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ABSTRACT: A disused electrical substation was the site of the spill of 40,000 L of Ascarel oil. However, none of the expected principal contaminants were chemically detected above established guidelines. Since the analysis of other possible contaminants is extremely expensive and impractical, the objective of this study was to indirectly evaluate possible contamination by determining the influence of physico-chemical parameters on the variation in soil mesofauna data and ecotoxicological testing. The tested substation is located in Ipanema National Forest in São Paulo State, Brazil. The area surrounding this site has several different soil uses and vegetation cover types. The square 200 m × 200 m collection area was defined by a grid comprised of 6 equally spaced lines along each axis. Soil samples were taken at each intersection save for 2, for a total of 34. Ecotoxicological testing was completed using *Enchytraeus crypticus*. The mesofauna was affected by multiple soil attributes that together accounted for 75.5 and 84.8% of the variability in samples taken from secondary forest and wooded pasture, respectively. However, variability in the *E. crypticus* testing was not strongly correlated to these physico-chemical attributes, possibly due to the tolerance of the tested species to the variations observed in this study. This provides strong evidence that no contaminants are affecting local biota. However, additional studies are needed to analyze more complex abiotic factors that might account for the observed variability.

Key words: ascarel; polychlorinated biphenyls; soil biota; soil contamination

Ecotoxicologia e mesofauna do solo em área com histórico de derramamento de óleo na Floresta Nacional Ipanema

RESUMO: Em uma subestação elétrica ferroviária desativada houve o derramamento de 40 mil L de óleo ascarel. Entretanto não se detectou no solo a presença dos contaminantes esperados acima dos valores orientadores. Como analisar quimicamente outros possíveis contaminantes se torna altamente custoso e inviável, o objetivo deste estudo é avaliar de forma indireta a possível presença de outros contaminantes, através do poder de explicação da variabilidade de dados da mesofauna do solo e de ensaios ecotoxicológicos por parâmetros químicos e físicos do solo. A subestação está localizada na Floresta Nacional de Ipanema, em São Paulo. No local foi definido um grid de coleta de 40 x 40 m com seis linhas. A mesofauna mostrou-se influenciada por alguns atributos do solo, que em seu conjunto explicou 75,5 % da variabilidade nos pontos de floresta secundária e 84,8 % nos pontos de pasto arborizado. A variabilidade dos resultados do ensaio com *E. crypticus* foi pouco explicada pelas características físico-química do solo. Com isso, há fortes indícios que não há contaminantes gerando impactos na biota do solo do local. Entretanto, verifica-se a necessidade de um estudo adicional com atributos abióticos de maior complexidade como fatores explicativos da variabilidade.

Palavras-chave: ascarel; bifenilas policloradas; biota do solo; solo contaminado

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Introduction

The extent to which humans introduce substances into the environment affects many features, including changes in the chemical, physical, and biological integrity of water and soil as well as the use of natural resources (Conama, 2009; Van Der Perk, 2012). However, National Forests are chosen based on their contribution to the continued biological diversity and genetic maintenance of natural ecosystems (MMA/Ibama, 2003), making it important to rule out all possible sources of soil contamination that have not yet been investigated. This need is highlighted by Plaza et al. (2010) who emphasize that chemical analyses must be completed for each isolated contaminant; indeed, there have been cases in which certain toxins, such as specific metabolites and biological derivatives, have not been detected, resulting in estimates that fail to account for the actual environmental risk.

The only known pathway for PCB degradation relies on the action of bacteria and other microorganisms, both aerobic and anaerobic (Beyer & Biziuk, 2009). Furthermore, incomplete degradation results in the formation and accumulation of metabolites that are more toxic than the original compound (Borja et al., 2005). Moreover, the possibility of additional contaminants was raised at the time of the oil spill by the Environmental Agency of the State of São Paulo (CETESB), with potential concerns including chlorobenzenes, aromatic compounds, furan compounds (which are degradation products of mineral oils), chlorinated hydrocarbons, and other variably volatile hydrocarbons. Evaluating the effects of possible chemical agents requires both ecotoxicological information relating to toxic effects and exposure conditions and ecological information about the biotic and abiotic features of the potentially exposed system.

