Applied Soil Ecology 123 (2018) 455–463

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Humus Index as an indicator of the topsoil response to the impacts of industrial pollution

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ARTICLE INFO

Keywords:
Humus forms
Forest litter
Organic matter decomposition
Earthworms
Soil fauna
Macroinvertebrates
Heavy metals
Soil contamination
Southern taiga
The Middle Urals

ABSTRACT

Industrial pollution by heavy metal has negative consequences for soil biota that, in turn, result in a change in the topsoil morphology. The Humus Index, which is based on the morphological description of topsoil horizons and classification of humus forms, can be used as a quantitative score to assess the biological activity of polluted soils. In our study, we examined the advantages and shortcomings of the Humus Index as an indicator of the biological activity of soils compared with other indicators when analysing the impact of industrial pollution. We analysed index changes in response to industrial pollution and estimated the variability of the index values at differing spatial scales.

The effect of air pollution from the Middle Urals Copper Smelter (Russia) on the spectrum of humus forms was examined in the southern taiga spruce-fir forests. Twenty-three study plots were located in background (30–20 km from the MUCS), buffer (7 km and 4–5 km) and impact (3–0.5) zones. At each study plot, 5–7 round miniplots were examined. Diagnosis of humus forms were performed in the field according to the European Humus Forms Reference Base (Zanella et al., 2011a, 2017a). The replacement of zoogenic Mull humus forms by nonzoogenic Mor humus forms (Humus Index increase) with increased pollution has been shown. Humus Index correlated with heavy metal concentrations and thickness of forest litter. The humus form spectra of background and impact areas did not overlap. The diversity of humus forms and the range of Humus Index values were low in the impact and background zones and were wide in the buffer zones at all investigated spatial scales: within a single miniplot (0.5–2 m scale), within study plots (tens of meters scale), and within the pollution zone (kilometre scale). The high informative value, reliability and low work input, compared with other methods of assessment of soil biological activity, allow the Humus Index to be an effective indicator of the impacts of industrial pollution on the soil biota and a useful tool for environmental monitoring.

1. Introduction

Industrial pollution by heavy metals, particularly from non-ferrous metal processing factories, poses a serious threat to the environment. Although in recent years, emissions have been reduced in Europe and North America due to factory closures or the use of improved technologies (Pacyna et al., 2007), emissions in other countries, such as China, have remained high or even increased. Metals are usually retained in the topsoil for a long time, exercising a lasting negative effect on the biota for many years after factory closure (Tyler, 1978; Barcan, 2002). For this reason, the environmental issue of soil pollution by heavy metals remains topical.

It is well recognised that heavy metal pollution (especially in combination with soil acidification), has dramatic consequences for soil biota. In particular, the abundance and feeding activity of soil macroinvertebrates are reduced (Filzek et al., 2004b), with some groups of macroinvertebrates (e.g. earthworms, potworms, snails) completely disappearing at high levels of pollution (Bengtsson et al., 1983; Vorobeichik, 1998; Nahmani and Lavelle, 2002). In addition, the abundance and diversity of soil microarthropods (Rusek and Marshall, 2000; Kuznetsova, 2009) and microorganisms (Ruotsalainen and Kozlov, 2006; Mikryukov et al., 2015), as well as soil enzymatic activity (Wang et al., 2007) declines. As a result of such negative processes, the rate of organic matter decomposition as a whole (Strojan, 1978; Freedman and Hutchinson, 1980; Berg et al., 1991; Zwoliński, 1994; McEnroe and Helmisaari, 2001; Kozlov and Zvereva, 2015) and cellulose decomposition in particular (Vorobeichik, 2007; Vorobeichik and Pishchulin, 2011) is reduced. One of the most considerable consequences that can be observed by the naked eye, may be the increased thickness of the forest litter (Strojan, 1978; Coughtrey et al., 1979; Freedman and Hutchinson, 1980; Vorobeichik, 1995), which is accompanied by an overall change in its structure and topsoil morphology

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http://dx.doi.org/10.1016/j.apsoil.2017.09.025
Received 30 January 2017; Received in revised form 15 September 2017; Accepted 17 September 2017
Available online 12 October 2017

(Kaigorodova and Vorobeichik, 1996).

Effective indicators of soil pollution by heavy metals are needed. Despite the broad range of available methods for soil health assessment, there are few indicators that are informative, reliable, easy to interpret, rapidly measurable and involve low cost (e.g. Stone et al., 2016).

A few previous studies have specifically studied soil profile morphology changes in response to industrial pollution (Kaigorodova and Vorobeichik, 1996; Dijkstra, 1998; Gillet and Ponge, 2002; Filzek et al., 2004a; Ciarkowska and Gambuś, 2005; Arocena et al., 2012). With the exception of one characteristic − the thickness of the forest litter − none of these previous reports have suggested the use of individual morphological characteristics as indicators of state of soil biota. This may be explained by two reasons: 1) each single morphological characteristic of soil may be of limited informative value individually, meaning that several morphological characteristics need to be assessed in combination to be useful as an indicator of a certain soil process; 2) morphological characteristics describe changes at a qualitative level and are therefore difficult to formalise for quantitative assessment.

