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1 **Environmental Pollution**

- 2 **New insights into wasteland ecosystems of the glass industry**
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Abstract

 Over the past two centuries, large amounts of waste have been released into ecosystems from glass manufacturing operations. Nowadays, an increasing number of abandoned slag heaps results from the cessation of these activities and may be contaminated with metallic trace elements (MTE) formerly used in glass and crystal products. However, recent findings emphasized that derelict lands should also be recognized as a resource delivering a wide range of ecosystem services. Therefore, the major purpose of this research was to provide new insights into wasteland ecosystems of the glass industry; while assessing the relative influence of edaphic factors on biotic communities, which is still poorly understood although closely related to the potential re-use of these sites. To that end, we investigated (i) Orthoptera, diurnal Lepidoptera, plant communities, and (ii) abiotic parameters in the topsoils.

 Multivariate analysis revealed strong correlations between potentially phytoavailable and pseudo-total lead (Pb), cadmium (Cd) and zinc (Zn). Arsenic (As) also co-varied significantly with DTPA-extractable Pb. Despite the severe Pb contamination reported in the topsoils, wastelands of the glass industry were able to support a substantially higher than excepted biodiversity with orthopteran assemblages similar to grasslands and a diurnal Lepidoptera diversity comparable to urban parks. We also noted a positive influence of plant richness on the diurnal Lepidoptera community structure. Hence, Pb contamination does not appear to be a primary limiting factor for the establishment of these communities regarding the low mobility of Pb due to the high amount of organic matter recorded in the topsoils under subalkaline conditions. We assumed that wasteland ecosystems of the glass industry were more affected by soil fertility or land management practices. To conclude, these sites are able to provide biodiversity ecosystem services, acting as wildlife sanctuaries for Orthoptera and diurnal Lepidoptera, and strategic metals by phytoextraction in a circular economy model.

1. Introduction

 With the closure of various industrial activities during the last century, many lands have been left abandoned throughout the world (Bradshaw and Wong, 2003). In France, large surfaces of brownfield sites have been actually recorded, which represents about 100,000 ha (ADEME, 2016). These derelict soils may be contaminated in metallic trace elements (MTE) due to former industrial activities (Tarradellas et al., 1996) like metal ore mining and smelting, coking plants, glass factories (Dudka and Adriano, 1997). In France, 322,400 potentially polluted areas were identified in the BASIAS database in July 2020 (BASIAS, 2021). These areas can be enriched in various MTE such as arsenic (As), cadmium (Cd), copper (Co), lead (Pb) and zinc (Zn) (Dudka et al., 1996; Jennings et al., 2002; Markiewicz-Patkowska et al., 2005; Gallagher et al., 2008; Hartley et al., 2009; Luo et al., 2012). Vacant lands are also characterized by strong spatial heterogeneities regarding their soil physico-chemical properties (Morel et al., 2005). Nonetheless, industrial wastelands share common edaphic features such as 46 elevated pH values (pH $>$ 7), high coarse fractions in soils ($>$ 2 mm) and may have high C/N ratios due to a lack of N in organic matter (Morel et al., 2005). Indeed, these industrial substrates can be depleted in nutrients (Dickinson, 2003).

 Wastelands, like urbans soils, are predominantly considered as surface areas to be converted or a potential threat to ecosystems due to their properties and pollution levels (Blanchart et al., 2019). In fact, the dispersal of soil particles in the atmosphere may directly affect human health through inhalation of dust and skin absorption. MTE can also indirectly damage organisms by contaminating the food through transfer to plants or metal leaching (Su et al., 2014). However, brownfields are not only contaminated lands and they should be recognized as a resource for land management (Morel et al., 2005; Lehmann and Stahr, 2007; Anderson and Minor, 2017). Indeed these abandoned sites could deliver various ecosystem services (ES) such as provisioning of biomass for the production of sustainable energy (Lord,

 2015) or bio-sourced metals through the cultivation of various herbaceous or ligneous hyperaccumulating plants (van der Ent et al., 2015; Corzo Remigio et al., 2020). On contaminated derelict lands, phytoremediation coupled with biomass production could provide new urban environments (e.g. green areas) that are attractive from a sustainable development perspective (Wilschut et al., 2013).

 At the same time, brownfields may record high levels of biodiversity (Bonthoux et al., 2014; Vincent et al., 2018). Following a certain time of abandonment (since the last anthropogenic actions), several vacant lands have shown a high degree of biodiversity with the early colonization of spontaneous flora and fauna communities on a short time scale. In an industrial settling pond managed by natural attenuation in Pompey (Lorraine, North-East France), 20 to 50 years were required to recreate a well-forested area (Lucisine et al., 2015; Huot et al., 2018). In the six derelict lands investigated by Vincent et al. (2018), 2 to 40 years were necessary to initiate recolonization by different biotic communities (e.g. microbial, mesofauna, macrofauna and plant components) that harbor a great biological diversity. According to Bonthoux et al. (2014), species-rich habitats can spontaneously establish themselves within a year to several decades in urban wastelands. In fact, some abandoned lands could play a crucial role in biodiversity conservation for a wide range of biotic components. In particular, several wastelands have demonstrated their ability to provide new habitats for endangered terrestrial arthropod species in Europe (Gardiner et al., 2013), while at the same time, a massive decline in terrestrial arthropod biodiversity was recorded in natural ecosystems throughout all compartments of the food web (Hallmann et al., 2017; Seibold et al., 2019). Further studies reported that some brownfields have the capacity to support a rich biodiversity and to host rare species such as Coleoptera (Eyre et al., 2003; Small et al., 2006), soil mesofauna (Joimel et al., 2017) and vascular flora (Robinson and Lundholm, 2012).

