



## Ecological risk assessment of pesticide residues in soils from vegetable production areas: A case study in S-Nepal



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### HIGHLIGHTS

- Risk quotient (RQ) > 1 was observed for 30% of the pesticides.
- Chlorpyrifos contributed to chronic toxicity of *F. candida*.
- About 16% of the fields showed risks due to chlorpyrifos and profenofos.
- Farmer's knowledge and behaviour was linked with RQ.

### GRAPHICAL ABSTRACT

Soil pesticides and their degradation products	3,5,6-TCP						
	Carbendazim						
	Chlorantraniliprole						
	Chlorpyrifos						
	DDT						
	Emamectin			NA	NA	NA	NA
	Imidacloprid						
	Metalaxyl						
	N-alanine			NA	NA		
	Profenofos						
Risk quotient (RQ)	RQ <sub>mx</sub>	RQ <sub>mn</sub>	RQ <sub>mx</sub>	RQ <sub>mn</sub>	RQ <sub>mx</sub>	RQ <sub>mn</sub>	
Soil depths (cm)	0-5		15-20		35-40		
No risk		Lower risk		Moderate risk		Higher risk	

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### ABSTRACT

Pesticides pose a serious risk to ecosystems. In this study, we used European Food Safety Authority methods, such as risk quotient (RQ) and toxicity exposure ratios (TER), to assess the potential ecological risks of 15 pesticide residues detected in agricultural soils in the Gaidahawa Rural Municipality of Nepal. The mean and maximum concentrations of the detected pesticide residues in the soil were used for risk characterization related to soil organisms. RQ<sub>mean</sub>, TER<sub>mean</sub> and RQ<sub>maximum</sub>, TER<sub>maximum</sub> were used to determine general and the worst-case scenarios, respectively. Of all the detected pesticides in soils, the no observed effect concentration (NOEC) for 27% of the pesticides was not available in literature for the tested soil organisms and their TER and RQ could not be calculated. RQ threshold value of  $\geq 1$  indicates high risk for organisms. Similarly, TER threshold value of  $\geq 5$ , which is acceptable trigger point value for chronic exposure, indicates an acceptable risk. The results showed that the worst-case scenario (RQ<sub>maximum</sub>) indicated a high risk for soil organisms from chlorpyrifos [RQ<sub>maximum</sub> > 9 at depths (cm) of 0–5, 15–20 and 35–40 soil layer]; imidacloprid (1.78 in the 35–40 cm soil layer) and profenofos (3.37 in the 0–5 cm and 1.09 in the 35–40 cm soil layer). Likewise, for all the soil depths, the calculated TER for both the general and worst-case scenarios for chlorpyrifos ranged from 0.37 to 3.22, indicating chronic toxicity to *F. candida*. Furthermore, the risk of organophosphate pesticides for soil organisms in the sampling sites was mainly due to chlorpyrifos, except for two study sites where the risk was from profenofos. Ecological risk assessment (EcoRA) of the pesticide use in the study area indicated that the EFSA soil organisms were at risk at some of the localities where farmers practiced conventional farming.

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## 1. Introduction

Over 4 million tons of pesticides are used annually worldwide (FAO, 2017). Unfortunately, this number is only expected to increase due to the burgeoning world population demanding more food from shrinking agricultural lands that suffer from declining soil quality. As if that wasn't enough, climate change and the emergence of new pests and diseases are throwing all kinds of new challenges into the mix (Brain and Anderson, 2019; Delcour et al., 2015; Xu et al., 2008). Modern farming methods rely on chemical pesticides to control insects and diseases, thereby improving food quantity. However, both the abundant use and in some cases, misuse of pesticides have contributed to soil pollution (Tsaboula et al., 2016). Research has discovered that pesticides can bioaccumulate and become biomagnified in soil, leading to even greater possible risks for the environment (Haj-Younes et al., 2015; Yuantari et al., 2015). There should be systematic monitoring of pesticide levels in soil that should include an evaluation of pesticide toxicity as well as an ecological risk assessment (EcoRA).

Many ecotoxicological studies have stated that pesticides can induce DNA injury, disturb hormone activity, decrease growth and survival rates, affect reproduction, alter individual food consumption, and diminish the density of earthworm communities (Jager et al., 2007; Uwizyimana et al., 2017; Wang et al., 2019). Pesticides in soils induced behavioural changes in some organisms thus affecting the environmental system and impairing predator-prey interactions (Dinh Van et al., 2014). A number of toxicological studies (Table S1) have confirmed that pesticides are harmful to soil fauna. According to the Regulation SANCO/10329/2002, the European Food Safety Authority (EFSA) recommended risk assessment methods such as risk quotient (RQ) and toxicity exposure ratios (TER) for soil organisms (EFSA et al., 2017). With these laws in place, ecotoxicological testing and systematic monitoring of pesticides in soil are being carried out in Europe and are slowly emerging from other parts of the world. Unfortunately, this testing is not yet a common occurrence in Nepal.

Pesticide application in Nepal has been increasing with the annual import going from 404 tons in 2012 to 635 tons in 2018 (CBS, 2019). The government of Nepal estimated the average application of pesticides to be about 396 g of active ingredients per ha in 2014 (PPD, 2014). About 80% of the imported pesticides were applied to vegetable fields (Adhikari, 2017). There were 169 types of active ingredients in pesticides registered and approved for use in agriculture in 2019 (GC and Neupane, 2019). Earlier studies have shown misuse and overuse of pesticides in agriculture (Aryal et al., 2014; Sharma et al., 2012), mainly in vegetable farming (Atreya et al., 2011; Chhetri et al., 2014). Bhandari et al. (2019) demonstrated human health risk due to consumption of vegetables in Nepal mainly due to organophosphate residues. Likewise, a similar study showed cancer risk in humans due to the presence of organochlorine residues in soils (Yadav et al., 2016). Unsustainable agricultural practices in Nepal expose soils to a mixture of pesticides that could decrease the country's rich biodiversity, which currently includes 17,097 known fauna species (MoFE, 2018). Soil biota such as bacteria, fungi, nematodes, earthworms, enchytraeids, microarthropods (springtails and mites), and insect larvae along with several other organisms help to maintain soil quality: structure and properties, pivotal functions and major ecosystem services. Soil health is of crucial importance as it determines the quality and quantity of food production, biodiversity, and resilience to climate change. Increased use of pesticides carries a greater risk to soil health that may destroy ecological cycles, including the breakdown of organic material, sequestration of carbon, cycling of nutrients, pest suppressiveness of soil and soil fertility (Keesstra et al., 2016; Lavelle et al., 2006).