To overcome such shortcomings in regard to morphological characteristics, it is expedient to use the Humus Index (Ponge, 2003; Ponge and Chevalier, 2006), that is based on the European morpho-functional humus form classification (Zanella et al., 2011b). Humus Index is the ordinal number of a given humus form in the arranged list of these forms. First, this index integrates manifold morphological characteristics of the topsoil, reflecting the features of the decomposition process; second, the index transforms narrative information into quantitative data. An important convenient feature of the European classification for operational purposes is that, unlike other systems, it has been developed for field use with clear diagnostic criteria for humus forms (Zanella et al., 2011a). The feasibility of using the Humus Index in environmental investigations is supported by a case study of stand and soil development under different forestry practices (Ponge and Chevalier, 2006).

Another case study that would be important for calibrating the index and solving other methodological issues, impact regions may be considered, i.e. areas close to sourse of pollution (Vorobeichik and Kozlov, 2012). Such studies, provided the correct experimental design is in place, offer the opportunity to investigate the impacts of pollution “in pure form”, not only with two contrasting impact levels (control and heavily pollution), but also across a broad range of gradually increasing doses of pollutant feed into ecosystems (Vorobeichik and Kozlov, 2012).

We have already shown the viability of using the Humus Index for one of the such impact region − around the Middle Urals Copper Smelter (MUCS) (Korkina and Vorobeichik, 2016). In the present study, we used much comprehensive data that allowed us not only to describe the index change trend with more certainty, but also allowed us to assess its variability and develop recommendations for sampling design and the required number of topsoil profiles to be studied in the test area.

The goals of our study were: 1) to analyse index changes in response to industrial pollution; 2) to analyse the level of spatial variability of the index values at differing spatial scales. We test the hypothesis that with increased pollution, the index grows considerably, i.e. Mull humus forms are replaced by Mor forms as the source of pollution is approached. Besides we examined the advantages and shortcomings of the Humus Index as an indicator of the biological activity of soils compared with other indicators when analysing the impact of industrial pollution.

2. Theoretical background

The main purpose of the Humus Index is to transform qualitative information into quantitative data. As suggested earlier (Ponge, 2003), the index is defined as follows: humus forms are arranged in a sequence according to the increasing role of large soil saprophages in organic matter decomposition and increasing decomposition intensity. The Humus Index is the ordinal number of the humus form in the sequence. Such sequences can be built for different series of forms, identified at the first level of European morpho-functional classification (i.e., by hydromorphic characteristics). However, the problem of the homology of such sequences arises. For example, Hydro or Para humus forms can be found among Terrestrial humus forms in the same studied plots. In such cases, humus forms similar in biological activity but in different series (Terrestrial humus forms and Para- or Hydro forms) should be assigned the same index.

Table 1
Correspondence between Humus Index value and humus forms in soils formed on non-calcareous parent material.

Index value	Terrestrial		Intergrades	
	systems	forms	Terrestrial and Para systems	Terrestrial and Histic systems
1	Mull	Eumull		
2		Mesomull		
3		Oligomull	Rhizo-Mull, Ligno-Mull, Bryo-Mull	Hydro Mull
4		Dysmull		
5	Moder	Hemimoder		
6		Eumoder	Rhizo-Moder, Ligno-Moder, Bryo-Moder	Hydro Moder
7		Dysmoder		
8	Mor	Hemimor		
9		Humimor		
10		Eumor	Rhizo-Mor, Ligno-Mor, Bryo-Mor	Hydro Mor

Therefore, a unified scheme for numbering humus forms is needed. As far as we know, the earlier Humus Index was applied only to single series of humus forms, namely for typical Terrestrial humus forms. We developed a modified version of the Humus Index that includes different classification branches (Table 1). A sequence of Terrestrial humus forms was used as the main series and Para- and Hydro Intergrades were then added into it. Next, homologues (i.e., forms that have the same index in different series) were determined according to a set of diagnostic horizons. While similar series can be built for Histo and Epihisto forms, the numbering of their indices is independent of Terrestrial forms because they are diagnosed by other horizons. This is our first attempt to build such a scheme; however, it has already been applied to Terrestrial and Hydro forms and tested in practice by carrying out the present research.

A multicriteria comparison of different biological indicators can typically be performed only as “rough” qualitative expert estimates (e.g., Stone et al., 2016). We provided such expert assessments of the features of some of the indicators (Table 2). If, as a criterion of a good indicator, the correctness of using the parameter for analysis of industrial pollution impact is used, then, as compared with other field methods of assessment of soil biological activity, the Humus Index possesses a number of undisputable benefits: 1) high informative value – impact areas will be clearly differentiated from background areas (this is demonstrated in our study); 2) transparent interpretation – it is closely linked by cause-and-effect relationships rather than just correlation relationships with agents, conditions and the results of decomposition processes (this is principle of humus form classification that based on morphological evidence of biological activity); 3) reliability – interfering factors have little influence; 4) conservatism – it assesses an average situation in space and time, but not individual points and not a single snapshot as provided by other methods; 5) low labor intensity (and thus low cost).

In general, the possibility of erroneous interpretation of the Humus Index in relation to pollution is low. This is one of the advantages of this index compared with other possible indices, where the possibility of such erroneous interpretation is high due to a number of reasons: 1. Bell

Table 2
Comparison of field methods for the assessment of soil biological activity as possible indicators of the impacts of pollution.