 In order to give indications on the potential re-use of wastelands, recent studies have focused on assessing the overall quality of these sites, not only in terms of pollution or soil fertility, but also in terms of their biological diversity. Godefroid et al. (2007) examined the influence of edaphic and microclimatic parameters on the community structure of vascular plants in 22 wasteland sites (in Brussels, Belgium). The results revealed that plant species assemblages were mostly governed by soil humidity, water pH, soil nutrient content and other environmental variables such as light intensity. On the other hand, Dazy et al. (2009) showed that Cd and Hg contaminations in soils were the main factors controlling the community structure of the spontaneous vascular flora in a former coking plant site (Homécourt, France). However, for other biotic components of brownfield ecosystems, Joimel et al. (2016, 2017) suggested that land management practices had probably a stronger effect on soil mesofauna diversity (e.g. Acari and Collembola) than edaphic parameters, such as MTE contamination or soil fertility. For instance, these authors reported that most industrial wastelands harboured a greater soil mesofauna diversity than in agricultural soils, despite the high concentrations in MTE found in some of them. In a recent work performed on six derelict lands, Vincent et al. (2018) revealed that the structure of microbial, soil fauna (e.g. mesofauna and macrofauna), and plant communities were more closely related to soil fertility proxies than to other edaphic parameters such as contamination in MTE or organic pollutants. Hence, the relative influence of environmental factors (e.g. soil contaminations, soil fertility, land management practices) on wasteland biodiversity is still poorly understood.

 The aims of this study are to provide new insights into wasteland ecosystems of the glass industry, which to our knowledge have never been studied. These derelict sites may include for instance Pb and Zn oxides as they are respectively used to induce transparency and elasticity in crystal products. Other elements can also enter into the composition of these materials for glass coloring (e.g. cobalt (Co), erbium (Er), gold (Au), manganese (Mn), neodymium (Nd), nickel

 (Ni), silver (Ag)). One sub-objective of this study is to assess the relative influence of soil fertility and trace metal contamination on wasteland biodiversity of the glass industry. For that purpose, several ecological inventories were performed on three distinct components of brownfield ecosystems: (i) vascular plant, (ii) Orthoptera, and (iii) diurnal Lepidoptera communities. According to the structure of fauna and flora communities, we also studied edaphic parameters (fertility and trace metal contamination) and we explored the links between abiotic variables. Then, we examined the relationship between plant assemblages, Orthoptera and diurnal Lepidoptera communities using ecological indices for an integrated description of spatial biodiversity patterns. Finally, we assessed the relative influence of soil fertility and trace metal contamination on wasteland biodiversity of the glass industry.

2. Methods

2.1. Site description

According to the BASIAS database (BASIAS, 2021), from the 18th to the middle of the $120 \,$ $20th$ century, the studied site was an industrial slag heap used as a dumping site for the waste of the glass industry (including glass fragments, refractory materials, and coke combustion residues) leading to the formation of a 10 m thick landfill. Until its closure in 1990, it received Pb3O4, acid waste, Pb-rich neutralization sludge for acid effluents and crystal fragments from the art glass industry. Over the last thirty years, a spontaneous vascular flora and a particular entomofauna have colonized this wasteland, which contributed to recreate a well-vegetated ecosystem (supplementary Fig. S1). Nowadays, this small artificial hill is covered by a 1200- 127 m² herbaceous layer at its top (about 60 m long and 20 m wide), surrounded by forested slopes. Since the 90's, this environment has been maintained semi-open by basic land management practices: some trees were pruned and a small path was partially mowed once a year in order to access to the top of the hill. The dumping site lies on clayed-marly formations of the Dogger (Middle Jurassic marly limestones) overlaid by a superficial layer of alluvia made of calcareous sands and gravels. The studied site is localized in the North-East of France (Lorraine), a region 133 with a climate under semi-continental influence. The mean annual temperature is 10.1°C with an average temperature range of 1.8°C in January to 18.8°C in July. The mean annual precipitation is 713 mm per year.

2.2. Sampling design for biotic components

 Several inventories were carried out on the herbaceous layer, located at the top of the hill. For vegetation sampling, a field study was conducted on July 2019. The identification of plant taxa was carried out down to the species level. Each species was classified according to its habitat preferences (inventory in supplementary Tab. S1). For the entomofauna sampling,

 an inventory of Orthoptera and diurnal Lepidoptera communities was performed during two days in July and August 2019 (inventory in supplementary Tab. S2). The month of July was 143 particularly dry with an average temperature of 21.1^oC (temperature range: 8.4^oC - 39.6^oC) and a cumulative rainfall of 20.8 mm. The month of August was slightly more humid with an average temperature of 20.6°C (temperature range: 8.7°C - 34°C) and a cumulative rainfall of 58.4 mm. Orthoptera and diurnal Lepidoptera were collected with nets for species identification and abundances were assessed by semi-abundance coefficients for all species.

2.3. Topsoil sampling and analyses

 Fifteen soil samples were taken from the topsoil layer (0-10 cm without the plant litter), in July 2019. Soil chemical analyses were conducted using French standard methods at the Laboratoire Sols et Environnement (Université de Lorraine, Vandoeuvre-lès-Nancy) on 2 mm- sieved and air-dried samples. Pseudo-total concentrations of MTE were evaluated after aqua regia soil digestion (NF ISO 11464 standard). Five hundred mg of crushed soil (at 250 μm) 154 were digested for 16 h in 6 mL HCl and 2 mL HNO₃ at room temperature. Then, mineralization took place in a DigiPREP system during 3 hours at 105°C. After cooling down, the solutions were diluted to 50 mL with ultrapure water and then filtered under vacuum at 0.45 μm before analysis on Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES, iCAP 6000 series, Thermo Scientific, Cambridge, UK). To study soil chemical fertility, several parameters were determined. Total C and N contents were measured by dry combustion of 160 crushed soil (at 250 µm) and quantification of the emitted volume of CO_2 and N₂ (NF ISO 10694). Organic carbon (Org C) was assessed by dry combustion after carbonate dissolution according to NF ISO 10694. To measure water pH, 5.0 mL of soils were added to 25 mL of ultrapure water according to NF ISO 10390. Electrical conductivity was also determined in a soil/water ratio 1:5 (v/v) according to NF ISO 11265. The effective cation exchange capacity (CEC) and the extraction of the exchangeable cations were implemented according to the NF

 ISO 31-130 standard. Exchangeable cations were then analyzed by ICP-OES, according to the NF ISO 22036 standard. Available P was assessed by the Olsen method (NF ISO 11263). To evaluate the potentially bio-available fraction of MTE, 5.0 g of soil were mixed with a 10 mL DTPA (Diethylene Triamine Pentaacetic Acid)-based extraction solution at pH 7.3, according to the NF ISO 13651 standard (solution prepared according to the NF ISO 31121 standard). DTPA-extractable major and trace elements were quantified by ICP-OES. Quality control assessment was done by inserting certified control soils as samples. To verify the validity of the ICP-OES results, a blank and a control sample, as well as a certified solution (EU-H3, SCP Science, Courtaboeuf, France) were inserted regularly into the batches to be analysed.