For the sustainable management and responsible application of pesticides, ecological risk assessment (EcoRA) is necessary. The EFSA's risk assessment procedure mainly involves an assessment of exposure and a characterization of risk (Fig. S1). The risks posed by pesticides depend on the exposure concentrations and intrinsic (eco) toxicity, expressed

as Toxicity-Exposure- Ratio (TER). The TER is interpreted using trigger standards as defined in the EU Regulation No. 546/2011 (EC, 2018). TERs are identified for single assessments and single organisms. The TER explains the toxicity of a pesticide and provides an impression of the exposure estimates for each species separately. A trigger value equal to 5 represents "safety factors" for earthworms and other soil organisms. TER values <5 and  $\geq 5$  indicate high risk (unacceptable) and low risk (acceptable), respectively. The ratio of a measured soil concentration (MSC) or a predicted soil concentration (PSC) to a predicted no-effect concentration (PNEC) is used to calculate RQ (EC, 2002; Palma et al., 2014; Vasickova et al., 2019) which represents no risk ( $RQ < 0.01$ ), lower risk ( $0.01 \leq RQ < 0.1$ ), moderate risk ( $0.1 \leq RQ < 1$ ) and higher risk ( $RQ \geq 1$ ). Earlier studies conducted elsewhere used both the TER and the RQ to define an EcoRA for pesticides (Thomatou et al., 2013; Vasickova et al., 2019; Wee and Aris, 2017). However, there are scant scientific studies examining the ecological risks of pesticides on soil organisms in Nepal.

This study aims 1) to investigate the potential risk posed by pesticide residues following the EFSA's guidelines for soil organisms [including earthworms (*Eisenia fetida*), enchytraeids (*Enchytraeus crypticus*), springtails (*Folsomia candida*), and mites (*Hypoaspis aculifer*) as well as nitrogen and carbon mineralization microorganisms, which are the recommended invertebrate subjects for ecotoxicological studies (EFSA et al., 2017; Jänsch et al., 2006)]; and 2) to compute the correlation between pesticide risk and a farmer's knowledge about pesticide use and behaviour in the environment. This study provides the first evidence of the ecological risk of exposure to the most commonly applied pesticides in Nepal. The findings can be useful in developing effective pesticide risk mitigation strategies and national pesticide policy.

## 2. Materials and methods

### 2.1. Pesticide residues in the study area

In a previous study (Bhandari et al., 2020) we studied pesticide residues in soils (3 depths) from 11 integrated and 38 conventional vegetable farms of the Gaidahawa Rural Municipality in the Rupandehi district, Nepal. Soil samples were collected from farms where pesticides were not sprayed for 7 days during the vegetable growing winter season in 2017. Of the 23 pesticides analysed in our previous study, residues of 15 different pesticides were detected frequently and heavily in soils from conventional farms (Bhandari et al., 2020). Except nine pesticides that are banned in Nepal, the other pesticides were applied much higher doses than the recommended (Bhandari et al., 2018), henceforth selected for monitoring in our past studies (Bhandari et al., 2020, 2019). Because of time and budget constraints for pesticide analytics, we included 23 pesticides. Details of these pesticide residues are included here in Table S2. In this study we focus on the assessment of ecological risk of these residues. Detailed descriptions of the study area including the soil sampling points and the residues detected are stated in our previous paper (Bhandari et al., 2020). To the best of our knowledge, a residual limit for pesticides in soil has not yet been developed in Nepal. Therefore, we compared the measured concentration of pesticide residues with the guidance values established for different countries (Li and Jennings, 2017). The guidance values are the maximum concentration of individual pesticide residues present in soils posing no ecological risk.

### 2.2. Risk assessment

Pesticide EcoRA included an assessment of exposure and ecotoxicity (effects) (Fig. S1). We used two common methods: a) the TER for 4 selected species of the EFSA soil organisms (EC, 2002), and b) the risk quotient (RQ) for each pesticide residue (Renaud et al., 2018). When the no observed effect concentration (NOEC) value for a pesticide was available via systematic review, the RQ and TER were estimated to assess

chronic EcoRA. When the value was not known, the risk could not be assessed. Additionally, we compared pesticide concentrations in soils with pesticide soil regulatory guidance values (PSRGVs) to see if findings using the TER and the RQ methods corresponded with the guidance values. The concentrations of banned pesticides such as DDT and its principal metabolites in soils were compared with the existing threshold values for soils. A farmer's field was denoted as a "site". The risk of organophosphate in pesticide mixtures was estimated by adding up all the individual pesticide risks with a common mode of action (Damodaran, 2019) based on the concentration addition (CA) technique (Bundschuh et al., 2014). The risk of pesticide mixtures for other chemical groups could not be computed because ecotoxicity data was not available.

### 2.2.1. Assessment of exposure

The concentration of pesticide residues detected in the 3 depths (0–5, 15–20 and 35–40 cm) (Bhandari et al., 2020) was used for the risk assessment. Majority of our soil samples had their concentrations below the detection limit (<LOD), therefore geometric mean would be appropriate for risk assessment of pesticides (WHO, 2009). The geometric mean and maximum pesticide levels detected at the studied sites were used as the mean measured soil concentration ( $MSC_{mn}$ ) and the maximum measured soil concentration ( $MSC_{mx}$ ). In the first case, the value gives a general scenario (GS) ( $TER_{mn}$  or  $RQ_{mn}$ ) and, in the second case, the value gives the worst-case scenario (WS) ( $TER_{mx}$  or  $RQ_{mx}$ ) (Palma et al., 2014).

### 2.2.2. Assessment of toxicity

The toxicity assessment was based on the available ecotoxicological data from i) the pesticide properties database (PPDB) <https://sitem.herts.ac.uk/aeru/ppdb/>; ii) the draft assessment reports (DARs) from the EFSA; and iii) a basic literature search using the Web of Science Core Collection and Scopus databases. The search terms included:

- (i) Pesticide AND soil AND \*toxic\*
- (ii) Pesticide AND soil AND organism

A systematic review of literature was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009) for the assessment of toxicity including the effect of pesticides on soil organisms (Supplementary material, Fig. S2 and Table S1).

In order to assess the ecological risks of pesticides, there are several ecotoxicological aspects that can be examined such as the no observed effect concentration (NOEC) and/or the lethal concentration at which 50% of the examined organisms exhibit mortality ( $LC_{50}$ ), as well as a median effective concentration ( $EC_{50}$ ) for organisms such as the earthworm (*E. fetida*), the enchytraeid (*E. crypticus*), the springtail (*F. candida*), the mite (*H. aculifer*) and the nitrogen and carbon mineralization microorganisms. These are organisms that need to be included in any study assessing pesticides for approval by the EFSA for the European Union. Data from past studies that were in agreement with the Organisation for Economic Co-operation and Development (OECD) standardized procedures for the organisms was considered for the current study. Since the toxicological dose descriptors listed above differed with their corresponding ecotoxicological output, they could not be compared. Although pesticides detected in soil have their  $LC_{50}$  as well as  $EC_{50}$ , the present study was based on the available NOEC endpoints. If multiple NOEC values were available for a single organism, the geometric mean was considered and used. Of the 15 pesticides (Table S2), the NOEC for DDD, DDE, quinalphos and triazophos were not available for the tested species thus, the TER and RQ for these compounds could not be calculated.