Indicator	Type of data	Sensitivity to intra and inter annual weather variability	Spatial variability at the scale of study plot	Probability of misinterpretation of relationship between index and pollution	Staff qualification	Necessity for measurements		Normal number of measurements per study plot	Labour intensity per study plot[a], man-hours		
						equipment	consumables		preparation of consumables	sampling or field assessment	cameral treatment
Humus index	ordinal	low	low	low	very high	no (yes)[b]	no	5–7	–	1.0	(0.5–1.0)
Thickness of forest litter	ratio	low	moderate	high	moderate	no	no	15–20	–	0.5	–
Standing litter stock	ratio	low	moderate	high	low	no	no	5–10	–	0.5–1.0	0.5–1.0[c]
Litter stock to annual litterfall ratio	ratio	low	moderate	low	low	no	yes	10–15	0.5	5.5–6.5	1.5–2.5 [c]
Abundance of earthworms and/or other macroinvertebrates (hand sorting)	ratio	very high	high/very high	moderate	moderate	no	yes	10–15	–	1.5–2.0	20.0–40.0[d]
Feeding activity of soil saprophagous (bait-lamina test)	nominal	high	high	high	moderate	no	yes	20–25	0.5	1.0–1.5	1.5–2.0
Rate of organic matter decomposition (litterbag study)	ratio	moderate	moderate	moderate	low	no	yes	10–15	2.5–3.0	1.0–1.5	2.5–3.0
Rate of cellulose decomposition (litterbag study)	ratio	moderate	very high	moderate	low	no	yes	10–20	1.5–2.0	1.0–1.5	1.5–2.0
Specific respiratory activity of forest litter	ratio	high	moderate	moderate	high	yes	no	10–15	–	1.0	0.5–1.0

[a] estimates are based on the experience of our research team and are given for the normal number of measurements (with an accuracy of half an hour).
[b] In parentheses – if measurements of the carbon concentration and pH are needed.
[c] Without separation of litterfall to fractions.
[d] Excluding the time for taxonomic identification of animals (if it is taken into account, it is necessary to add an additional 10–20 h).

shape form of parameter dependence on pollution. For example, as pollution initially increases, forest litter thickness and litter stock also increase due to decomposition retardation, but these subsequently start to decline in the industrial barren either due to water or wind erosion, or due to a reduction in litter-fall input (Vorobeichik, 1995); 2. Method errors, that are especially strongly manifested when working with pollution gradients. For example, in background areas, scores for the feeding activity of soil macroinvertebrates as determined by the bait-lamina test may appear underestimated due to an extended exposition period, and the choice of such an extended period is due to low feeding activity in polluted areas. Another example is that in litterbag studies in polluted areas, the decomposition rate may be low due to the presence of mineral particles in the bags or the decomposition rate in polluted areas may be high due to the mechanical loss of materials through large mesh as a result of erosion; 3. Strong influence of interfering factors, that manifests itself to different degrees in control and polluted plots. For example, the specific respiratory activity of forest litter depends not only on microbial respiration, but also on root respiration, and is therefore affected by the root stock in the forest litter, which may differ in control and polluted areas (Smorkalov and Vorobeichik, 2016).

The disadvantages of the Humus Index are not so numerous: 1) ordinal scale, so many standard statistical methods cannot be applied; 2) some risk of wrong diagnosis of humus forms by different operators due to subjective assessments in profile descriptions; 3) the need for highly skilled operators, so theoretical and hands-on training of operators is needed.

We recognise that the limitations of using the ordinal scale are important, because, if neglected, incorrect conclusions may be drawn. In particular, the arithmetic mean cannot be used for assessment of the central tendency, but a median should be calculated instead (median and mode usually coincide, at least in not very skewed frequency distributions). Indeed, mistakes are possible if the arithmetic mean was to be used. For example, if in the test plot in two soil pits the Mesomull form was encountered (index equals 2), in one − Dysmull (4), in two − Dysmoder (7) and in four − Hemimor (8), it will be correct to say that on average the Dysmoder is represented in this area (index median equals 7), but not the Eumoder form (6), which is in fact absent from the given area, although arithmetic mean of indices equals 6.

There are at least two limitations regarding the use of the index as a pollution impacts indicator. 1. Background areas feature an abundance of large saprophages, which is why Mull humus forms predominate. If, with no pollution, Moder forms predominate, the index resolution (i.e. its informative value) is low and if Mor forms dominate, the use of the index can hardly be justified. 2. Humus Index changes lag behind changes in the environment, including the growth or reduction of pollution. Most likely, the index will gradually change in response to an increase or decrease in the abundance of large saprophages, that will, in turn, respond to environmental changes with a lag. So the use of the index may not be justified for the monitoring of fast changes in an ecosystem.

3. Materials and methods

Studies were performed in the territory affected by the long-term (since 1940) air pollution of the MUCS, located near the town of Revda (50 km from Ekaterinburg, Russia, N56°51′ E59°54′). This territory belongs to the low-mountain province of the Middle Urals (with elevations of 100 to 450 m a.s.l.), in the southern taiga subzone, where primary coniferous (*Picea obovata, Abies sibirica*) and secondary birch (*Betula pendula*) and aspen (*Populus tremula)* forests prevail. The climate is continental, with an average annual precipitation of 500 mm. The average annual temperature is +1°C, with average monthly temperatures −16 °C in January, and +16 °C in July (Prokaev, 1976). The predominant soil-forming material is eluvo-deluvium of metamorphic rocks (shales, sandstones, quartzites, silicified limestone).