2.4. Biodiversity assessment method

 Alpha diversity was determined on vascular plant, Orthoptera and diurnal Lepidoptera communities with species richness (S), whereas species abundances were evaluated only for Orthoptera and diurnal Lepidoptera communities. The following biodiversity indexes were determined for diurnal Lepidoptera and Orthoptera communities.

 The Shannon-Weaver diversity index (H') was computed according to the following relation (Shannon and Weaver, 1963):

182
$$
H' = -\sum_{i}^{S} p_i \ln(p_i)
$$
 (1)

183 with
$$
p_i = \frac{n_i}{N}
$$
 (2)

184 where ln is the natural logarithm, n_i is the species abundance of the species "i" obtained from the median value of the semi-abundance coefficients assigned for each species, N corresponds to the species abundance of each community and S is the species richness index. Thus, H' considers the species abundance in the assessment of biodiversity. The maximum value of H'

 is reached when all individuals of each species are equally distributed. It is represented as: 189 H'max = ln(S). Hence H' is in the range [0; ln(S)]. The Simpson diversity index (\tilde{D}) was calculated according to the following formula (Grall and Coïc, 2006):

191
$$
\widetilde{D} = 1 - \sum_{i}^{S} \frac{[n_i(n_i-1)]}{[N(N-1)]}
$$
 (3)

 This index may be interpreted as the probability that two randomly selected individuals belong 193 to different species (Grall and Coïc, 2006). Thus, \tilde{D} is in the range [0; 1] and its value increases with evenness given by the Pielou index (J) which is computed from the Shannon-Weaver diversity index as following (Pielou, 1966):

$$
196 \t J = \frac{H'}{H'max} \t(4)
$$

Therefore, J is in the range [0; 1] (from dominance of one of the species to even distribution).

2.5. Chemical risk assessment method

 To assess the level of MTE contamination in the topsoils (0-10 cm), an integrated chemical risk index was calculated using a Triad-like approach (Jensen and Mesman, 2006). In this method, MTE pseudo-total concentration is compared to its respective soil screening level (SSL). For each MTE, SSL vas calculated as three times the local anomalous threshold (vibriss) coming from Saby et al. (2018) dataset (Tab. S3). Only pseudo-total As, Cd, Cu, Pb and Zn contents were included into this index because their values were in the range or above the local threshold values. The effect of a global toxic pressure (PAF – Potentially Affected Fraction) was computed using SSL as environmental quality criteria. This chemical risk index is in the range [0; 1] (1 was assigned for a maximum risk).

 All tests were performed with R software v 4.1.1 (R Development Core Team, 2015). 212 In order to evaluate significant differences ($p < 0.05$) among edaphic parameters between the three zones, one-way ANOVA was performed, followed by Tukey's HSD test from the "multcomp" package in parametric conditions. The adequacy of the data with respect to the ANOVA models was verified by examining the residuals for independence (Durbin-Watson test, "car" package), normality (Shapiro test) and homogeneity (Levene test). If the latter assumptions were not validated after "sqrt" or "log" transformation of the data, a Wilcoxon pairwise test with a Holm correction of the p-value was used. The relationships between the variables describing i) the soil fertility, ii) total soil element content and iii) available soil element content were assessed through a multiple-factor analysis (MFA) followed by an ascendant hierarchical classification (HCPC) with the "FactomineR" package. For the three zones together, Spearman's correlation coefficients were calculated to determine the relationships between the studied parameters. Results are presented as a heatmap with a dendrogram added.

3. Results

3.1. Vascular plant assemblages

227 The vascular plant inventory was conducted on July $11th$, 2019. The 1200-m² studied area was delimited into three zones (Fig. 1) for several reasons: (i) the vegetation was rather distinct between these areas with a denser plant cover observed in zone 3, the southern part of the plot, (ii) zone 2 differed from zone 1 and zone 3 in terms of light intensity as it was shaded by forested slopes, and (iii) a 200-m² mown area separated zone 1 from zone 2. Hence, the size of the inventoried surface was reduced to 1000 m²: zone 1 covered an area of 350 m²; zone 2 represented 320 m²; and zone 3 was 330 m² in size (Fig. 1).

 According to Tab. S1, 54 vascular plant species were identified in the 1000-m² studied zone. The plant species composition was rather homogeneous with 30, 33, and 36 species recorded respectively in zones 1 to 3 (Fig. 2). Half of the species was common to the three zones revealing some uniformity in the assemblage of plant species on a site scale. Nevertheless, more than a third of the species in zone 3 was not observed in zone 1 and zone 2, and more than a quarter of the species in zone 2 was not reported in other parts of the area. By contrast, only *Achillea millefolium*, *Linaria vulgaris* and *Torilis japonica* were endemic to zone 1 (Tab. S1). Regarding Fig. 2, zone 1 recorded the highest ratio of plant species (37 %) shared between two zones: six species were shared with zone 2 and five species were shared with zone 3 (Tab. S1).