Using the NOEC value, we derived the predicted no-effect concentration for the most sensitive species ( $PNEC_{mss}$ ). To overcome issues such as insufficient toxicity data, errors and inaccuracy related to the

conservative approach, the  $PNEC_{mss}$  value was estimated as the lowest long-term NOEC divided by the assessment factor (AF). The most susceptible organism for each pesticide was selected to obtain the  $PNEC_{mss}$  with an AF to account for potential chronic risks. The selection of the AF was based on the guidance document of the EU (EC, 2002) and could range from 10 to 1000: (i) an AF of 1000 was used in a case where at least one  $LC_{50}$  at one ecological level was available; (ii) an AF of 100 was used in a case where data from a long term assay was available; and (iii) an AF of 50 and 10 were used in the cases where two and three or more NOECs were available, respectively. In the present study, based on available long-term NOECs, we used an AF of 100, 50 and 10 (Table 1).

### 2.2.3. Risk characterization of a single pesticide

The most commonly used methods for assessing ecological risk are the Toxicity-Exposure- Ratio (TER) (EC, 2002) and the Risk Quotient (RQ) (Renaud et al., 2018). TERs based on NOECs for single test organisms, which included *E. fetida*, *E. crypticus*, *F. candida*, *H. aculifer* as well as nitrogen and carbon mineralization microorganisms were considered.

Based on EC (2009), the TER approach relates toxicity and exposure. As mentioned earlier, in the cases denoted as "≥ value" or "< value", the given value was used. The TER for each pesticide was estimated by using the TER for the test organisms ( $TER_{species}$ ) and the following Eq. (1).

$$TER_{species} = \frac{NOEC_{species}}{MSC_{maximum\ or\ mean}} \quad (1)$$

where, NOEC = No observed effect concentration and MSC = Measured pesticide concentration in soil.

The EC (2002) defined cut-off (trigger point) values of 5 and 10 for chronic and acute toxicity for soil organisms, respectively. Pesticide risk was considered negligible if the TER exceeded the cut-off values. TER values of  $\geq 10$  or  $\geq 5$ , which are acceptable trigger point values for acute and chronic exposure, respectively, indicated an acceptable risk for the organisms (Jaabiri Kamoun et al., 2017).

The risk quotient of a pesticide i ( $RQ_i$ ) provided an index for the risk of a single pesticide and was calculated as described in Eq. (2).

$$RQ_i = \frac{MSC_{soil}}{PNEC_{mss}} \quad (2)$$

where, MSC = Measured pesticide concentration in soil and  $PNEC_{mss}$  = Predicted no-effect concentration for the most sensitive species.

The classification of the risk quotient was based on the previous existing studies (Sánchez-Bayo et al., 2002; Vryzas et al., 2011): no risk ( $RQ < 0.01$ ), lower risk ( $0.01 \leq RQ < 0.1$ ), moderate risk ( $0.1 \leq RQ < 1$ ) and higher risk ( $RQ \geq 1$ ).

### 2.2.4. Risk characterization of pesticide mixtures-concentration using addition model

The widely accepted concentration addition (CA) approach was used to calculate the toxicity of pesticide cocktails (Vasickova et al., 2019; Wee and Aris, 2017). Multi-pesticide exposures can lead to additive actions. The mixture RQ ( $RQ_{mix}$ ) of organophosphates (OP) was estimated by adding up the individual  $RQ_i$  of each pesticide that belongs to the OP group. Furthermore, the total risk of multiple pesticide residues of a site ( $\sum RQ_{site}$ ) was estimated using the concentration addition (CA) based on the mixture risk assessment method (Bundschuh et al., 2014). CA, the most suitable model to use in ecotoxicological studies (Chen et al., 2014), is based on the assumption that all pesticides in a cocktail have the same mode of action and can be stated as in Eq. (3).

$$\sum (RQ_{site\ or\ RQ_{mix}}) = \sum_{k=1}^n RQ_i = \sum_{k=1}^n (MSC_i / PNEC_i) \quad (3)$$

where,  $RQ_{site}$  = Risk quotient of a site;  $RQ_{mix}$  = Risk quotient of pesticide mixtures;  $RQ_i$  = Risk quotient of a pesticide i;  $MSC_i$  = Measured

**Table 1**

Ecotoxicology (NOEC and NSDE in  $\mu\text{g}/\text{kg}$ ) of pesticides for *E. fetida* (earthworm), *E. crypticus* (enchytraeid), *F. candida* (springtail), *H. aculeifer* (mite), and N and C mineralization organisms extracted from different sources (see details in footnote). Degradation products of chlorpyrifos and metalaxyl such as 3,5,6-TCP and N-alanine indicated 3,5,6-trichloro-2-pyridinol and N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine, respectively. The N-alanine was referenced as CGA 62826 in the EFSA document and hence its NOEC was used in the calculation. PNEC = Predicted no-effect concentration; NOEC = No observed effect concentration; AF = Assessment factor.

Group	Compound	<i>E. fetida</i> , NOEC	<i>E. crypticus</i> , NOEC	<i>F. candida</i> , NOEC	<i>H. aculeifer</i> , NOEC	NSDE for N C mineralization microorganisms	Critical concentration	PNEC AF	PNEC <sub>mss</sub>
AD	Chlorantranilprole	1000000 <sup>d</sup>	na	390 <sup>d</sup>	100000 <sup>d</sup>	700 <sup>a,d</sup>  700 <sup>a,d</sup>	390	10	39
BD	Carbendazim	1000 <sup>a,d</sup>	100 <sup>b,i</sup>	na	na	4800 <sup>d,k</sup>  4800 <sup>d,k</sup>	100	10	10
MOD	Emamectin	2000 <sup>d</sup>	na	na	na	400 <sup>d,k</sup>  na	2000	50	40
NND	Imidacloprid	178 <sup>a</sup>	1000 <sup>g</sup>	1250 <sup>d</sup>	$\geq 2670$ <sup>d</sup>	na	178	10	17.8
OC	DDT	280000 <sup>a,h</sup>	na	176000 <sup>a,h</sup>	na	na	176,000	50	3520
OP	Chlorpyrifos	12700 <sup>a</sup>	5000 <sup>e</sup>	65 <sup>c</sup>	na	na 4800 <sup>a,d</sup>	65	10	6.5
	Profenofos	na	na	<50 <sup>f</sup>	na	250 <sup>j</sup>  na	50	50	1
PA	Metalaxyl	40000 <sup>a,d</sup>	na	125000 <sup>d,l</sup>	na	1350 <sup>d,k</sup>  1350 <sup>d,k</sup>	40,000	10	4000
UNC	3,5,6-TCP	4600 <sup>a,d</sup>	na	na	na	4150 <sup>d,k</sup>  4150 <sup>d,k</sup>	4600	50	92
	N-alanine	500000 <sup>d</sup>	na	na	na	na	500,000	100	5000

Data stated as "≥"number or "<"number, the given number was used in a calculation.