The main components of the emissions from the smelter are sulphur dioxide and polymetallic dust containing Cu, Pb, Zn, Cd, Fe, Hg and As, among others. Total MUCS emissions were 150–225 Gg/year in the 1980s, but decreased to 3–5 Gg/year after 2010 (Vorobeichik et al., 2014). Information regarding emission dynamics was published by Kozlov and colleagues (Kozlov et al., 2009). Despite recent reductions in emissions there is no evidence of ecosystem recovery in the highly polluted areas (Vorobeichik et al., 2014), or of reduced metal content in the topsoil (Vorobeichik and Kaigorodova, 2017).

For the MUCS region there is detailed information on soil contamination levels, soil morphology and chemistry (Kaigorodova and Vorobeichik, 1996; Vorobeichik and Kaigorodova, 2017), thickness of forest litter (Vorobeichik, 1995), state of soil organic matter (Meshcheryakov and Prokopovich, 2003; Prokopovich and Kaigorodova, 1999), ground vegetation layer (Vorobeichik et al., 2014), soil microbocenosis structure (Mikryukov et al., 2015) and its functional activity (Smorkalov and Vorobeichik, 2011), abundance and structure of communities of soil-dwelling macroinvertebrates (Vorobeichik, 1998; Vorobeichik et al., 2012), soil microarthropods (Kuznetsova, 2009), and ground running macroinvertebrates (Ermakov, 2004).

Twenty-three study plots (SP), 25 × 25 m in size, were located on the flat hill slopes in the spruce-fir forests to the west of the smelter, these included: background (3 SP at a distance of 30 km from the MUCS, 2 SP at 20 km), buffer (5 SP at a distance of 7 km, 5 SP at 4–5 km) and impact (5 SP at 3–1 km, 3 SP at 0.5 km) zones. These zones characterised the successive stages of technogenic degradation of an ecosystem. The following changes in vegetation were notable when approaching the smelter: the stand density decreased, the portion of dead standing trees increased, the abundance and species richness of ground vegetation reduced. Silt loam Leptic Retisols and Albic Retisols were the common soils at the plots, but near the smelter these soils transformed into Stagnic Retisols (IUSS, 2014). Within the zone, the mean distance between the SP was 600 m (300–1000 m between neighbouring plots). Since the buffer zone is highly heterogeneous by soil contamination (see Section 4.1) it was subdivided into two subzones: buffer (7 km) and buffer (5–4 km).

At each study plot, 5–7 round miniplots were examined. At each miniplot, 3–5 small pits to a depth of about 15–20 cm were made in the centre of the miniplot, radiating at a distance of 0.5–1 m from the centre in different directions, so the final area of the miniplot was 2–3 m^2. In total 364 pits were made. Soil profiles of each pit were described in the field. Miniplots were distributed randomly, but sites with visible disturbances of the soil and tree-base areas were avoided. The mean distance between miniplots was about 10 m.

Identification and description of horizons and diagnosis of humus forms were performed in the field according to the European Humus Forms Reference Base (Zanella et al., 2011a, 2017a,b). A detailed description of each pit included: 1) identification of horizons: OL (fresh entire or slightly transformed needles and leaves), OF (partly decomposed fragmented litter), OH (well-humified organic layer), organic-mineral horizon A horizon; 2) measuring the thickness of intact horizons by ruler with an accuracy of 3 mm; 3) for organic horizons − the proportion of recognisable plant remains and humic components, botanical composition of dominant plant residues, changing of their entirety, colour, softness, the preservation of fibres of plant tissues; for the humic component − their colour, structure (granular, powdered, microfiber, presence of animal droppings); characteristics of the layers − compactness, density, the arrangement of the components relative to one another, presence of mycelium; 4) for mineral horizons (A) − structure, colour, features of organic matter (presence of un-, partly-, well-decomposed organic materials, involvement into soil aggregates or binding with mineral particles), texture, density, redoximorphic characteristics, presence/abundance of roots and stones; 5) character of transition between organic and organic-mineral horizons.

In some cases, the structure of organic-mineral horizon did not correspond to any type proposed in the humus form classification. In such cases, the structure was estimated according to FAO (2006), and was considered non-zoogenic. For example, in the soils of the impact and the buffer zones, lumpy, cloddy (with unequal shape and size) and blocky angular structures were noted that were thought to be due to the loss of the primary granular and blocky subangular structure of the A-horizon by compaction.

In each study plot, five samples of forest litter and five samples of A-horizon were taken (each of them was composed of five individual samples) to estimate the concentration of heavy metals and acidity. In the laboratory, dried samples were ground and sieved (2 mm). The pH was measured potentiometrically in water suspension with a ratio of soil:water of 1:2.5 for the A horizon and 1:25 for the litter. The concentrations of acid-soluble forms of the metals were determined by an atomic absorption spectrometer AAS 6 Vario (Analytik Jena, Germany) after extraction with 5% HNO_3 (ratio of substrate:acid was 1:10 by mass). In 5 individual samples, the total carbon content was determined by the dry combustion method on a Multi N/C 2100 analyser (Analytik Jena) to distinguish the OH and A horizons, since their field identification was difficult.