Ecotoxicological testing methods have been developed to integrate exposure and effects into a single analysis and describe the ecotoxicological potential of a substance when added to the soil. It is use to combine a conventional version of chemistry analysis and the progress of bioremediation projects (Chapman et al., 2010; ABNT NBR ISO 17616, 2010; Rodrigues et al. 2011). These typically analyze multiple characteristics of a contaminant, including particularity, potential toxicity, toxicity of an agent that had not been analyzed separately, but which was contained in the substance, the toxicity of the mixture and the bioavailability (Chapman et al., 2010).

The assays are made with representative living organisms, which together show different types of responses as well as varied functional activities, in order to measure the actual toxicity of a substance or mixture present in the environmental samples of the contaminated site (Mesman et al., 2006; Environmental Agency, 2008; Fernandez & Tarazona, 2008; ABNT NBR ISO 17616, 2010), and with the final objective of carrying out a risk assessment (Landis & Yu, 2003).

The selection of the appropriate bioassay is fundamental to determine the potential of contaminated soil to cause damage (Environmental Agency, 2008), and is summarized by Fernandez & Tarazona (2008) in lethal and sublethal (inhibition of growth or reproduction). Thus, for example, considering a pollution situation that involves a long exposure time at low concentrations, chronic (reproduction) tests usually represent the situation more realistically. However, the avoidance tests can be used as a valuable tool for evaluation of soil contamination (Loureiro et al., 2005).

The environmental analysis evaluating the effects on biological communities of the soil, such as changes of the structure, function, sensitivity and vulnerability (Lange et al., 2010), depending on the spatial distribution of contamination (Salminen & Sulkava, 1997). The characterization of ecological effects is certainly the most critical aspect of the risk assessment process, as it is a more realistic condition and includes multiple species and additional exposure routes (Landis & Yu, 2003). These data provide information about the structure and function of the soil, directly linked to the objective of biodiversity protection, complementing and providing ecological relevance (Niemeyer et al., 2010).

In the scientific literature the largest amount of reports in this field is related to the microbial community of the soil. Changes in soil conditions due to contamination by heavy metals, for example, has a large negative effect on the activity and composition of soil microorganisms (Oliveira & Pampulha, 2006; Wang et al, 2007), the microbial biomass loss of their functional aspect and inhibition of processes performed as the mineralization of organic matter and (Frey et al., 2006). In this subject Niemeyer et al. (2012) found that the contamination causes detrimental effects on soil properties and the quality of the deposited organic matter, causing negative impacts on soil microorganisms and consequently affecting the ecosystem processes they measured.

Ecological analyses completed on a communal level have already provided site-specific information relating to the actual bioavailability of contaminants (Antunes et al., 2013). These contaminants, when present, cause changes in soil biota, specifically with respect to their abundance, distribution, biodiversity, growth rate, interactions, and overall relation on the food chain (Van Der Perk, 2012).

While the number of potential contaminants makes a complete analysis extremely expensive and impractical, especially in the previously described case, it might be possible to link ecological and ecotoxicological results to natural soil characteristics, which in turn would strongly suggest that no contaminants are present. Therefore, the objective of this study was to evaluate the mesofauna community of the soil in this area, complete *ex situ* ecotoxicological testing, and compare the results with the chemical and physical parameters of the soil.

Materials and Methods

The Ipanema National Forest extends for more than 5,000 hectares, within the municipalities of Iperó, Araçoiaba

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da Serra, and Capela do Alto in the southeast region of the state of São Paulo. The altitude of the forest ranges between 550 and 917 m, highlighting the mountains of Araçoiaba, the defining feature of the region (MMA/Ibama, 2003).

It includes sections of semideciduous forest and rain forest, as well as areas of cerrado *sensu lato* (MMA/Ibama, 2003). Its climate is classified as Cfa under the Köppen classification system. The average annual precipitation is 1,400 mm, while the minimum and maximum values are 800 mm and 2,200 mm, respectively (MMA/Ibama, 2003).