 In terms of habitat preferences, Tab. 1 shows the distribution patterns of vascular plant species for each zone. It was shown that grassland, wasteland and edge species particularly dominate. The presence of seven bio-indicators of calcareous soils and seven woody plant species should also be noted (Tab. S1). On a site scale, the plant species common to the three zones were the most relevant bio-indicators for habitat characterization. According to Tab. S1, ubiquitous species were: *Arrhenatherum elatius*, *Cirsium arvense, Dactylis glomerata*, *Elytrigia repens* subsp. *repens*, *Ervilia hirsuta*, *Galium album*, *Hypericum perforatum* var. *perforatum*, *Origanum vulgare* subsp. *vulgare*, *Pastinaca sativa* subsp. *urens, Plantago lanceolata*, *Poa pratensis*, *Prunus spinosa*, *Trigonella alba* and *Urtica dioica*. Regarding these 14 species, wasteland and grassland species were still dominant followed by an edge species prevalence. Two calcareous plant species (*Galium album* and *Origanum vulgare* subsp. *vulgare*) and one thicket species (*Prunus spinosa*) were identified in the three zones (Tab. S1).

 For each zone, the order of the main habitats followed the general trend described on a site scale with a prevalence of wasteland, grassland, edge and calcareous plant species (Tab. 1). However, some differences were observed between the three zones. First, zone 1 comprised the highest number of grassland species and the smallest number of edge and thicket species. According to Tab. S1, *Prunus spinosa* was the only shrub species inventoried in zone 1 while zone 2 and zone 3 contained three thicket species each: *Cornus sanguinea*, *Hedera helix*, *Prunus spinosa* and *Crataegus monogyna*, *Prunus spinosa, Rosa canina*, respectively. Then, the largest number of calcareous plant species and the smallest number of wasteland species were found in zone 2. Last, on the contrary, zone 3 was characterized by a dominance of wasteland and edge species.

 3.2. Spatial distribution and biological diversity of Orthoptera and diurnal Lepidoptera communities

268 The first inventory began at 10:00 a.m. on July $16th$, 2019. The weather was sunny with 269 no cloud cover. No precipitation fell that day and the wind was light $(5 \text{ to } 7 \text{ km h}^{-1})$. Temperature was 20°C with 40% of moisture during the day. The second inventory began at 9:30 a.m. on August $22nd$, 2019. The weather was sunny, and the wind was blowing at 8 km h 272 ¹. No precipitation fell that day. Temperature was 14° C with 75% of moisture during the day. Both inventories were combined in Tab. S2.

 On a site scale, 8 species of Orthoptera and 13 species of diurnal Lepidoptera were recorded (Tab. S2). The number of butterflies was estimated to be at least 50 specimens. The most abundant species were *Pieris rapae* and *Pyronia tithonus*. The number of grasshoppers and crickets was estimated to be at least 35. The most abundant species were *Bicolorana bicolor* and *Gomphocerippus rufus*.

 Spatial patterns of Orthoptera and diurnal Lepidoptera communities are presented in Fig. 3. Regarding Orthoptera (Fig. 3a), twice as many species and individuals were counted in zone 1 and zone 2 compared with zone 3. According to Tab. S2, *Bicolorana bicolor*, *Gomphocerippus rufus* and *Phaneroptera falcata* were common to the three zones. Zone 1 and zone 2 comprised the same ratio of endemic species, whereas no endemic species were found in zone 3. *Chrysochraon dispar* was only shared between zone 1 and zone 2. Regarding diurnal Lepidoptera abundances (Fig. 3b), twice as many specimens were reported in zone 3 compared to zone 1 and zone 2. The distribution of butterfly species was highly heterogeneous with six, three and twelve species identified in zone 1, 2 and 3, respectively. According to Tab. S2, *Melanargia galathea, Pieris rapae* and *Pyronia tithonus* were common to the three zones. Almost 60% of the species recorded in zone 3 were not inventoried in zone 1 and zone 2. On the contrary, no endemic species were found in zone 2. Only one species was endemic to zone 1: *Cupido argiades* (Tab. S2).

 Further characterization of Orthoptera and diurnal Lepidoptera communities was done with the calculation of several ecological indices to assess biological diversity (Tab. 2). On a site scale, the Shannon-Weaver diversity (H') index was 1.70 for Orthoptera. The Simpson 295 diversity (D) and the Pielou evenness (D) indices reached elevated numbers with 0.79 and 0.82, respectively. Regarding the Orthoptera diversity in the three zones, all ecological indices were higher in zone 2. In particular, the Pielou index reached a high value in zone 2 (0.91) in comparison with the one reported in zone 1 (0.68). Shannon-Weaver diversity (H') index increased in the following order: zone 3 (0.81) < zone 1 (1.22) < zone 2 (1.62). A similar pattern 300 was observed for the Simpson diversity (\widetilde{D}) index: zone 3 (0.52) < zone 1 (0.65) < zone 2 (0.81) . On a site scale, the Shannon-Weaver diversity (H') index was 2.01 for diurnal Lepidoptera. The 302 Simpson diversity (D) and the Pielou evenness (D) indices reached high values with 0.84 and 0.79, respectively. Regarding the diurnal Lepidoptera diversity in the three zones, all ecological indices were higher in zone 3. A lower Pielou evenness (J) was computed in zone 1 (0.66) compared to zone 3 (0.79). Zone 1 displayed an intermediate Shannon-Weaver diversity (H') number (1.18) but much closer to the one determined in zone 2 (0.83) than in zone 3 (1.95). For 307 the Simpson diversity (\tilde{D}) index, a similar result was found with close values in zone 1 (0.59) and in zone 2 (0.55), that were substantially lower than the one calculated in zone 3 (0.83).