The Humus Index for each pit was assigned on the basis of humus form diagnosis (Table 1). Based on these 3–5 scores, we estimated the median index for each miniplot, and, in turn, from these scores the median and minimum index was estimated for each SP. In the sequence of Terrestrial forms there are 10 gradations of biological activity of soils (the index varies from 1 to 10). From Mull forms (HI from 1 to 4) to Moder forms (HI 5–7) and to Mor forms (HI 8–10) Humus Index increases with retardation of the rate of decomposition, decrease in the activity of soil macroinvertebrates in transformation of plant residues, the change of the dominant decomposers (from anecic, epigeic and endogeic earthworms in Mull to arthropods, epigeic earthworms and enchytraeids in Moder and to fungi in Mor) and increase in accumulation of organic matter in organic horizons.

Because number of pits per pollution zone was not equal, we calculated the expected number of humus forms per uniform number of pits for all zones by individual based rarefaction with PAST ver 3.15 software (Hammer et al., 2001). Comparisons between the pollution zones were performed by the Kruskal-Wallis test for Humus Index and one-way ANOVA for thickness of forest litter, number of humus forms, pH and log-transformed metal concentrations. False discovery rate (FDR) control for multiple hypothesis testing was performed by Benjamini–Yekutieli procedure. Multiple comparisons were made by the Dunn's test (for Humus Index) or by Tukey test (for forest litter thickness, number of humus forms, pH and metal concentrations). The relationship between the Humus Index and litter thickness, pH and metal concentrations was estimated by Kendall's tau coefficient.

4. Results

4.1. Soil acidity and heavy metal concentration

In response to contamination, the acidity of the soil significantly increased in the impact zone compared with the background zone (Table 3). The concentration of metals in the forest litter of the impact zone significantly exceeded the background level by 93.4, 36.6, 6.9, 3.4 and 3.4 times for Cu, Pb, Cd, Zn and Fe, respectively. In the A-horizon, these increases were significant, but with less contrast for Cu, Pb and Fe (38.9, 7.5 and 1.7 times) and more contrast for Cd and Zn (8.2 and 4.1 times). Most metal concentration and acidity in the buffer zone (5–4 km) were significantly higher than those in the background zone, and only copper and lead concentration were significantly lower than those in the impact zone. At a distance of 7 km, the pH in the A-horizon and litter increased compared with the buffer zone at 5–4 km and did not differ from the background values. The heavy metal content in the A-horizon (except for Fe) and Cd and Zn in the litter, by contrast, significantly exceeded only the background level and did not differ from the buffer zone (5–4 km). Further, the concentration of Cu and Pb in the litter significantly differed both from the background zone and from the buffer (5–4 km) and impact zones. These data demonstrate the high heterogeneity of the buffer zone in terms of soil contamination and acidity as well as the specificity of the sites at 7 km (different parameters were closer to either the background zone or the impact zone).

4.2. Humus forms and Humus Index

In all study plots, 11 humus forms were encountered. Nearly entire spectrum of variants in the sequence of soil biological activity from Mesomull to Eumor was represented along the pollution gradient. The most widespread were Dysmull (117 times in all pits), Eumor (116) and Humimor (51); less frequent were Mesomull (17), Oligomull (18), Hemimoder (12) and Eumoder (15); Dysmoder (6), Hydro Mor (8) and Hemimor (3) were relatively rare; and there was only one profile of Rhizo-Moder.

We provided a description of the typical profiles for each zone (Supplementary, Table S1–S4, Figs. S2, S3). The structure of both organic and mineral horizons changes noticeably on approaching the smelter: organic horizons without signs of zoogenic plant residue transformation appear, their thickness increases, zoogenic organic horizons gradually disapper and the structure of the A-horizon changes from granular and subangular blocky to powdery-crumby, cloddy or massive (Figs. S4, S5).

The humus forms of the Mull types were typical in the background zone (Mesomull − 6% of all the pits in this zone, Oligomull − 14%, Dysmull − 77%, Hemimoder − 2%, Rhizo-Moder − 1%). The more frequent profile structure in this zone was vOL/zoOF/meA (Fig. 1a), indicating the rapid decomposition of plant residues with the involvement of large saprophages (earthworms). The humus forms in the buffer zone (7 km) varied from the high-zoogenically active form Mesomull to the non-zoogenic form Eumor (Mesomull − 12%, Oligomull − 3%, Dysmull − 39%, Hemimoder − 11%, Eumoder − 16%, Dysmoder − 6%, Hemimor − 2%, Humimor − 8%, Eumor − 3%). Although the Moder forms did not dominate, they were more frequent in this zone compared with the other zones. The appearance of the OH horizon in the profile of the Moder (OL/zoOF/OH/miA) (Fig. S2) indicates the retardation of litter decomposition, but soil macroinvertebrates (epigeic earthworms, arthropods, enchytraeids) continue to be the main decomposers. At a distance of 5–4 km, the forms of the Mull and Moder humus systems disappeared (Hemimor − 2%, Humimor − 70%, Eumor − 28%). In the widespread Humimor profile (vOL/nozOF1/zoOF/OH/meA) (Fig. S3), signs of the activity of soil fauna were found only in the lower part of the organic layer. The extreme Eumor profile prevailed in the impact zone (Humimor − 9%, Eumor − 84%, Hydro Mor − 7%). Its structure (OL/nozOF/nozA) (Fig. 1b) indicated the lowest rate of biodegradation in the absence of soil macroinvertebrates. Thus humus forms shifted from Mull types to Mor types when approaching the source of emissions.