The study area is located at the edge of the Vanhargem water reservoir, at 23°25'S 47°35'W and at an elevation of 560 m. This reservoir provides water to administrative buildings and houses in São João de Ipanema, a community within the National Forest. This area is characterized by its diverse soil use, owing to the presence of pastures, secondary forest fragments, roads, railways, and domestic buildings. This limited an assessment of the abiotic characteristics of the soil, thereby preventing a complete understanding of the physical and chemical features.

In Brazil, contaminated soil management begins with a preliminary evaluation of the historic use of the site and a field inspection. This is followed by sampling and chemical analysis (Conama, 2009).As a research initiative, this procedure was applied at a defunct electrical substation located within a National Forest, resulting in the detection of 5,000 m² of soil contaminated by 40,000 L of the oil ascarel (MMA/Ibama, 2003). The historical analysis, which was performed at the time the contamination was detected, revealed the presence of both polychlorinated biphenyls (PCBs), the principal toxic agent in Ascarel, and metallic mercury. However, the chemical analysis, completed in 2014, did not detect PCBs (IT 06-07.141 ver.01 method) or polycyclic aromatic hydrocarbons (SW-846 Update IVA method), which are typically found in mineral oils. Meanwhile, barium, chromium, copper, lead, nickel, selenium, and zinc levels (SMWW 3120 B method) were below the quality benchmark (Conama, 2009). Therefore, according to the protocol, no further action must be taken.

The square 200 m × 200 m collection area was defined by a grid comprised of 6 equally spaced lines along each axis. Soil samples were collected at each intersection save for 2, for a total of 34, in June 2014. No sample could be obtained at point 27, given that the soil at this location is primarily composed of gravel due to the presence of railroad lines. Sample collection was also not possible at point 33 because this area was on private property and was therefore inaccessible (Figure 1).

At each collection point, three soil samples were taken at depths of 0.00–0.10 m. The samples were then transported to the laboratory. The composite samples were then submitted to a sieve analysis and were assessed for organic matter content, soil humidity and pH (KCl 1 mol L⁻¹) (Table 1).

The mesofauna was sampled using a modified Berlese-Tullgren funnel. In order to accomplish this, 4 samples of 0.1 kg each were taken from each point at depths of 0.00–0.10
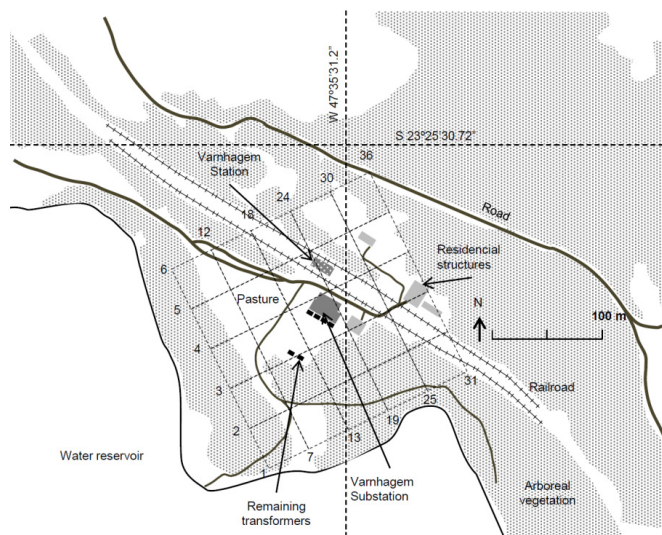

Figure 1. A map of the 200 x 200 m collection grid overlaid on the study area.

m, after which they were placed in sealed plastic containers and labeled. Then, in the laboratory, each sample was placed in a plastic container with a 2-mm mesh at its base. These samples were then placed under an incandescent 20-W bulb for 7 days so that the generated heat would prompt organisms in the soil to move through it, eventually passing through the bottom and falling into the collection bottle containing 1% formaldehyde.

These collected organisms were counted and broadly grouped taxonomically. The number of individuals was used to calculate total and average richness. This was followed by the Shannon-Weaver diversity index (H = -∑ p_i log p_i) and the Pielou equation (e = H log R^{-1}), where p_i is the relative frequency of individuals within each taxonomical group and R is richness, defined as the number of different taxonomical units collected in each area that was evaluated. Occurrence was also determined based on the presence or absence of certain groups.