3.3. Abiotic parameters in the topsoils

 Each zone was characterized by five soil samples taken from the topsoil layer (0-10 cm). Soil pseudo-total element composition is presented in Tab. 3. It was dominated by Ca, Fe, Al, 312 K, Mg and Pb (content exceeding 1 g kg^{-1} dry soil). Significant differences between zones have been only observed for Ca and Fe, with zone 2 having respectively highest and lowest values. All the topsoils collected were highly contaminated with Pb and Cd, which pseudo-total concentrations that were respectively at least 30 times and 2.5 to 4 times greater than the local geochemical background (Tab. S3). In comparison with the local average soil composition, all samples contained large amount of pseudo-total As, Cu and Zn. By contrast, all the topsoils were strongly depleted in Co, Cr and Ni. Hence, the trace metal pollution in the topsoils was largely dominated by Pb and Cd then, by a lower degree, by Cu, As and Zn. The concentration of Pb in the topsoils was rather distinct between the three zones with values in the following 321 order: zone 3 (3.1 g kg⁻¹) > zone 1 (2.6 g kg⁻¹) > zone 2 (1.8 g kg⁻¹); even if values were not significantly different. Zone 3 also displayed the most severe contamination in As (95.1 mg kg⁻ ¹; p < 0.05) and Cd (5.7 mg kg⁻¹), these two elements behaving like Pb. On the contrary, the

324 highest Zn level was measured in zone 2 with 276 mg $kg⁻¹$ of dry soil. In zone 1, Cu concentrations were close to the SSL limit value. Regarding the chemical risk index, the values 326 for the toxic pressure increased in the following order: zone $2(0.940) <$ zone 3 $(0.969) <$ zone 1 (0.973).

 Regarding the parameters of soil fertility, no statistical differences have been observed between the three zones (Tab. 4). Topsoils present a water-pH slightly alcaline (7.6-7.7) and a 330 mean electrical conductivity of 0.153 dS m⁻¹. The cation exchange capacity (CEC) ranges from 331 24.2 cmol⁺ kg⁻¹ in zone 3 to 27.1 cmol⁺ kg⁻¹ in zone 1, and is always saturated by Ca^{2+} with 332 Ca/CEC ranging from 0.83 to 0.90. Both K^+ and Mg^{2+} represent each about 3.5 % of the CEC with thus a K/Mg ratio near 1. The content of topsoils in organic carbon increases in the following order: 6.6% in zone 3 < 7.7% in zone 2 < 8.1% in zone 1. As total nitrogen is rather homogenous among the three zones, the C/N ratio decreased from 27.5 in zone 3 to 24.5 in zone 1. Finally, the bio-available fraction of phosphorus is rather low with a mean value of 17.8 $mg \, kg^{-1}$.

 Cobaltihexammine (CEC-exchangeable) and DTPA extractions were used for respectively assess the actual and potential MTE availability (Tab. 4). Regardless of the zone and the element considered, CEC-exchangeable concentrations were all below the analytical detection limits. Concerning the potential bioavailability of MTE, significant differences between the three zones were only found for Fe. For Pb and Cd, DTPA-extractable content in zone 2 was respectively about and 1.5 and 1.3 times lower than that in zone 1 and zone 3. For Fe, Mn and Zn, an opposite pattern was found with DTPA concentrations about 1.3 (Mn) and 1.5 (Fe, Zn) times higher in zone 2 compared to zones 1 and 3. Cu and Ni have a similar pattern with the highest DTPA-extractable content in zone 1. At the site level, the percentage of the pseudo-total content that was extracted by DTPA decreased in the following order: 45% (Pb), 27% (Cd), 14% (Cu), 9.2% (Zn), 2.3% (Ni), 1.9% (Mn), 0.1% (Fe). It should be noted that the

- distribution of DTPA-extractable Cd, Pb and Zn contents in the topsoils of the three zones (Tab.
- 4) followed the same trend as that described by their pseudo-total concentrations (Tab. 3).
- *3.4. Relationships between abiotic parameters in the topsoils*

 The first MFA axis explained 29.1% of the variance and was mainly explained by both groups of variables determined as available and total elements soil contents (Fig S2). Thus, this axis was positively correlated with total As and Pb and DTPA-extractable Pb for the element content parameters and CEC and exchangeable K and Mg for the fertility parameters (Fig. 4A). Only element content parameters (DTPA-extractable Cu, Fe and Mn, and total Mg and Mn) were negatively correlated to the first axis. This axis allowed a separation of soil samples along a gradient of both As and Pb pollution and soil exchange properties. This is confirmed by the ascendant hierarchical classification that allowed to separate the soil samples in four clusters of which clusters 1 and 4 are the most correlated with axis 1 (Fig. 4C). Indeed, cluster 1 was 361 characterised by lower average levels of total Pb $(1471 \text{ mg kg}^{-1})$ and DTPA-extractable Pb (629 m) 362 mg kg⁻¹) and total As (50.2 mg kg⁻¹) than the overall average. Similarly, DTPA-extractable Cd 363 had a lower average content than the general average $(0.654 \text{ mg kg}^{-1})$. On the other hand, cluster 364 3 was characterised by average contents above the general average of total Pb $(3248 \text{ mg kg}^{-1})$ 365 and DTPA-extractable Pb $(1456 \text{ mg kg}^{-1})$ and total As $(101.7 \text{ mg kg}^{-1})$. Cluster 4 was characterised by higher levels of total Cu. The second MFA axis explained 18.6% of variance. It was positively correlated with both soil fertility (exchangeable P and Ca and total N) and element content (total S, Na, Mg, Zn, and B and DTPA-extractable Mn and Zn) parameters (Fig. S2 and Fig. 4A). This axis allowed a separation of soil samples along a gradient of Zn pollution and P and N fertility. Thus, cluster 2 was characterised by higher average contents of 371 total Zn (364 mg kg⁻¹), DTPA-extractable Zn (39 mg kg⁻¹), and P Olsen (24.59 mg kg⁻¹) than the general average (Fig. 4C). When comparing the representation of individuals on the factorial map according to their zone or cluster of origin (Fig. 4B and 4C) we did not observe a full

 match. However, zone 3 was mostly characterized by cluster 3, whereas zone 2 was mainly characterized by both clusters 1 and 2.

 The heatmap of the Spearman correlation coefficients between soil parameters (Fig. S3) highlighted that DTPA-extractable metal concentrations were significantly positively correlated to their respective pseudo-total concentration. Pseudo-total As and Pb and DTPA-379 extractable Pb presented high significant positive correlations with each other $(R > 0.9)$ but 380 significant negative correlations with DTPA-extractable Fe and Mn and total Ca $(-0.71 < R < -1)$ 0.5). DTPA-exchangeable Pb was also significantly positively correlated with CEC-382 exchangeable K and Mg and total K $(0.5 < R < 0.58)$. Pseudo-total and DTPA-exchangeable 383 Zn were significantly positively correlated with P Olsen $(0.57 < R < 0.64)$ and total P $(0.35 <$ R < 0.49), whereas pseudo-total and DTPA-exchangeable Cd were significantly negatively 385 correlated with DTPA-extractable Fe $(-0.62 < R < -0.56)$.

