude higher than the unity. According to the assumptions of the substance-based approach, these results lead to the need for further site-specific assessment of each inorganic chemical considered. With the exception of Sulfur, the analyzed inorganics are metals. The resulting soil SVs of Aluminum, Antimony, Arsenic, Barium, Beryllium and Iron are derived, according to TGD_EU [8], by the Equilibrium Partitioning method due to the lack of terrestrial toxicity data. Moreover, no SVs for metals take into account the background concentrations characterizing the sampled soil. These facts could have consequences as an underestimation of the magnitude of the derived metallic soil SVs resulting in “unrealistic” HQs. However, the derived HQ for Cu and S could be considered realistic. In fact, high amounts of these chemicals were applied as plant protection products (PPPs), as reported in Table 2, thus resulting in specific PECs values high enough to justify the need of a further site-specific assessment. On the other hand,
the concentrations of organic chemicals and PPPs do not show risk or need of further assessments. This could be due to the very low concentrations detected or assumed. The majority of the derived soil SVs for these chemicals was derived through the Assessment Factor method, based on terrestrial toxicity data. The SV for dieldrin was instead established through the Equilibrium Partitioning Method, due to the absence of terrestrial toxicity data about this compound. Two exceptions have been recognized. The first is the case of dieldrin, as its derived HQ was higher than unity. This could be justified by the fact that the resultant SV was derived through the Equilibrium Partitioning method, which could have overestimated the value when extrapolating terrestrial sensitivity from freshwater toxicity data. Otherwise, the derived PEC could be set to an unrealistic high value thus leading to an overestimation of the specific HQ. The second exception is represented by the HQ for fluopicolide, which presents a value higher than unity. In this case, the specific PEC was only detected by the laboratory analysis and thus set at a more realistic value.

3.2. Matrix-based approach risk characterization

Seed Germination Bioassay (SGB) and Seed Germination Eluate Bioassay (SGB_E) showed that the analyzed soil cannot be considered as risky to a screening phase according to the assumed criteria for risk acceptability using the matrix-based approach. Both tests did not show germination index percentage (GI %) lower than 50%. SGB tests showed values around 80% for soil concentrations lower than 50% and higher than 100% for concentrations higher or equal to 50%. SGB_E tests resulted in values ranging between 80% and 130% in the investigated eluate concentration interval. It is worth mentioning that both series of results do not show any monotony.

The results based on Earthworm bioassay indicated that the tested soil cannot be considered as risky. No monotony can be detected in both series of measurements (survival and growth percentages); thus no further assessment needs to be made. Earthworm Chronic Bioassay (ECB_a) showed a survival percentage around 100% in the whole soil concentration interval; the minimum value, 95%, were obtained for concentrations of 2 and 3%. Earthworm Chronic Bioassay (ECB_b) showed a growth percentage around 80% for soil concentrations lower than 10% and in the remaining concentration range, the growth value were around 90–100%.

Collembola Bioassays and Nematode Bioassays 1 and 2 did not show any harm to these animals and the results agree with the previous tests considering the tested soil as not risky. In fact, there were no differences in the mortality and development of the tested species in the tested soil compared to the individuals living in the control soil. For all three species tested, percentage survival values corresponding to an exposure of 100% w/w of sampled soil was not recorded below
50%. The survival values remain approximately constant to 100% for all the concentrations except the concentration of 100% where a survival percentage of 90% was recorded.

3.3. Comparison of the two approaches

The two procedures used to generate screening risk assessments exploiting have generated conflicting results regarding the actions to be applied to the investigated soil.

The screening ERA implementing the substance-based approach highlights the need for a further site-specific assessment of two plant protection products (dieldrin and fluopicolide) and for all the inorganic compounds (metals and sulfur). This is probably due to the derived soil SVs which were characterized by a high level of conservatism since they do not take in account background concentrations of metals. The “Guidance Document on deriving Environmental Risk Limit” (RIVM) [9] proposes a solution to overcome this problem. SVs for metals should be derived as sums of the specific PNECs and the specific natural background concentrations. Moreover, the majority of soil SVs for inorganic compounds were derived according to the Equilibrium Partitioning Method based on aquatic toxicity because of a lack in terrestrial toxicity data as established by TGD_EU [8]. The extrapolation from aquatic toxicity to adverse toxic effects occurring in the soil compartment could have led to a much higher level of conservatism. This fact resulted in consequent need of a bigger terrestrial toxicity database about inorganic compounds (mostly about metals). Therefore, the derived results could not be taken as representative of site-specific conditions and thus they lead to an unrealistic estimation of risk, mainly for metallic compounds. On the other hand, the screening matrix-based ERA does not show the need for further level 2 risk assessment on the sampled soil. The defined SVs for this kind of assessment could probably be considered not conservative enough to preserve ecological functions of the soil compartment. These values were originally proposed by the authors to derive SVs “as much operative as possible”, i.e. easy to compare with results coming from ecotoxicological tests characterized by being cost-effective and easily implemented. However, despite a level of conservatism that could be criticized, the derived results were considered more realistic and site-specific due to the inherent properties of the approach (possibility of accounting for effective bioavailability, interactions between substances, etc.). Furthermore, it was considered as a starting point to deepen the scientific discussion about setting a soil SV for matrix-based ERAs that effectively protects the environment.