For ecotoxicological testing, 4 samples of 0.1 kg each were taken from each point at depths of 0.00–0.10 m, after which they were combined into a composite sample. In the laboratory, they were sieved through a 2-mm mesh and subjected to 2 alternating cycles of freezing and thawing, each for 24 h, with the goal of completely removing all soil fauna.

Testing with *E. crypticus* followed the required and recommended standards of ABNT NBR ISO 16387 (2012). The organisms were cultured in agar gel petri dishes at 20 ± 2 °C. The soil was pre-moistened with distilled water to between 40 and 60% of its water retention capacity. Testing was completed in an incubation chamber at a controlled temperature of 20 ± 2 °C, a photoperiod of 16/8 h day/night, and a luminous intensity of 400–800 lx. Soil humidity was controlled weekly by comparing the initial weight of the test containers to the instantaneous weight and adding distilled water to account for the difference. The organisms were also tested using the standardized "artificial tropical soil"

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Table 1. Type of vegetation cover and physico-chemical characterization of soil at the various sampling points.

Point	Vegetation cover	Clay	Sand	Silt	Organic material	Humidity	pH
				(%)			
1	Secondary forest	23.1	48.7	28.2	4.1	16.1	5.6
2	Secondary forest	18.7	49.4	31.9	3.4	12.6	6.1
3	Secondary forest	20.1	67.3	12.6	2.3	9.1	5.7
4	Wooded pasture	8.1	51.2	40.7	1.9	6.2	5.9
5	Wooded pasture	12.3	79.4	8.3	2.2	12.1	5.9
6	Wooded pasture	12.9	51.4	35.7	5.1	10.1	6.7
7	Pasture	17.3	48.8	33.9	2.8	13.5	5.2
8	Pasture	23.7	63.5	12.9	1.7	18.3	6.0
9	Secondary forest	13.1	58.5	28.4	2.3	5.9	5.8
10	Pasture	15.6	71.6	12.8	2.6	13.3	5.7
11	Pasture	12.4	68.3	19.3	3.2	14.6	5.7
12	Pasture	11.1	71.5	17.4	1.6	12.0	6.6
13	Secondary forest	6.5	68.8	24.7	2.0	10.4	5.3
14	Secondary forest	18.4	68.1	13.5	3.6	10.8	5.3
15	Pasture	6.8	62.5	30.7	4.0	15.1	6.7
16	Pasture	10.5	70.9	18.6	1.1	10.8	6.4
17	Pasture	11.2	69.0	19.8	0.6	14.1	7.5
18	Pasture	9.6	63.7	26.7	7.2	14.7	5.8
19	Secondary forest	16.0	67.7	16.3	3.3	9.8	5.4
20	Secondary forest	12.1	75.9	12.1	2.7	13.6	5.4
21	Pasture	11.4	69.4	19.2	5.0	23.0	6.0
22	Pasture	5.1	66.9	28.0	2.8	12.5	5.9
23	Pasture	13.5	77.2	9.4	1.7	13.0	5.8
24	Pasture	5.6	76.3	18.1	1.2	8.3	5.8
25	Secondary forest	1.3	52.1	46.6	2.2	16.2	5.2
26	Wooded pasture	11.1	55.9	33.0	3.3	26.1	5.0
28	Wooded pasture	13.0	75.7	11.3	2.4	7.9	6.0
29	Pasture	14.4	83.2	2.4	0.9	8.8	5.2
30	Wooded pasture	20.4	72.9	6.7	1.4	11.2	6.2
31	Wooded pasture	9.7	54.5	35.8	3.2	18.3	5.9
32	Wooded pasture	9.2	57.5	33.3	3.3	12.1	5.7
34	Pasture	2.1	62.5	35.4	1.3	12.1	6.3
35	Wooded pasture	21.5	72.4	6.1	3.3	14.4	5.7
36	Secondary forest	60.7	27.1	12.2	3.2	7.2	5.9

substrate (ABNT NBR 15537, 2014) in order to evaluate the overall adequacy of the tested environmental conditions. Each trial was performed 5 times.