PNEC<sub>mss</sub> = the lowest long-term NOEC of the most susceptible species/AF.

na = Information on toxicity was not available in the refereed databases.

NSDE = No significant adverse effect.

In the case of metalaxyl, NSDE data for ridomil gold was used.

Pesticides group: AD = Anthranilic diamide; UNC = Unclassified; OP = Organophosphate; NND = Neonicotinoid; OC = Organochlorine; BD = Benzimidazole; PA = Phenylamide; MOD = Micro-organism derived.

<sup>a</sup> (Lewis et al., 2006).

<sup>b</sup> (Novais et al., 2010).

<sup>c</sup> (Herbert et al., 2004).

<sup>d</sup> Data collected from the EFSA documents that are available online.

<sup>e</sup> (Carniel, 2019).

<sup>f</sup> (Liu et al., 2012).

<sup>g</sup> (de Lima et al., 2017).

<sup>h</sup> (RIVM, 2015).

<sup>i</sup> Data for *Enchytraeus crypticus* was not found in literatures, hence NOEC of carbendazim is used for *Enchytraeus albidus*.

<sup>j</sup> Nitrification rate.

<sup>k</sup> <25% effect considered as NSDE.

<sup>l</sup> Toxicity data of metalaxyl to *Folsomia candida* was not available, hence the data of ridomil was used in the calculation.

soil concentration of a pesticide  $i$ ; PNEC <sub>$i$</sub>  = Predicted no-effect concentration of a pesticide  $i$ ;  $n$  = number of pesticides.

The classification of RQ<sub>mix</sub> was based on Sánchez-Bayo et al. (2002) and Vasickova et al. (2019) as mentioned for the RQ above.

To correlate the classified RQ at sites where the pesticide knowledge and behaviour of farmers was known, we developed a summative score based on farmer's replies to the survey questions (Table 2).

The contribution of each RQ <sub>$i$</sub>  to RQ<sub>site</sub> or RQ<sub>mix</sub> was derived following Eq. (4).

$$\% \text{ contribution} = \left( \frac{RQ_i}{\sum (RQ_{\text{site}} \text{ or } RQ_{\text{mix}})} \right) \quad (4)$$

where, RQ <sub>$i$</sub>  = Risk quotient of a pesticide  $i$ ; RQ<sub>site</sub> = Risk quotient of a site; RQ<sub>mix</sub> = Risk quotient of pesticide mixtures.

**Table 2**

Variables ( $n=6$ ) for farmers pesticide use KNB score. Results of the questionnaire survey among the farmers was based on our previous study (Bhandari et al., 2018). Commercial vegetable farmers from different villages were selected for the survey conducted in 2017. Of the 183 farmers, this study included the scores of 49 farmers that were selected randomly.

Variables	Description	KNB values (1 or 0)
ANIMALS	Do pesticides negatively affect animals?	0=no, 1=yes
BIRDS	Do pesticides negatively affect birds around you?	0=no, 1=yes
FISHES	Do pesticides negatively affect fishes?	0=no, 1=yes
HONEYBEES	Do pesticides negatively affect honeybees?	0=no, 1=yes
PESCONT	Do you throw pesticides container at field after use?	0=yes, 1=no
UNUSEDPES	Do you throw unused/leftover pesticides at field?	0=yes, 1=no

### 2.3. Statistical analysis

Data analysis on the concentration of pesticides  $\geq$ LODs was performed. Data entries where pesticide concentrations were <LODs were left empty and excluded from the study (Sun et al., 2016). We used the Spearman's rho correlation coefficient to calculate the linear correlation of the risk quotient (RQ<sub>site</sub>) calculated for the soil depths (0–5, 15–20 and 35–40 cm) and the knowledge and behaviour (KNB) of farmers. A  $p$ -value less than 0.05 was considered significant. The correlation was also used to demonstrate if there was a positive relationship between the risks of pesticides at different sites and the farmers' pesticide KNB scores. The score was based on the sum of values from each variable listed in Table 2.

## 3. Results

### 3.1. Risk assessment

#### 3.1.1. Ecological risk based on the TER approach

Data from ecotoxicological tests conducted following the ISO/OECD procedures were considered for this research. Most ecotoxicological information about pesticides included data for only a single organism. Toxicological information for several detected pesticides was unavailable and thus assessment of their potential ecological risk was not possible. Data on the NOEC for *E. fetida* was available for 60% of the 15 detected pesticides (Table 1). In Nepal, ecotoxicological studies have not been carried for many of the pesticides that have been approved for agricultural use. However, ecotoxicological studies from other countries have been used (Table S1).

The toxicity exposure ratio (TER) for a general scenario (GS) and a worst-case scenario (WS) are presented in Tables 3a–3c. For both

scenarios, chronic risk for *E. fetida*, *E. crypticus*, and *H. aculifer* was negligible for all assessed pesticides at all the depths of soil, except chlorpyrifos. Both  $TER_{mx}$  and  $TER_{mn}$  calculated for different depths were above the cut-off value of 5 for chronic toxicity. Pesticides such as carbendazim, chlorantraniliprole, DDT, emamectin, metalaxyl and N-alanine notably showed high TERs at different depths. Of all pesticides, chlorantraniliprole showed the highest TER ( $TER = 494,430$ ) at 35–40 cm for *E. fetida* under the GS.

The calculated TER of chlorpyrifos for GS and WS at all depths of soil was lower than the corresponding trigger value which indicated potential risk ( $TER < 5$ ) to non-target soil organisms. It has been observed that  $MSC_{mx}$  and  $MSC_{mn}$  of chlorpyrifos posed a risk to *F. candida* based on  $TER_{mx}$  and  $TER_{mn}$ , respectively. Furthermore, chlorpyrifos showed the lowest TER ( $TER = 0.37$ ) at 0–5 cm for *F. candida* under the WS.

### 3.1.2. Ecological risk based on RQ approach

The risk quotient (RQ) values under GS and WS for the studied pesticides are shown in Table 4. Chlorpyrifos in all the depths showed RQs  $> 1$  for both MSCs geometric mean and MSCs maximum. Profenofos showed RQs  $> 1$  for both MSCs at 0–5 cm and 35–40 cm depths. In addition to chlorpyrifos and profenofos, our results suggested a potential risk of imidacloprid to soil organisms at 35–40 cm under the WS. Due to the higher RQ values at all depths and scenarios, the risk posed by chlorpyrifos was worrisome. The top 3 pesticides ranked in decreasing order of their toxicity to the in-soil organisms were: chlorpyrifos  $>$  profenofos  $>$  imidacloprid, indicating the highest toxicity for organophosphates (OPs).