The index increased from the background zone to the impact zone, with the background and impact zones forming non-overlapping spectra (Fig. 2). The differences between the zones in terms of the index medians estimated for the study plots were significant ($H = 17.71$, $p < 0.0005$). The use of median or minimum values within study plots yielded similar results. The spectra of minimum values within background and impact zones did not overlap, and differences between the zones were significant and more pronounced ($H = 18.96$, $p < 0.0003$).

The lowest level of diversity among the humus forms was detected in the impact zone, slightly higher diversity was observed in the background areas, and the greatest diversity was found in the buffer zone (Table 4). The difference between the zones in the diversity of humus forms was significant ($F(3;18) = 9.5$, $p = 0.0005$). Despite the unequal number of pits in the different zones, the trend for the observed diversity of humus forms coincided with the trend for the expected diversity for the equal number of pits. It can thus be concluded that the

Table 3
Acidity (pHwater) and acid-soluble heavy metal concentration (μg/g) in the forest litter and the soil from zones with different levels of pollution (mean ± SE).

Element	Zone (distance from smelter, km)				Results of ANOVA	
	Background (30–20) n[a] = 5	Buffer (7) n = 5	Buffer (5–4) n = 5	Impact (3–0.5) n = 8	$F_{3;19}$	p[b]
	Forest litter					
pH	5.9 ± 0.1 a	5.6 ± 0.1 a	5.1 ± 0.1 b	4.9 ± 0.1 b	25.4	0.00001
Cu	37.3 ± 4.3 a	481.6 ± 48.0 b	1137.7 ± 90.1 c	3484.3 ± 543.1 d	176.6	< 0.00001
Pb	67.3 ± 8.3 a	512.8 ± 39.7 b	903 ± 22.4 c	2462.5 ± 327.0 d	147.2	< 0.00001
Cd	2.4 ± 0.2 a	12.0 ± 1.1 b	13.7 ± 1.8 b	16.6 ± 2.6 b	25.3	0.00001
Zn	191.8 ± 11.4 a	562.1 ± 43.7 b	507.7 ± 75.8 b	650.5 ± 82.3 b	14.7	0.00016
Fe	2465.8 ± 211.1 a	1632.9 ± 155.4 a	2898.5 ± 483.2 a	8364.7 ± 1112.2 b	23.4	0.00001
	Organic-mineral horizon A (the upper 5 cm)					
pH	5.1 ± 0.1 a	5.1 ± 0.1 a	4.8 ± 0.1 b	4.8 ± 0.1 b	9.8	0.00139
Cu	28.4 ± 3.3 a	222.4 ± 25.5 b	254.1 ± 53.3 b	1104.4 ± 281.2 c	40.4	< 0.00001
Pb	25.9 ± 1.1 a	79.9 ± 13.9 b	71.2 ± 16.7 b	193.4 ± 44.3 c	10.6	0.00095
Cd	0.6 ± 0.1 a	2.7 ± 0.4 b	2.8 ± 0.7 b	4.9 ± 0.7 b	24.2	0.00001
Zn	41.8 ± 1.8 a	117.2 ± 19.6 b	116.5 ± 21.6 b	173 ± 20.3 b	14.2	0.00018
Fe	5773.9 ± 356.7 a	5535.3 ± 1164.2 a	9428.9 ± 681.0 b	9559.7 ± 839.5 b	4.5	0.04536

Different letters (a, b) indicate statistically significant differences (Tukey test, $p < 0.05$).
[a] Number of study plots. Study plot was considered as a statistical unit; previously for each study plot arithmetic mean of 5 samples was calculated.
[b] FDR adjusted p-value.

expected diversity at 7 km is significantly higher than that in the other zones, while none of the other zones significantly differ from each other (based on 95% confidence intervals overlapping).

As well as humus forms diversity, range of index values was highest in the buffer zone (7 km) within all spatial scales, i.e. within a single miniplot (0.5–2 m scale), within a study plot (tens of meters scale) and within the pollution zone (kilometre scale) (Table 4). The lowest index range was identified in the impact zone for all spatial scales. In the background zone, the index range was slightly higher than in the impact zone.

4.3. Metal concentration, litter thickness and Humus Index relationships

A strong positive relationship was found between the Humus Index values and the concentrations of metals in the forest litter (for different metals Kendall's tau coefficient was in the range 0.37 … 0.75, FDR corrected p-value = 0.04541 … 0.00002) and in the A-horizon (0.41 … 0.61, p = 0.02359 … 0.00051). The relationship between the Humus Index and pH was also strong, but negative (for the litter the Kendall's tau coefficient was −0.65, FDR corrected p = 0.00028, and for the A-horizon −0.61, p = 0.00042).