These tests used sealed cylindrical transparent 40 mL containers containing 30 g of humid soil and 50 mg of thin oat flakes meant to serve as a food source. Each of these contained 10 individual organisms selected using a stereomicroscope. One additional replicate was performed without any added food or organisms for both the natural and artificial soil trials. This was used to evaluate pH and humidity at the end of the experiment. The organism trials were restocked with the same amount and type of food after 14 days. All tests lasted 28 days in total. At the end of the experiments, the containers were filled with 70% alcohol solution and 3 drops of 1% rose bengal stain, after which both adult and adolescent organisms were counted under a stereomicroscope.

Data analysis began by evaluating homogeneity of error variance using the Cochran test and normality using the Lilliefors test. Averages were compared using the Kruskal-Wallis and Tukey's tests, while correlations were tested using the Spearman and Pearson tests; for both of these, the first

test was used for non-parametric data, while the second was used for parametric data ($p \leq 0.05$). Variance between the mesofauna and biotic ecotoxicological data and the abiotic physico-chemical soil parameters was evaluated using principal component analysis (RDA).

Results and Discussion

Overall, 1578 individual organisms were observed in the mesofauna analysis. These were assigned to 24 groups, with the most common being Acari, accounting for 60% of all individual on average, followed by Entomobryomorpha, Formicidae, Poduromorpha, Symphyla, and Protura (Table 2).

Generally, points with secondary forest vegetation showed greater average richness and number of individuals from the Acari, Entomobryomorpha, and Protura groups when compared to those from pastures. This is the result of changes to the forest environment resulting from being part of an agroecosystem, which changes abiotic soil conditions and decomposing organic material (Henneron et al., 2015). In addition, forestry systems have greater plant litter and

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Table 2. Characterization of soil mesofauna at the individual sites by average number of individuals within the primary groups, total number of individuals, richness, and diversity indices.

Point	Acari	Entom.	Formicidae	Poduromorpha	Symphyla	Protura	Individuals	Average richness	Total richness	Shannon	Pielou
					Secondary forest						
1	82.0	7.3	3.5	5.3	1.0	2.5	112	8.5	14	1.66	0.43
2	54.3	11.5	0.5	7.3	1.5	4.3	84	8.0	13	1.82	0.49
3	40.3	6.3	1.8	6.5	0.3	1.8	60	7.3	13	1.75	0.47
9	37.3	9.8	5.3	2.8	0.3	3.3	71	8.8	14	2.36	0.62
13	22.8	8.5	3.0	1.3	0.3	8.8	46	6.0	10	2.09	0.63
14	13.3	4.5	11.3	0.5	0.3	0.5	31	4.5	7	1.77	0.63
19	57.5	12.5	12.8	2.8	0.5	7.3	96	6.5	10	1.87	0.56
20	9.5	4.0	2.3	0.3	0.0	0.5	18	3.8	7	1.90	0.68
25	16.3	5.8	3.0	2.8	1.8	0.3	33	7.0	11	2.36	0.68
36	14.0	0.8	2.3	0.8	0.0	0.0	26	5.5	8	2.03	0.68
Average	34.7 a[2]	7.1 a	4.5 b	3.0 a	0.6 a	2.9 a	57 A[1]	6.6 A	19	2.14	0.50
					Wooded pasture						
4	16.8	1.8	1.8	0.8	0.0	0.3	24	5.5	13	1.78	0.48
5	25.5	2.5	0.5	1.0	0.8	0.5	33	5.0	9	1.34	0.42
6	11.5	2.0	0.0	3.0	0.5	0.3	20	3.5	8	1.94	0.65
26	58.3	12.3	53.0	1.8	2.0	12.5	146	7.8	11	2.08	0.60
28	12.3	0.5	1.3	1.5	0.3	0.0	16	3.5	6	1.25	0.48
30	14.5	0.8	0.5	0.0	0.3	0.0	18	4.0	9	1.25	0.39
31	23.3	9.8	6.0	4.8	2.3	0.5	53	7.3	10	2.47	0.74
32	38.5	11.3	0.8	0.8	1.3	0.5	56	6.3	12	1.56	0.44
35	13.0	2.0	2.0	2.0	0.0	0.0	20	3.0	5	1.54	0.66
Average	23.7 ab	4.8 ab	7.3 ab	1.7 a	0.8 a	1.6 b	42 A	5.1 B	16	2.16	0.54
					Pasture						
7	86.3	17.5	3.3	6.0	0.0	4.8	122	6.8	10	1.51	0.45
8	29.8	2.8	0.0	1.8	0.5	0.0	39	5.8	12	1.46	0.41
10	3.3	1.0	2.0	0.8	0.3	0.0	7	3.0	5	1.93	0.83
11	5.3	0.3	0.0	0.8	0.0	0.0	8	2.3	5	1.45	0.62
12	4.0	0.3	0.3	1.3	0.0	0.0	8	3.0	7	2.14	0.76
15	11.5	4.0	1.3	8.8	0.3	0.8	29	6.0	10	2.29	0.69
16	7.3	1.0	1.3	3.5	0.0	0.0	15	4.3	7	2.03	0.72
17	10.5	2.3	2.8	1.8	0.5	0.0	20	4.3	8	2.12	0.71
18	27.0	8.0	104.0	8.5	2.8	0.5	164	10.0	17	1.92	0.47
21	22.5	8.0	0.8	2.8	0.0	0.0	36	4.0	7	1.56	0.56
22	18.0	1.0	0.3	1.3	0.0	0.0	22	3.0	6	1.07	0.42
23	19.3	13.8	2.3	4.0	0.5	1.0	43	5.8	10	2.05	0.62
24	37.5	1.0	3.0	2.8	1.3	0.3	55	7.0	11	1.77	0.51
29	25.3	1.0	0.0	0.8	0.0	0.0	30	3.5	7	0.99	0.35
Average	21.9 b	4.4 b	8.6 a	3.2 a	0.4 a	0.5 b	45 A	4.8 B	22	2.21	0.50