The two approaches can be considered comparable from an economic and time point of view. The chemical analysis for the “substance-based” approach can require 2–3 weeks with costs around 1,000 Euro, while the ecotoxicological tests
for the “matrix-based” approach can be completed within 4 weeks with costs around 800 Euro. Both approaches need to better address both temporal and spatial variability of the sampled soil to be tested. This means that derived soil ecological SVs should take in account appropriate exposure time for the toxicity test data. In fact, even if a good sampling design addresses spatial heterogeneity, temporal variations in concentrations represent a major challenge for ERAs. This is particularly important when assessing risk to the ecosystem characterized by frequent chemical inputs (as the case of the agroecosystems). For instance, exposures to PPPs are not dependent on constant but on pulsed concentrations, the peak and duration of which depend on the interactions between the PPPs and the soil [40, 41]. The current study removed the upper sward before collecting the soil to test, according to the proposed guidelines. However, this practice could have removed some sources of adverse effects to ecosystems, i.e. herbicide applied on vineyard upper soil. Moreover, vertical migration is a way of maintaining a balance between the possible mortality in upper layers (drought, predation) and reduced reproductive output resulting from less favorable feeding conditions in the lower layers [29]. Thus, upper soil and vertical movements especially directed to the soil upper layers should be accounted for when characterizing the ecotoxicologically relevant type of exposure concentration for in-soil organisms. Therefore, the characterization of the removed sward should be significantly addressed by future studies.

4. Conclusions

This study aimed to fill the gap in Italian legislation about contaminated soils and the use of a scientifically based procedure for deriving soil Screening Values (SVs) to be applied to Ecological Risk Assessments (ERA), especially for agricultural land-use. Two approaches were compared (i) the substance-based approach and (ii) the matrix-based approach. For the former, soil SVs were derived for single individual substances according to the methodology proposed by TGD_EU while for the latter, SVs were derived by the authors using a scientific approach that considered the whole-matrix. The derived screening values were then used to compare results coming from two screening ERAs applying the two approaches using a case study assessing agricultural soil sampled in a North-Eastern Italian area (in the province of Venice). The resulting screening risk assessments lead to results that are in contrast and show that the substance-based approach is much more conservative. Finally, it is possible to state that the “matrix-based approach” differentiates intrinsically from the institutionalized “substance-based approach”, since the latter can be defined as a “tabular-based” approach while the former can be defined as a “performance-based” approach.

Operatively, the substance-based approach characterizes the risk by just assessing a comparison between each derived PEC and a specific threshold (SV) that refers to a tabulated value established “a priori”. It means that no site-specific effect is
assessed. On the other hand, the matrix-based approach determines the risk by assessing the specific performance of the tested matrix which relies on causing a defined real (site-specific) effect. In conclusion, this work suggests the idea that the “matrix-based” approach can be efficiently implemented in Italian legislation for the ecological risk assessment of agriculture soils.

This method, if compared to the institutionalized “substance-based” approach:

- is comparable from an economic and time consumption point of view;
- is more site specific, assessing the real effect of the investigated soil to a battery of bioassays;
- accounts for phenomena that may radically modify the exposure of the organisms to the totality of contaminants (e.g. exposure to minor pollutants, chemicals partially adsorbed on soil particles, interactions between substances, etc.);
- can be considered sufficiently conservative for the environmental protection.

However, future developments could involve the need of ecotoxicological tests able of screening more trophic levels, interactions and different endpoints, in order to increase ecological significance and consider more ecosystem services. In this context, specific soil SVs could be derived for endpoints characterizing microorganism functions.

Declarations

Author contribution statement

Alberto Pivato, Maria C. Lavagnolo, Barbara Manachini: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stefano Vanin: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Roberto Raga: Analyzed and interpreted the data; Wrote the paper.

Giovanni Beggio: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.
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