Litter thickness also significantly increased in response to pollution

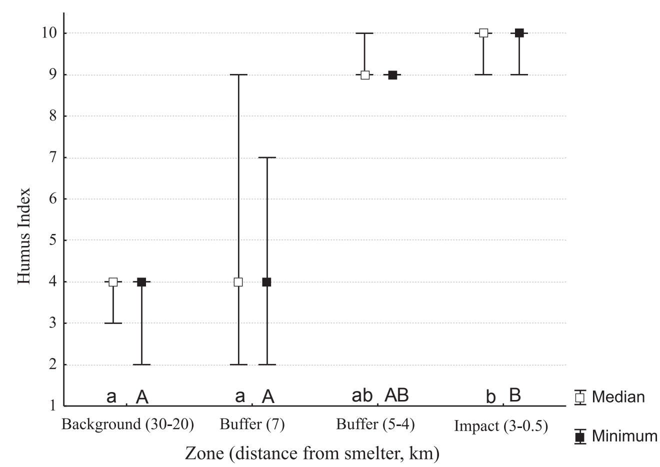

Fig. 2. Humus Index values in zones with different pollution levels. Median (empty) and minimum (filled) of Humus Index were estimated for each study plot, then, based on these scores the median (squares), maximal and minimal values (whiskers) were estimated for each zone. Number of study plots per zone is given in Table 4. Different letters indicate statistically significant differences (Dunn's test, $p < 0.05$): lowercase (a, b) for median, uppercase (A, B) for minimum.

Fig. 1. Topsoil profiles in background and impact zones. Humus form (zone, distance from the smelter, km): a – Dysmull (background zone, 30); b – Eumor (impact zone, 1). Description of profiles see in Supplementary materials, Table S1 and S4.

Table 4
Diversity of humus forms in forest soils from zones with different pollution levels.

Scales of averaging	Zone (distance from smelter, km)			
	Background (30–20) n[a] = 5	Buffer (7) n = 5	Buffer (5–4) n = 4	Impact (3–0.5) n = 8
	Number of humus forms			
Within the study plot				
Mean ± SE	2.6 ± 0.5 ab	3.8 ± 0.4 a	2.0 ± 0.4 b	1.4 ± 0.2 b
minimal and maximal	1–4	3–5	1–3	1–2
Total in zone	5	9	4	3
Expected in 30 pits in zone (95% CI)[c]	3.7 (2.1–5.2)	7.7 (6.0–9.4)	3.4 (2.1–4.7)	2.9 (2.2–3.5)
Total in region	11			
	Range (maximum minus minimum) of Humus Index value			
Within single miniplot				
maximal	2	3	1	0
median	0	2	0	0
Within study plot[b]				
maximal	2	4	1	1
median	0	2	0	0
Within zone on the miniplots[b]	2	8	1	1
Within zone on the study plots[b]	1	7	1	1

Different letters (a, b) indicate statistically significant differences (Tukey test, $p < 0.05$).
[a] Number of study plots.
[b] On the median for miniplots.
[c] Expected numbers of humus forms were estimated by individual based rarefaction, 95% confidential interval was calculated as 1.96*SE for unconditional variance estimators.

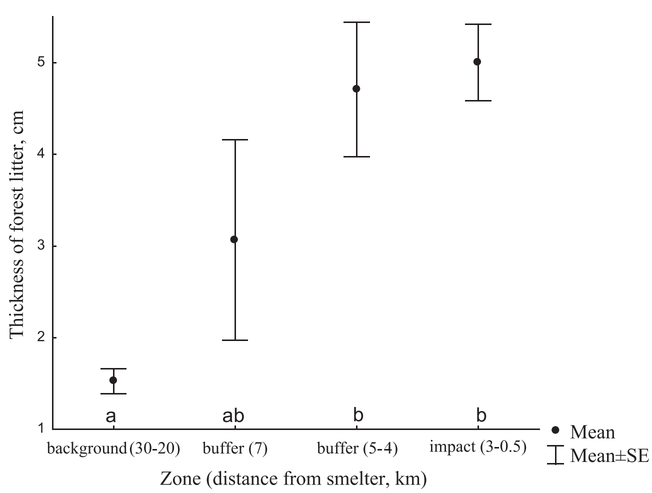

Fig. 3. Thickness of forest litter in zones with different pollution levels. Number of study plots per zone is given in Table 4. Different letters (a, b) indicate statistically significant differences (Tukey test, $p < 0.05$).

($F(1, 19) = 6.3$, $p = 0.0039$), (Fig. 3). A positive correlation was established between litter thickness and the Humus Index median (Kendall's tau coefficient was 0.55, $p = 0.00035$).

5. Discussion

Humus Index growth with increased pollution (accompanied by replacement of Mull forms by Mor forms) coincides with changes in the abundance and structure of soil macroinvertebrate communities. It has previously been established that the abundance of earthworms in background areas of MUCS is high (261 ind/m^2), but closer to the factory reduces sharply (212 ind/m^2 at a distance of 7 km and 39 ind/m^2 at 5–4 km) and, ultimately, earthworms disappear altogether (Vorobeichik et al., 2012). The territory within the radius of several kilometres of the smelter is referred to as the “lumbricid desert” (Vorobeichik, 1998; Vorobeichik and Nesterkova, 2015). The abundance of enchytraeids changes similarly (168, 125, 30 and 0 ind/m^2 in background, buffer (7 km), buffer(5–4 km) and impact zones, respectively) (Vorobeichik et al., 2012). The abundance of macroinvertebrates is strongly suppressed by heavy metals (Bengtsson et al., 1983; Filzek et al., 2004b; Nahmani and Lavelle, 2002), so received relationship between the Humus Index and heavy metals was expected and well-explaned.