4. Discussion

 4.1. Biodiversity assessment of vascular plant, Orthoptera and diurnal Lepidoptera communities in wastelands of the glass industry

 In the 1000-m² studied site, vascular plant assemblages were dominated by wasteland, grassland and edge species. On the one hand, with 54 identified species, we observed about half the vascular plant species inventoried in three calcareous grasslands (between 96 and 116) located in the northwestern Swiss Jura mountains (Baur et al., 1996). However, these sites were 10 to 20 times larger (1-2 ha) and according to Bonthoux et al. (2014), the species richness index increases with the size of a delimited area. Nonetheless, it should be noted that Melliger et al. (2017) found that plant species richness did not increased with the size of grasslands located in the urban region of Basel (Switzerland). Hence the plant diversity was considered to be rather low compared to natural ecosystems such as calcareous meadows. On the other hand, the species richness of vascular plants in the studied site was typical of the one recorded in small clearings growing on a former settling pond managed by natural attenuation in Pompey (Lorraine, North-East France) with more than 30 species identified (Lucisine et al., 2015). According to Dazy et al. (2009), our results (54 species identified) were even slightly higher than the ones reported in the vegetation of coking plants sites (27 to 46 species detected in seven zones of the Homécourt wasteland).

 Regarding orthopteran assemblages, our results (8 species identified) were quite comparable to the species richness of grasshoppers and crickets harboured by 1-2 ha dry grasslands in the Swiss Jura mountains (10 to 16 species; Baur et al., 1996) and 9-10 ha meadows (10 to 14 species; Báldi and Kisbenedek, 1997). Melliger et al. (2017) reported than Orthoptera species richness increased with habitat size in meadows (in the urban region of Basel, Switzerland). Hence, the number of Orthopteran species recorded in the 1000-m² studied was high given the size of the area. On the contrary, the species abundances found in the plot (35 individuals) were much lower than the ones recorded (64 to 175 individuals) in Báldi and Kisbenedek (1997). Nonetheless, the Shannon-Weaver diversity index (H') measured in our study (1.70) was in the range of the ones calculated by Baur et al. (1996) for the three calcareous grasslands (1.41 to 1.81). According to Báldi and Kisbenedek (1997), the Shannon index in the wasteland site (1.70) was higher compared to those determined for one meadow (0.73 to 1.02) and similar to the ones reported in the other meadow (1.71 to 2.07). In the studied site, the 418 Simpson diversity (\tilde{D}) and the Pielou evenness (J) indices (0.79 and 0.82, respectively) tend to show a great heterogeneity in the community structure of Orthoptera. In addition, both ecological indices were more elevated than the ones measured for the two meadows (0.17 to 0.72 for the Simpson diversity index and 0.32 to 0.79 for the Pielou evenness) in Báldi and Kisbenedek (1997). The Pielou index (0.82) was in the range of the ones computed by Baur et al. (1996) for the three 1-2 ha calcareous grasslands (0.70 to 0.86). Therefore, this wasteland ecosystem must be considered as attractive for grasshoppers and crickets.

 With 13 identified species, the assemblages of diurnal Lepidoptera remain different from the ones found in less disturbed ecosystems such as typical calcareous grasslands wherein the butterfly diversity reached 32 to 46 species in three sites of 1 to 2 ha in size (Baur et al., 1996). In comparison with urban areas, our results (13 species identified) were in the range of the species richness observed in eight parks of New York (7 to 22 species reported between June and September 2002; Giuliano et al., 2004). The number of day-active butterfly species was also more elevated than that observed in a business district and an office park near Palo Alto, in California (3 and 7 species, respectively), but was similar to those identified in a residential neighborhood (11 species), a golf course (12 species) and an open-space recreational area in the same city (14 species; Blair and Launer, 1997). However, in the studied site, diurnal Lepidoptera abundances (50 individuals) were below the ones measured in the residential neighborhood (65 individuals), the golf course (60 individuals) and the open-space recreational area (95 individuals) but considerably higher than that counted in the business district (8 individuals) and the office park (27 individuals; Blair and Launer, 1997). Nevertheless, the Shannon-Weaver diversity index (H') determined in our study (2.0) was higher than those calculated in the residential neighborhood (1.6) and in the golf course (1.8) but lower than that reported in the open-space recreational site (2.2; Blair and Launer, 1997). Furthermore, the 442 Simpson diversity (\tilde{D}) and the Pielou evenness (J) indices (0.84 and 0.79, respectively) tend to show a great heterogeneity in the community structure of diurnal Lepidoptera. To conclude, the biodiversity of day-active butterflies in the 1000-m² wasteland ecosystem of the glass industry may be considered as correct regarding the diurnal Lepidoptera diversity of moderately undisturbed areas to highly developed urban landscapes. However, it is worth noting that

 Orthoptera and diurnal Lepidoptera inventories were conducted between July and August, thus comparison with other full-year studies is more difficult as spring species were not recorded.