En – Entom.; [1]Capital letters within columns indicate no significant difference shown with Tukey's test (p ≤ 0.05). [2]Lowercase letters within columns indicate no significant difference shown with the Kruskal-Wallis test (p ≤ 0.05).

a more developed root system. Both of these features increase carbon and nitrogen soil content, thereby improving conditions for fauna (Manhães et al., 2013).

The Acari, Entomobryomorpha, and Protura groups were found in different types of soil cover. These groups are very important for decomposition and in maintaining soil fertility, and are usually present in greater number and diversity in environments like forest fragments, which have more soil cover, greater richness of vegetable species, and superior microclimate conditions (Manhães et al., 2013; Meloni & Varanda, 2015).

There was a significant correlation between the mesofauna and the physical and chemical attributes of the soil. The most pertinent soil characteristics in this case were the percentage of sand, organic matter content, and pH (Table 3).

Soil texture is an important characteristic both in its own right and with respect to humidity and organic matter content (Lavelle & Spain, 2001). In this case in particular, increased sand content negatively affected the abundance of certain groups, as well as average richness and the Shannon Weaver index (the last of which, along with the Pielou equation, showed no dependence on vegetation type). Other authors (Lavelle & Spain, 2001; Gagnarli et al., 2015) have corroborated this observed influence; however, on the whole, it has not been seriously addressed in the literature.

Only organic matter showed any positive correlation with the mesofauna groups and the overall number of individuals. This relationship has been connected to the influence of decomposition and the overall quality of vegetation cover (Araújo et al., 2015; Yunfeng et al., 2015). Among the favored

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Table 3. Correlations between the mesofauna and the physical and chemical attributes of the soil.

	Clay	Sand	Silt (%)	Organic material	Humidity	pH
Acari	0.15	-0.35*	0.22	0.18	0.03	-0.41*
Entomobryomorpha	0.06	-0.34*	0.33	0.40*	0.31	-0.41*
Poduromorpha	-0.08	-0.36*	0.40*	0.25	0.28	0.16
Formicidae	-0.05	-0.24	0.21	0.21	0.10	-0.50*
Symphyla	-0.14	-0.24	0.33	0.19	0.29	-0.09
Protura	0.01	-0.35*	0.33	0.35*	0.03	-0.47*
Individuals	0.01	-0.32	0.32	0.48**	0.31	-0.41**
Average richness	0.04	-0.43**	0.41**	0.33	0.11	-0.28
Shannon	-0.05	-0.35**	0.40**	0.12	0.12	0.14
Pielou	0.01	-0.07	0.06	0.01	0.12	0.22

*Spearman correlation ($p \leq 0.05$); **Pearson correlation ($p \leq 0.05$).

groups, springtails play a considerable role in the production, modification, and movement of organic matter, principally through their residual egg matter, feces, and dead biomass. The resulting matter could serve as a binding agent, leading to the formation of aggregates (Maaß et al., 2015).