Furthermore, 84% of the sites represented no risk ( $\sum RQ_{site} < 0.01$ ) and 16% showed a higher risk ( $\sum RQ_{site} \geq 1$ ). The highest risk based on  $RQ_{site}$  was 30.60 for a conventional farmer's site with the identification code F53 and was indicated for the depth of 15–20 cm (Table S3). All IPM sites such as F4, F5, F7, F32, F36, F56, F62, F102, F109, F143 and F158 showed no risk ( $RQ < 0.01$ ). The contribution for the RQ was 98% and 2% for chlorpyrifos and profenofos, respectively. Table S3 presents pesticide contributions measured at 8 sites with a higher risk ( $\sum RQ_{site} \geq 1$ ) to the overall risk. Although multiple pesticide residues were observed at a single site, this doesn't infer that pesticides contributed equally to the overall risk posed by the pesticide cocktail. Table S3 shows that chlorpyrifos contributed higher than profenofos to the overall risk at one site ( $\sum RQ_{site}$ ). The RQ of OPs at depths (cm) 0–5, 15–20 and 35–40 ranged from 0 to 11, 0 to 31 and 0 to 9, respectively (Table 5).

Overall, both the RQ and TER methods seemed conservative as the  $PNEC_{mss}$  applied an assessment factor of 10–1000 due to the scarcity of NOEC values and thus considered as the worst-case scenarios. For example, in this study, we considered the  $RQ_{mn}$  with geometric mean of positives only, and the  $RQ_{mx}$  with maximum measured soil concentration. Among the 15 compounds that were detected (Table S2), a higher risk ( $RQ_{mx} > 1$ ) was observed for imidacloprid, profenofos and

chlorpyrifos, while a higher risk for sites ( $\sum RQ_{site} > 1$ ) was posed by chlorpyrifos and profenofos only (Table S3).

### 3.2. Perspectives on pesticide risk and farmers' knowledge and behaviour (KNB)

Data on farmers' knowledge and behaviour originated from our previous study (Bhandari et al., 2018). The knowledge score (mean  $\pm$  SD) of farmers about the effect of pesticides on animals, birds, fishes and honeybees was  $0.84 \pm 0.37$ ,  $0.51 \pm 0.51$ ,  $0.59 \pm 0.50$  and  $0.51 \pm 0.51$ , respectively. Likewise, the behaviour score (mean  $\pm$  SD) of farmers concerning pesticide waste management and correct application was  $0.45 \pm 0.50$  and  $0.73 \pm 0.45$ , respectively. The knowledge score ranged from 1 to 4 and the behaviour score ranged from 1 to 2. About 45% of the farmers managed their pesticide packets/containers by burning the waste at a designated area, while 73% of the farmers kept the leftover pesticides and reused them. During a visit to a farmer's field, packets and containers of pesticides were observed simply discarded on the ground, which was unsafe and indicated poor hygiene in fields (Fig. 1). The total KNB score (mean  $\pm$  SD) for farmers was  $3.63 \pm 1.81$ , ranging from 1 to 6. The score indicated a level of awareness of farmers related to pesticide effects and waste management.

The Spearman's rho correlation between the KNB score and the risk quotient (RQ) of pesticides at different farm sites is shown in Table 5. The pesticide risk at sites was negatively correlated ( $p < 0.01$ ) with the KNB score, and the correlation coefficients at depths (cm) 0–5, 15–20 and 35–40 were  $-0.44$ ,  $-0.60$  and  $-0.38$ , respectively. A significant positive correlation was observed among RQs of organophosphate at depths (cm) 0–5 and 15–20 as well as 15–20 and 35–40.

## 4. Discussion

### 4.1. Pesticide residues in the soils

Although EFSA equations have been used for estimating predicted pesticide environmental concentrations (PEC) (EFSA et al., 2017), the use of real measured concentrations in the agricultural fields (MSC) instead of modelled (PEC) for the pesticide EcoRA provides significant benefits since it gives an accurate measurement of pesticides and includes the inherent heterogeneity of ecosystems (ECOFRAM, 1999). The use of modelled PEC data in the pesticide evaluation process has also been criticized due to its limitation on reflecting the dissipation of pesticides in the environment (Vasickova et al., 2019). Due to higher application rates of pesticides, PEC of most pesticides were much higher than their MSC, indicating that the risk of pesticides could be overestimated due to the use of PEC in the risk assessment processes (Bhandari et al., 2020).

**Table 3a**

Maximum and mean toxicity exposure ratios ( $TER_{mx}$  and  $TER_{mn}$ ) for soil organisms at 0–5 cm. TER values  $< 5$  (in bold) and  $> 5$  indicated risk and no risk, respectively. WS = worst scenario and GS = general scenario.  $MSC_{mx}$  and  $MSC_{mn}$  indicated the maximum and mean concentration of pesticides in soils (in  $\mu\text{g}/\text{kg}$ ), respectively. DDT represented the sum of p, p'-DDT and o, p'-DDT concentrations. "NA" = not applicable. Degradation products such as 3,5,6-TCP and N-alanine indicated 3,5,6-trichloro-2-pyridinol and N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine, respectively.

Pesticides	$MSC_{mx}$	$MSC_{mn}$	E. fetida Chronic		E. crypticus Chronic		F. candida chronic		H. aculeifer chronic	
			$TER_{mx}$ (WS)	$TER_{mn}$ (GS)	$TER_{mx}$ (WS)	$TER_{mn}$ (GS)	$TER_{mx}$ (WS)	$TER_{mn}$ (GS)	$TER_{mx}$ (WS)	$TER_{mn}$ (GS)
3,5,6-TCP	57.4	8.69	80.1	529	NA	NA	NA	NA	NA	NA
Carbendazim	6.45	1.62	155	617	15.5	61.7	NA	NA	NA	NA
Chlorantraniliprole	14.2	3.23	70,423	309,723	NA	NA	27.5	121	7042	30,972
Chlorpyrifos	177	32.5	71.8	391	28.3	154	<b>0.37</b>	<b>2.00</b>	NA	NA
DDT	5.41	3.53	51,756	79,330	NA	NA	32,532	49,865	NA	NA
Emamectin	3.30	3.30	606	606	NA	NA	NA	NA	NA	NA
Imidacloprid	13.8	3.94	12.9	45.2	72.5	254	90.6	317	193	678
Metalaxyl	6.49	2.44	6163	16,382	NA	NA	19,260	51,194	NA	NA
N-alanine	2.49	2.49	200,803	200,803	NA	NA	NA	NA	NA	NA
Profenofos	3.37	1.74	NA	NA	NA	NA	14.8	29	NA	NA

**Table 3b**

The TER at 15–20 cm. The pesticide concentration in µg/kg. N-alanine and profenofos residues were not detected at the depth, hence did not appear here. TER values <5 (in bold) and >5 indicated risk and no risk, respectively.