4.2. Spatial biodiversity patterns based on vascular plant and diurnal Lepidoptera communities

 In the studied site, zone 3 recorded the highest plant diversity, with more wasteland and edge species, and displayed the greatest level of endemicity (Fig. 2). Richness is obviously connected with endemicity, but the number of plant species does not vary enough between areas to fully explain the specificity reported in that zone. Zone 3 also shown the highest day-active lepidopteran and the lowest orthopteran biodiversity levels (Fig. 3). Conversely, more grassland plant species were identified in zone 1, the area with the lowest plant species richness. This was concomitant with a butterfly community structure characterized by a very low degree of endemicity and the lowest abundances, similarly to that observed in zone 2 (zone 1 and 2 harbored the lowest day-active Lepidoptera biodiversity). Such relationship between the species richness in both vascular plants and lepidopteran is consistent with Erhardt (1985) who found that species richness of diurnal Lepidoptera was often positively influenced by plant species diversity in cultivated and derelict grasslands. In urban environments, Giuliano et al. (2004) observed that plant species richness was also positively correlated with butterfly species richness and abundances in eight parks of New York. The mechanisms behind these patterns must be related to the pollination function of adult Lepidoptera in ecosystems (Hammond and Miller, 1998). Indeed, some studies have observed an increase in butterfly species richness with the abundance (Kuussaari et al., 2007) and diversity of nectar plant species (Kitahara et al., 2008). In addition, the richness of nectar flower species was closely connected with plant species richness (Kitahara et al., 2008). Hence, we assumed that day-active lepidopteran biodiversity was clearly related to the plant species richness found in the studied site as many 470 plants were at the flowering stage during the vascular flora inventory, in particular in zone 3 (Fig. S1, southern part of the plot).

4.3. Influence of trace metal contamination in the topsoils on the biodiversity of wastelands of the glass industry

474 In the studied site, Pb was a major element (content exceeding 1 g kg^{-1}) as reported in the blast furnace sludge from a derelict land located in North-Rhine Westphalia, Germany (Mansfeldt and Dohrmann, 2004). Pseudo-total Pb concentrations in our study were comparable to the levels recorded in several topsoils contaminated by Pb-Zn smelting activities (810-1695 478 mg Pb kg⁻¹ and 2920-7894 mg Pb kg⁻¹ at site 1 and site 4, respectively, in Rabitsch, 1995; 140- -2600 mg Pb kg⁻¹ measured in five sites near two Pb-Zn smelters, studied by Stone et al., 2001). Regarding the chemical risk index, each zone was highly contaminated with trace metal elements: all topsoils were characterized by high pseudo-total Pb and Cd contents. These two elements are considered to be toxic agents for plants: (i) vascular flora can be affected by Pb 483 when a level of 500 mg kg^{-1} is reached in the soils and, (ii) Cd is known to affect most biological components even at low concentrations (Kabata-Pendias and Pendias, 2001). For instance, Dazy et al. (2009) reported that Cd contamination in soils was one of the main factors influencing the community structure of plants in a former coking plant site (Homécourt, France) as significant negative correlations were found between the plant species richness (S) and high 488 levels of Cd contamination $(4 \text{ to } 28 \text{ mg Cd kg}^{-1})$ in the most polluted areas).

 Despite the high Pb and Cd pseudo-total contents reported in the topsoils, the wasteland site of the glass industry was attractive for Orthoptera, and the biodiversity of diurnal Lepidoptera was considered as attractive in the 1000-m² studied plot. Therefore, grasshoppers, crickets, and day-active butterflies did not seem to be adversely affected by the high Pb and Cd contamination in the topsoils. For the vascular flora, the diversity was considered to be rather low on a site scale, suggesting an influence of Pb and Cd contamination in topsoils on the plant species richness. However, regarding the distribution patterns of biodiversity in each zone, our results were not consistent with this previous assumption. Firstly, zone 3 was the most contaminated area with Pb and Cd although it recorded the greatest level of plant and diurnal Lepidoptera biodiversity. Secondly, pseudo-total and available Pb concentrations in topsoils and chemical risk indices were similar between zone 1 and zone 3, whereas the two zones differed in terms of plant species richness, endemicity and habitat preferences. Hence, trace metal contamination in topsoils (Cd, Pb) does not appear to be a critical limiting factor for vascular plant, Orthoptera and diurnal Lepidoptera biodiversity in this study.

 Moreover, Pb often has a low bioavailability in soils, especially under alkaline conditions (Kabata-Pendias and Pendias, 2001). In the superficial soils studied, DTPA-Pb concentrations were high and negatively correlated with the total Ca content while the pH was rather high (7.6-7.7). In the studied site, the high pH values are mainly reflected in negligible CEC-exchangeable As, Cd, Cu, Pb and Zn contents compared to the major cations (Tab. 4). Other authors have observed that a spontaneous forest can develop on former industrial sites heavily contaminated with metals with a plant community comparable to that of similar natural ecosystems (Lucisine et al., 2015) and a high diversity of meso- and macrofauna in the topsoil layer (Huot et al., 2018). In comparison with this study, the Pb contents of the surface horizon 512 amounted to > 3 g kg⁻¹ total Pb, 2060 mg kg⁻¹ DTPA-exchangeable Pb and 0.1 mg kg⁻¹ CEC- exchangeable Pb (Huot 2013; Huot et al., 2018). It has been shown in urban soils that Pb was mainly found in stable forms such as organic complexes, thus limiting its bioavailability (Ge et al., 2000). Therefore, the high total and DTPA-exchangeable Pb contents of the studied surface soils probably have little influence on the ecological recovery of glass industry wastelands.

4.4. Influence of topsoil fertility on the biodiversity of wastelands of the glass industry

 In the studied site, the topsoils displayed high organic carbon contents regarding grasslands (2.5%) and soils of urban, industrial, traffic, mining and military areas (SUITMA) (3.5- 4.5%; Joimel et al., 2016; Mao et al., 2014). However, our results were of the same order of magnitude as those measured in coking plant soils (7-10 %; Biache et al., 2008). All C:N ratios were elevated compared to C:N values from typical meadows (around 10; Ponge et al., 2013; Stauffer et al., 2014), thus corresponding to moder-like non-biologically active types of humus that are commonly found in forests on non-calcareous soils. High C:N ratios can be related to low N contents. In the studied topsoils, our results were similar to the values recorded for coking plant soils (Biache et al., 2008) and typical of the high ratios found in industrial soil substances, suggesting a technogenic origin for organic matter (Morel et al., 2005). Soil electrical conductivity was rather low, suggesting that topsoils were not affected by salinity, a problem often encountered on brownfield sites. Indeed, the topsoils were strongly depleted in pseudo-total Na and K, when compared to the average compositions of surface soils (Sposito, 1989). CEC was almost in the range of values found in some urban park or garden soils (32 532 cmol+ kg⁻¹; Santorufo et al., 2014) or even the same as reported in meadows (35 cmol⁺ kg⁻¹; Stauffer et al., 2014). With a slightly alkaline pH and a high CEC saturated by calcium, the studied site shared common features with natural carbonated soils as reported by Huot et al. (2013) for the topsoils of a settling pond developing on iron industry by-products. Alkalinization might be induced by the presence of gravel and concrete in the studied materials (Morel et al., 2005; Rokia et al., 2014). From the point of view of agronomic soil fertility, the studied soils presented high CEC-exchangeable K and Mg contents (Schvartz et al., 2005). 539 However, Olsen P concentrations were very low according to SUITMA's (45 mg kg^{-1}) and 540 grasslands soils (31 mg kg^{-1}) ; Joimel et al., 2016). To conclude, as measured in this study, the topsoils showed a medium fertility in comparison with other soils.