Increasing pH correlated with a decrease in total population for the majority of organisms. This relationship has previously been observed for mites and springtails, in which case it is most pronounced in more conserved sites (Birkhofer et al., 2012; Heiniger et al., 2014; Mueller et al., 2016; Silva et al., 2016). However, this pattern is not consistent for all organisms given that changes in pH affect other pertinent factors, including the dominance of tree species, nutrient availability, and soil humidity (Mueller et al. 2016). In addition, different species interact very differently with their environment, with euedaphic organisms actually favoring low pH environments (Silva et al., 2016).

On the other hand, RDA of all the mesofauna and physico-chemical soil attribute data determined that just 25.9% of the variability was explained by the abiotic data set. However, grouping points by their vegetation cover (Tables 1 and 2) increased the share of mesofauna variability to 75.5% (35.2% for the x-axis) for secondary forests, 84.8% (60.5% for the x-axis) for wooded pastures, and 48.5% (49.4% for the x-axis) for pastures (Figure 2).

This shows that soil characteristics are closely linked with vegetation cover, making this the defining set of properties that determines the structure of the mesofauna community. This significant effect of soil properties on the variance of biotic abundance and diversity has also been observed by Birkhofer et al. (2012). The weaker correlation observed for pastures likely results from the fact that this land type is more heavily influenced by humans, making artificial effects more pronounced.

The observed differences in vegetation cover result from the different effects that soil properties exert on living organisms (Luo et al., 2014). In secondary forests, organic matter content showed little effect when compared with those of other factors; for example, humidity and pH heavily influenced the Poduromorpha and Symphyla groups. On the other hand, organic matter content and humidity were the factors with the most pronounced influence in wooded pastures. Similarly, organic matter content was especially
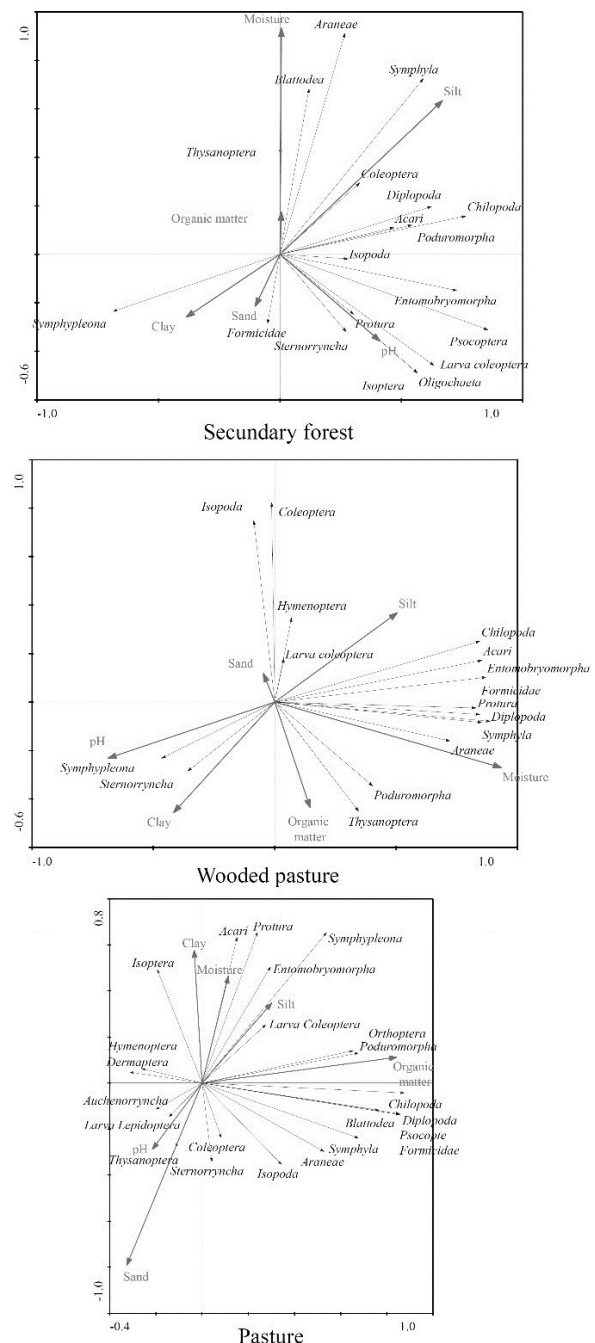