Pesticides	MSC <sub>mx</sub>	MSC <sub>mn</sub>	E. fetida Chronic		E. crypticus Chronic		F. candida Chronic		H. aculeifer Chronic	
			TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)
3,5,6-TCP	15.9	7.26	289	633	NA	NA	NA	NA	NA	NA
Carbendazim	5.55	2.01	180	498	18.0	49.8	NA	NA	NA	NA
Chlorantraniliprole	4.22	2.06	236,967	485,546	NA	NA	92.4	189	23,697	48,555
Chlorpyrifos	68.4	20.2	186	629	73.1	248	<b>0.95</b>	<b>3.22</b>	NA	NA
DDT	29.8	7.98	9402	35,088	NA	NA	5910	22,055	NA	NA
Imidacloprid	7.57	2.06	23.5	86.6	132	486	165	608	353	1299
Metalaxyl	4.23	2.67	9456	14,961	NA	NA	29,551	46,752	NA	NA

Of all pesticides, chlorpyrifos showed the highest concentration at all the depths (177 µg/kg). DDT, an organochlorine insecticide has been banned for use in Nepal since 2001 (PQPMC, 2019), however its residues were detected at all the depths as they degrade very slowly in the environment (Boul, 2010). A study conducted in Pakistan showed the highest concentration (125,000 µg/kg) of ∑DDT in top soils (Ullah et al., 2019). Residues of HCH and DDT were detected at concentrations 6.12 µg/kg and 1.85 µg/kg, respectively in bottom soils from Hong Kong (Zhang et al., 2006). At the 35–40 cm depth, residues of N-alanine were more frequently detected. Risk was due to higher concentrations of DDT and endosulfan, the frequently occurring residues in soil (Yadav et al., 2016). Most frequently detected pesticides in Chinese agricultural topsoil were imidacloprid and emamectin benzoate (Tan et al., 2020). The presence of several pesticides in soil increased the ecotoxicity and caused several effects (Table S1), including the death of non-target soil organisms (Cang et al., 2017; Tiwari et al., 2019).

#### 4.2. Ecological risk based on TER, RQ including threshold and guidance values

Ecotoxicological data is a prime requisite when performing studies on EcoRA (Frampton, 2000). The ecotoxicity was especially high due to chlorpyrifos residues in soils (Tables 3a–3c). Thomatou et al. (2013) demonstrated a non-acceptable ecological risk due to chlorpyrifos methyl in Greece. In the same study, pesticides with the highest ecotoxicity were organophosphates (OPs). The major contributors to ecotoxicity in many different studies were triazoles (Vasickova et al., 2019), OPs (Wee and Aris, 2017), triazines and OPs (Palma et al., 2014), and triazoles, carbamates and neonicotinoids (Xu et al., 2020). In our study, OPs most notably contributed to the ecological risk, while the other groups didn't.

The ecological risk based on TER under general scenario (GS) and worst-case scenario (WS) is shown in Tables 3a–3c. Of all pesticides and soil organisms, chlorpyrifos exhibited higher risk under both scenarios for *F. candida* due to its longer persistence in a tropical soil (Watts, 2012). The higher risk might be due to higher concentrations

of chlorpyrifos and its lower NOEC. Chlorpyrifos was indicated as one of the most acutely as well as chronically toxic pesticides for soil organisms (USEPA, 2009). In Table 3c, the TER<sub>mx</sub> value of *E. fetida* at 35–40 cm under the WS (i.e. 5.63) was observed closer to its trigger point value (i.e. 5) which might be due to its chronic exposure to imidacloprid (EC, 2011). In a multi-level ecotoxicological study, Wang et al. (2019) also demonstrated a toxicity of imidacloprid for *E. fetida*. Of all pesticides and organisms examined in a previous study (de Lima et al., 2017), imidacloprid was found to be the most toxic compound to *F. candida*, which is known to be very susceptible to pesticides and is one of the most susceptible organisms among soil invertebrates (Fountain and Hopkin, 2005). Although chlorantraniliprole was applied in higher doses than recommended on vegetables such as chillies (Bhandari et al., 2018), it showed greater TER values (Tables 3a–3c), indicating negligible risk at all depths of soil. However, the risk assessment of the pesticide applied to a fruiting vegetable by EFSA presented a chronic risk to *F. candida* (TER = 3) (EFSA, 2013). In the same study, the ecotoxicity of chlorantraniliprole was observed for *F. candida* in fruits such as grapes (TER = 1.9) and pomes (TER = 1.4).

Our study presented higher risks (RQs > 1) for individual compounds such as chlorpyrifos and profenofos for both scenarios: general and worst-case. In a previous study on risk assessment, chlorpyrifos presented a similar trend (Wee and Aris, 2017). Profenofos contributed higher risk for 3 sites and the risk ranged from 2 to 100%. However, the major contribution to the higher risk for 6 sites was only for chlorpyrifos and ranged from 98 to 100%. A similar study (Chen et al., 2020b) indicated that chlorpyrifos and butachlor were the main pollutants. In the arable soils of the Czech Republic, Vasickova et al. (2019) identified that 11% of sites had no risk and 35% of sites had higher risk. One study reported a higher risk in 29% of the sites, mainly due to the use of chemical pesticides that have been banned in Europe (Iturburu et al., 2019). In our study, chlorpyrifos significantly contributed to the overall risk of a site (∑RQ<sub>site</sub>), a finding similar to a previous study (Wee and Aris, 2017). Chlorpyrifos, profenofos and imidacloprid have an ability to persist in soil (Lewis et al., 2006) and were used in higher doses than the recommended (Bhandari et al., 2018); all of which

**Table 3c**

The TER at 35–40 cm. The pesticide concentration in µg/kg. TER values <5 (in bold) and >5 indicated risk and no risk, respectively.