 Regarding the distribution patterns of biodiversity in each zone, great differences were noted in the structure of Orthoptera and diurnal Lepidoptera communities and in plant endemicity whereas topsoil fertility was similar between the three zones. However, in detail, zone 3 displayed the lowest chemical fertility in the topsoils although it recorded the greatest level of plant and diurnal Lepidoptera biodiversity. In fact, plant species richness was probably

 related to the low amounts of N and available P reported in the topsoils of zone 3, limiting the prevalence of some species (Heinze et al., 2015). We also noted a possible influence of the calcareous status of topsoils on the plant species composition in the studied site. Our results in zone 2 are consistent with this previous assumption as the area recorded the largest number of calcareous plant species while pseudo-total Ca contents were more elevated in that zone. The calcareous status and nutrient contents (total N and P Olsen) in topsoils have probably an impact on plant assemblages (species richness and habitat preferences), and thus an indirect influence on the diurnal Lepidoptera biodiversity. Thus, biodiversity could be more influenced by soil fertility parameters than by trace metal contamination in the studied topsoils, as reported by Vincent et al. (2018) for several derelict soils.

 Nonetheless, as measured in this study, topsoil fertility proxies are probably not the most impacting variables for plant, Orthoptera and diurnal Lepidoptera biodiversity. Other environmental factors could have an influence on wasteland biodiversity of the glass industry. On the one hand, the plot was surrounded by well-forested borders that may be involved in the spontaneous colonization of the site by vascular flora and insect communities. These wooded areas could also protect diurnal Lepidoptera from the wind, leading to the rather high diversity reported in the investigation zone. On the other hand, biodiversity levels could be explained by the poor land management practices carried out on the plot. For instance, Zerbe et al. (2003) also reported a higher plant species richness in less disturbed areas and Erhardt (1985) indicated that day-active Lepidoptera diversity was greater in undisturbed meadows than in intensively managed grasslands.

5. Conclusion

 In our study, wastelands of the glass industry displayed a higher biological quality than expected regarding the high Pb and Cd contamination reported in the topsoils. Indeed, the 1000 m² studied plot was attractive for Orthoptera and the biodiversity of diurnal Lepidoptera was correct. Thus, several ecosystem services were already provided by wastelands of the glass industry, giving habitats for some terrestrial arthropods communities that harbor a great Orthoptera diversity. According to the distribution patterns of biodiversity and topsoil contamination in each zone, the results highlighted that the most contaminated area recorded the greatest level of plant and diurnal Lepidoptera biodiversity. This can be accounted for by the low bioavailability of Pb under sub-alkaline conditions and the positive influence of plant species richness on diurnal Lepidoptera biodiversity. Hence, trace metal contamination in the topsoils does not appear to be a main limiting factor for vascular plant, Orthoptera and diurnal Lepidoptera communities. Our results showed that wastelands of the glass industry had a medium chemical fertility, in accordance with the biodiversity levels recorded in this study. Moreover, the least fertile area was characterized by the highest plant and day-active Lepidoptera diversity, suggesting a positive effect of soil nutrient depletion (N, P) on plant species richness. Therefore, we assumed that biodiversity in wastelands of the glass industry was more influenced by soil fertility parameters than by trace metal contamination in the topsoils. To conclude, these sites are able to provide biodiversity ecosystem services, acting as wildlife sanctuaries for Orthoptera and diurnal Lepidoptera, and strategic metals by phytoextraction in a circular economy model.

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806 **Tables**

807 **Tab. 1.** Spatial distribution of vascular plant species regarding their habitat preferences.

808 Habitats represented by only one species were not mentioned. The numbers were computed

809 from the data listed in Tab. S1.

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⁸¹² determined in each zone for Orthoptera and diurnal Lepidoptera communities, from Tab. S2.

ECOLOGICAL INDICES	ZONE ₁	ZONE ₂	ZONE ₃	SITE
ORTHOPTERA				
Shannon-Weaver index (H')	1.22	1.62	0.81	1.70
Simpson diversity index (\widetilde{D})	0.65	0.81	0.52	0.79
Pielou evenness index (J)	0.68	0.91	0.74	0.82
LEPIDOPTERA				
Shannon-Weaver index (H')	1.18	0.83	1.95	2.01
Simpson diversity index (\widetilde{D})	0.59	0.55	0.83	0.84
Pielou evenness index (J)	0.66	0.75	0.79	0.79

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⁸¹¹ **Tab. 2.** Shannon-Weaver diversity (H'), Simpson diversity (\tilde{D}) and Pielou evenness (J) indices

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841 **Fig. 1.** Subdivision of the 1000-m² studied area into three zones.

 Fig. 2. Plant species assemblage in the three zones. Species richness (S) and the ratios were computed from the data listed in Tab. S1.

 Fig. 3. Differences in the community structure of Orthoptera (a) and diurnal Lepidoptera (b) reported between the three zones. Species richness (S), abundances (A) and the ratios were computed from the data listed in Tab. S2.

 Fig. 4. Multiple-factor analysis (MFA) performed on abiotic parameters in the topsoils. A. Correlation circle of variables. B and C. Representation of individuals on the factorial map according to their zone or cluster of origin.