Previously we have demonstrated that Humus Index value growth is also in agreement with other parameters, reflecting the reduction in soil biological activity when approaching MUCS, in particular, with decrease in the abundance of soil microarthropods, feeding activity of soil saprophages, cellulose decomposition rate, specific respiratory activity of the litter and increase in thickness and stock of forest litter (Korkina and Vorobeichik, 2016).

Changes in the humus profiles following heavy metal pollution of nonferrous smelters have previously been reported (Gillet and Ponge, 2002; Filzek et al., 2004a). A shift in humus forms along the pollution gradient from Mull to Mor types also reportedly coincides with the reducing abundance of earthworms, culminating in their disappearance near a Zn smelter (Gillet and Ponge, 2002).

In the buffer zone, the diversity of humus forms is great (9 forms), almost the same as the general diversity in the whole of the pollution gradient (11 forms). Indicatory of this fact is that at the distance of 7 km from the factory, high variation in earthworm abundance is observed (Vorobeichik, 1998; Vorobeichik et al., 2012). This highlights the transitional nature of this zone. Soil contamination at a distance of 7 km from the smelter was greater than the background level and was similar to that at 5–4 km except the two-fold lower Cu, Pb and Fe concentrations in the litter (Table 3). However, at a distance of 5–4 km from the source of pollution Mor forms dominate, and at a distance of 7 km Mor forms decrease (in 14% of pits) and Mull forms become more widespread (53%). High variability in large soil saprophage abundance and the predominance of biologically active humus forms in the buffer zone (7 km) are likely connected to changes in metal concentration and the performance of the soil biota. Site features (such as physical and chemical soil parameters, quality and content of organic matter) could mitigate the negative effects of metals, for example, low soil acidity that is close to background level (Table 3) could result in decreased bioavailability of metals to soil invertebrates (Spurgeon and Hopkin, 1996; Van Gestel and Koolhaas, 2004). It is probable that diverse combinations of metal concentrations and site conditions result in the high spatial heterogeneity of humus forms in the buffer zone.

Soil de-structuring in the impact zone led to soil physical property deterioration, i.e. increase of bulk density and concomitant decrease of porosity (Meshcheryakov and Prokopovich, 2003). This, in turn, became one of the reasons for technogenic soil gleying and the appearance of Hydro forms, that are not typical for conspecific forest biotopes in background areas. Other most likely reasons for disturbance in the hydric regime that activated soil gleying in the impact zone are reducing soil desiccation due to the stand inhibition and the influence of engineering constructions (Kaigorodova and Vorobeichik, 1996).

The spatial variability of the Humus Index within the study plots is relatively small, which allows sampling efforts to be minimised. However, it is clear that sampling efforts must be differentiated depending on the pollution level. The number of examined profiles can be kept to a minimum in background and impact areas (3 pits per miniplot, and 3 miniplots per study plot), but should be increased in the buffer area (3–5 pits per miniplot, 5–7 miniplots per study plot). However, the final decision on the number of pits and miniplots will be made by the investigating operator and will depend on the diversity of humus forms on the site.

It is interesting to note that both median values and the minimum value of HI for the study plot can be equally well used as indicators. Minimum HI values reflect the maximum achieved level of soil biological activity and the rate of organic matter transformation in the some area. To avoid uncertainty in subsequent data analysis, the number of soil pits, miniplots and study plots for the zone must be odd (with numbers being even, the median will be not established for ordinal data). If the number did appear to be even (generating two medians, i.e. left hand and right hand values), it is logical to use the left hand median (the lower one).

6. Conclusion

In this study, the Humus Index changed greatly in response to industrial pollution, with the humus form spectra of background and polluted areas not overlapping. The humus form shift occurred due to strong changes in large saprophage activity, as evidenced by the abundance of earthworms. The working hypothesis was therefore confirmed: zoogenic Mull humus forms are replaced by nonzoogenic Mor forms on approaching the factory.

The Humus Index allows for clear differentiation between strong polluted territory (impact zone) and control territory (background zone). In the buffer zone, the humus form spectrum was broad, ranging from those typical for the background zone to those typical for the impact zone. This illustrated the transitional nature of this area.

Comparison of the Humus Index with other methods of soil biological activity assessment have highlighted its high informative value, reliability and low work input. Therefore, the Humus Index can be recommended as an indicator of soil biota and organic matter state. However, further testing of the index in vicinity to other factories and in different natural environments is needed (whilst taking into consideration its main limitations, i.e. the high rate of the biological turnover and the dominance of Mull forms in control areas). Further index testing is also required for hydromorphic sites (i.e. for Hydro and Histo humus forms).

The question of how quickly the Humus Index responds to biota perfomance changes still remains. To date, there has been no assessment of the lag magnitude in the index change in response to degradation or rehabilitation of macroinvertebrate communities during increased or decreased pollution.

Acknowledgements

The authors wish to thank S.Y. Kaigorodova for soil type diagnosis, A.V. Shchepetkin for measurement of the heavy metal concentration and K.G. Khorosh for help with the field work. We are grateful to anonymous reviewers and Guest Editor A. Zanella for their valuable comments on the manuscript. The analysis of heavy metals data provided by E.L. Vorobeichik was supported by the Russian Science Foundation (grant no. 16-16-00071).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.09.025.

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