Pesticides	MSC <sub>mx</sub>	MSC <sub>mn</sub>	E. fetida Chronic		E. crypticus Chronic		F. candida Chronic		H. aculeifer Chronic	
			TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)	TER <sub>mx</sub> (WS)	TER <sub>mn</sub> (GS)
3,5,6-TCP	31.1	7.26	148	634	NA	NA	NA	NA	NA	NA
Carbendazim	3.41	2.23	293	448	29.3	44.8	NA	NA	NA	NA
Chlorantraniliprole	6.52	2.02	153,374	494,430	NA	NA	59.8	193	15,337	49,443
Chlorpyrifos	60.6	26.4	210	481	82.5	189	<b>1.07</b>	<b>2.46</b>	NA	NA
DDT	81.1	13.5	3454	20,734	NA	NA	2171	13,033	NA	NA
Imidacloprid	31.6	3.35	5.63	53.2	31.7	299	39.6	373	84.5	798
Metalaxyl	8.97	2.41	4459	16,582	NA	NA	13,935	51,818	NA	NA
N-alanine	1.56	1.32	320,513	379,967	NA	NA	NA	NA	NA	NA
Profenofos	1.09	1.09	NA	NA	NA	NA	45.9	45.9	NA	NA

**Table 4**

The Risk Quotient (RQ) for the most sensible organisms at different depths of soil. RQ values for an individual pesticide at the depth calculated as a ratio of the measured soil concentrations [(MSC<sub>mx</sub> or mn) µg/kg] divided by the PNEC<sub>ms</sub> µg/kg (reported in Tables 3a–3c and 1). WS = worst scenario and GS = general scenario. The calculated values of RQ were categorised into 4 risk levels: no risk (RQ<0.01), lower risk (0.01≤RQ<0.1), moderate risk (0.1≤RQ<1) and higher risk (RQ≥1). RQ values in bold indicated higher risk. DDT represented the sum of p, p'-DDT and o, p'-DDT concentrations. "NA" = not applicable. Degradation products such as 3,5,6-TCP and N-alanine indicated 3,5,6-trichloro-2-pyridinol and N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine, respectively.

Pesticides	Risk quotient (RQ)					
	0–5 cm		15–20 cm		35–40 cm	
	RQ <sub>mx</sub> (WS)	RQ <sub>mn</sub> (GS)	RQ <sub>mx</sub> (WS)	RQ <sub>mn</sub> (GS)	RQ <sub>mx</sub> (WS)	RQ <sub>mn</sub> (GS)
3,5,6-TCP	0.62	0.09	0.17	0.08	0.34	0.08
Carbendazim	0.65	0.16	0.56	0.20	0.34	0.22
Chlorantraniliprole	0.36	0.08	0.11	0.05	0.17	0.05
Chlorpyrifos	<b>27.23</b>	<b>4.99</b>	<b>10.52</b>	<b>3.10</b>	<b>9.32</b>	<b>4.06</b>
DDT	<0.01	<0.01	0.01	<0.01	0.02	<0.01
Emamectin	0.08	0.08	NA	NA	NA	NA
Imidacloprid	0.78	0.22	0.43	0.12	<b>1.78</b>	0.19
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N-alanine	<0.01	<0.01	NA	NA	<0.01	<0.01
Profenofos	<b>3.37</b>	<b>1.74</b>	NA	NA	<b>1.09</b>	<b>1.09</b>

might have caused moderate to higher individual ecological risk (RQ). Li et al. (2018) also estimated lower to moderate ecological risks due to simazine. As described by Montuori et al. (2016), the RQ values may be less relevant in other regions because of the variation in seasons, agricultural practices, contamination levels, and distribution of pollutants. For all the pesticides that showed higher risk (RQ > 1), a future site-specific risk assessment is required to better understand the risks of specific pesticides (Pivato et al., 2017). It is noteworthy to mention that all of the sites that were from the integrated farming had no risk ( $\sum RQ_{site} < 0.01$ ); however, 21% of the sites from conventional farming had high risk ( $\sum RQ_{site} \geq 1$ ).

The threshold value of DDT in soil (µg/kg) for the safety of soil organisms is 10 (Jongbloed et al., 1996). The Dutch ecological limit for DDT as well as DDD and DDE in soil is 10 µg/kg (RIVM, 2015), the maximum permissible concentration in soil based on direct ecotoxicology. The DDT (o, p'-DDT + p, p'-DDT) mean concentration in 35–40 cm from our study area was above 10 µg/kg (Table 3c), indicating the higher ecotoxicity associated with DDT. Furthermore, the mean concentration of p, p'-DDD at the same depth was slightly greater than the Dutch standard for DDD in soil (Table S2), which might affect soil organisms. However, the mean concentration of p, p'-DDE in soils did not exceed the standard, indicating no risk. The high TER and low RQ values for DDT in Tables 3a–3c and 4, respectively, further supported that the risk was either negligible or lower. Our previous study (Bhandari et al., 2020) indicated that fungicides such as carbendazim and metalaxyl and insecticides such as chlorpyrifos and imidacloprid had concentration in soils below the guidance values (PSRGVs). However, the current

**Table 5**

Correlation matrix between risk quotient (RQ<sub>site</sub>) of organophosphates (OPs) and knowledge and behaviour (KNB) score at different depths (cm) of soil (n=49). Descriptive statistics of the variables in parenthesis. Values in bold represented either positively or negatively correlated.

Variables (min–max; mean±SD)	RQ_0–5	RQ_15–20	RQ_35–40	KNB
RQ_0–5 (0–11; 0.34±1.57)	1	<b>0.57*</b>	0.27	<b>–0.44*</b>
RQ_15–20 (0–31; 0.96±4.45)		1	<b>0.62*</b>	<b>–0.60*</b>
RQ_35–40 (0–9; 0.25±1.36)			1	<b>–0.38*</b>
KNB (1–6; 3.63±1.81)				1

\* Correlation is significant at the 0.01 level (2-tailed). OPs included in the analysis were profenofos and chlorpyrifos.

EcoRA showed moderate to high risks to soil organisms from the aforementioned pesticides and did not converge with the guidance values, except for metalaxyl which showed negligible risks. Different methods and databases used in the EcoRA may preclude the effective comparison among the estimated risks (Wang et al., 2009), henceforth our results are less consistent. In the PSRGVs, Ukraine considered 1 µg/kg as a benchmark for the concentration of profenofos in soil. The profenofos concentrations in 3% of our soil samples were above this benchmark, indicating that ecological risk associated with profenofos pollution should be considered. Furthermore, the PSRGVs for 3,5,6-TCP, N-alanine and chlorantraniliprole were not found, hence their estimated risk (TER and RQ) could not be compared.

Pesticide residues and their mixtures in soil have adverse effects on plants and animals. Removal of these residues from agricultural soils requires time, budget and technology. Sun et al. (2018) summarized pollution remediation sustainable methods (i.e. physical, chemical and biological) in agricultural soils. The efficiency of such methods depends upon several factors such as pesticide properties and their concentrations, climatic conditions and soil types. Developed nations have adopted such technologies for the reclamation of degraded lands (Ashraf et al., 2019; Ayangbenro and Babalola, 2021). Research works on pesticide residues are scarce and there are no any regulations and frameworks for the reclamation of polluted sites in developing countries like Nepal. Therefore, it is hard to recommend suitable reclamation methods for the remediation of pesticides pollution in agricultural soil. Nevertheless, this study recommends future studies focusing on the distribution of pesticide residues and their ecological interactions in Nepalese environment. Furthermore, the country should initiate research on in-situ bioremediation methods such as composts, microorganisms and plants for pollution prevention and control.