Figure 2. RDA analysis between soil mesofauna and physico-chemical soil attribute by vegetation cover.

Table 4. Ecotoxicological testing of *E. crypticus* using the various soil samples.

Point	Adult survival (%)	Reproduction	Point	Adult survival (%)	Reproduction
1	98	358	18	78	387
2	96	473	19	80	123
3	84	543	20	66	90
4	96	465	21	94	247
5	90	435	22	76	117
6	48	152	23	88	301
7	98	463	24	76	96
8	98	567	25	34	39
9	58	204	26	2	1
10	92	560	28	80	198
11	94	504	29	90	561
12	82	364	30	62	54
13	92	556	31	50	132
14	92	621	32	52	277
15	96	348	34	92	588
16	74	510	35	90	213
17	36	49	36	84	274

important in pastures, particularly for the Formicidae and Symphyla groups.

Despite this, a certain percentage could not be accounted for solely with the physico-chemical parameters. Other factors must be responsible in this case, possibly including human interference, habitat structure (Heiniger et al., 2014), and pollutants originating from the previously mentioned transformer oil spill. However, if the soil were contaminated, there should be tremendous changes in soil biota, specifically with respect to their abundance, distribution, biodiversity, growth rate, interactions, and overall relation on the food chain (Van Der Perk, 2012); in such a case, the correlation showed by the physico-chemicals characteristics should be much lower. As discussed above, the inclusion of vegetation data in RDA helps to highlight the situation, as it improves the explanatory power of the variability analysis with respect to mesofauna; this is because such a small-scale study provides information on local information relating to specific habitats (Birkhofer et al., 2012).

The survival and reproduction of *E. crypticus* placed in the soil samples showed significant variation between sampling points, with values ranging from 2 to 98% survival and 1 to 621 living adolescents (Table 4). Meanwhile, the artificial soil trials confirmed that the lighting and temperature of the test conditions had no effect on the results.

The physico-chemical parameters showed no significant correlations with *E. crypticus* survival and reproduction. While the reason for the difference is not clear (Chelinho et al., 2011), other authors have demonstrated a correlation with these variables, especially for pH, conductivity, clay and sand content, and the carbon/nitrogen ratio (Chelinho et al., 2011; Luo et al., 2014; Vašíčková et al., 2015). Any lack of correlation observed might be because the selected organism can effectively survive and reproduce within the range of conditions found in the sampled points; in this case, pH values fell between 4.3–8.2, organic content measured at 1.2–42%, and clay concentrations were between 1–29% (Table 1) (ABNT NBR ISO 16387, 2012; Vašíčková et al., 2015).

Soil parameters accounted for 38.0% of the overall variability. Although soil properties have previously been shown to be responsible for the survival and reproduction of *E. crypticus* (Vašíčková et al., 2015), these results show that the source for a significant percentage of the variability remains unaccounted for. This situation, together with the lack of any significant correlations in the soil testing, leaves two possible conclusions with respect to the initial hypothesis. On the one hand, it is possible that some unknown contaminant(s) is heavily influencing the environment tested in the study. However, it is also possible that the low sensitivity of *E. crypticus* to the soil features disqualifies its use in ecotoxicological testing in this case.

Conclusions

The variability in mesofauna data is largely accounted for by physico-chemical parameters, especially in environments with individual trees. This indicates that other factors play only a minor role, or that no contaminants are present. However, *E. crypticus* testing suggests that the opposite is the case. The lack of conclusive results might only be resolved by quantifying any potential contaminants. With everything considered, it seems likely that there are no contaminants in the study area, despite the Ascarel oil spill.

Acknowledgment

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