#### 4.3. Risk quotient and its relationship with farmers' knowledge and behaviour (KNW)

Farmers are the end-stakeholders of pesticides. Their KNW about safe use of chemical pesticides prevents the release of hazardous pesticides and pesticide containers to the environment. Farmers KNW about safe disposal of pesticides waste can play a crucial role in increasing the environmental safety (Yang et al., 2014). Farmers who had poor knowledge about pesticides and associated risks showed improper waste management methods in Iran (Sharafi et al., 2018). A significant correlation ( $p < 0.01$ ) existed in our study between pesticide risk (RQ) at a farm site and the farmer's KNW related to the effects and management of pesticides. Farmers who were aware of the ecological effects of pesticides and the appropriate waste management measures that should be taken had a negligible pesticide risk at their sites. Although demonstrating no relationship between pesticide risk and knowledge (Lekei et al., 2014), many other studies have highlighted the significance of a farmer's knowledge regarding pesticide effects and management (Mohanty et al., 2013; Wang et al., 2017a; Wang et al., 2017b; Yang et al., 2014). Farmers were at high occupational risk due to the lack of knowledge and training related to pesticides (Akter et al., 2018; Atreya et al., 2012). A few farmers randomly disposed of pesticide containers in fields after carrying out pesticide applications (Fig. 1). A similar disposal method for pesticide packaging was common among farmers in Tanzania (Lekei et al., 2014) and Ghana (Okoffo et al., 2016). Furthermore, unacceptable levels of exposure to organophosphates caused high ecological risk (RQ > 1) in Malaysia (Wee and Aris, 2017). In Costa Rica, an RQ > 1 was observed and believed to be due to exposure to pyrethroids (Fournier et al., 2018) and in China, the same can be said for organochlorines (Chen et al., 2020a). Organophosphate pesticides are commonly used in Nepal (Aryal et al., 2014). This study demonstrated that there was a high risk of organophosphate exposure at farm sites and the risk was linked to poor knowledge and behaviour related to chlorpyrifos and profenofos exposure and waste management.



Fig. 1. Unsafe disposal of pesticide packets and containers at fields.

#### 4.4. Uncertainty and variability related to risk assessment

In this section, uncertainty and variability regarding the EcoRA performed are recognized and discussed. For every EcoRA, uncertainty and variability is inevitable and the risk cannot be estimated with absolute certainty (USEPA, 2004). Of course, the EcoRA rests on and is limited by the availability of data and handling (i.e. strength and excellence) (Wee and Aris, 2017). Pesticides were not detected in most of the soil samples (due to the limits of detection) (Table S2). In our study, data on pesticide concentrations  $<LOD$  were excluded which could have led to an overestimation of the mean concentration of pesticides and their associated risks. However, the  $TER_{mx}$  and  $RQ_{mx}$  of pesticides (values in bold of Tables 3a–3c and 4) allows us to say that, considering the worst-case scenario, the ecological risk of several pesticides is notably high. Variability in endpoint data and risk assessment models accelerate the uncertainty (Chen, 2005). Uncertainty related to ecotoxicity data and models can be anticipated since we used the NOEC data from studies with known ISO/OECD procedures and the models for risk assessment were based on EFSA methods. However, EFSA's risk assessment approaches are used internationally, including non-EU countries. These approaches are even adopted by the FAO and WHO that might be due to the similar environments.

For reducing uncertainties due to variations in soil quality, Dutch  $EC_{50}$  values were corrected for location-specific differences in samples, considering the organic content and clay material in soils (Rutgers et al., 2008). Therefore, the use of NOEC values in this study without any correction for differences in backgrounds such as with Nepalese soil properties, pesticide application practices and meteorological parameters has brought a higher level of uncertainty. Furthermore, the use of international pesticide soil regulatory guidance values for comparisons with measured pesticide concentration (MSC) in our soil samples without corrections could also increase uncertainty. The aforementioned properties, practises and parameters may vary within the country. Generalization of results from the site-specific research conducted in previous studies may not represent the current scenario of risks, thus conclusion should be drawn with caution when comparing findings to other areas of Nepal. Nevertheless, information from this study provides a baseline of pesticide EcoRA for policy makers of Nepal.

Ecotoxicological information about chemical pesticides and their degradation products are not always available and should be incorporated in future risk assessments. For instance, the EcoRA of N-alanine should be considered more in testing protocols because of its persistence in soils. Furthermore, the ecotoxicity of the degradation product of chlorpyrifos was higher than chlorpyrifos itself (Baskaran et al.,



2003). Higher tier risk assessment methods directed at improving risk assessments should also be considered to better understand pesticide risk rising from the current (first tier) risk assessment. For comprehensive EcoRA, future studies should also consider acute toxicity which can be derived from using toxicity data such as EC<sub>50</sub> and an assessment factor of 1000 (Wang et al., 2020). Our study dealt with a few pesticides and limited ecotoxicological information (Table 1), henceforth results should be interpreted with caution. Furthermore, we collected limited soil samples from a small area in winter season, henceforth results are not comparable to other regions and seasons. It isn't likely that it provided a complete assessment of pesticide risks in the environment. The present risk assessment depends upon deterministic methods and conservative approaches. Various elements including the test organisms, the regional meteorology and hydrology, and the local soil qualities can directly influence pesticide ecotoxicity (Huguier et al., 2015; Jegede et al., 2017). Consequently, studies need to be conducted with recent soil samples and the findings of these studies need to be integrated into Nepalese pesticide risk assessments after developed models are validated.

## 5. Conclusions

We found limited ecotoxicological studies on pesticides in Nepal. This is the first case study that assessed the ecological risk of pesticide residues in 3 depths of soil from vegetable production areas of Nepal. The risk assessment was based on TER and RQ methods under two scenarios: general and worst-case. For both the scenarios, our results identified chlorpyrifos as the main soil pollutant and contributed significant ecological risks at all depths, particularly for *Folsomia candida*. For the worst-case scenarios, chlorpyrifos, profenofos and imidacloprid posed a potential risk to soil organisms, hence application of these pesticides in agriculture should be reduced. Future studies should include models for assessing cocktails risk due to either antagonistic or synergistic effects of pesticides. Since the knowledge and behaviour of farmers can reduce ecological risk of pesticides, a crucial step can be increasing programs such as awareness and training related to pesticide effects and waste management. Ecological risk of a few pesticides could not be assessed in this study due to inadequate toxicity data (i.e. NOEC). Our findings argue for a comprehensive EcoRA in future research, which would benefit by considering and evaluating more pesticides and collecting toxicity data on the sensitive species in a given country so as to make a standard test species.

## CRedit authorship contribution statement

**Govinda Bhandari:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. **Kishor Atreya:** Supervision, Writing – review & editing. **Jana Vašičková:** Writing – review & editing. **Xiaomei Yang:** Supervision, Writing – review & editing. **Violette Geissen:** Supervision, Writing – review & editing.

## 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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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.147921>.

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