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Hematological profile as a crude oil exposure-related marker in wild rodents

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ABSTRACT

The toxicity of petroleum components is well described in the literature, especially with regard to mutagenic and carcinogenic effects. In some groups of animals, such as birds, oil exposure seems to alter blood parameters, while this relationship is poorly understood in rodents. The study aimed to investigate alterations in hematological profile in the wild rodent *Calomys laucha* exposed to crude oil contaminated soils. In this study, males specimens of *Calomys laucha* were exposed for 14 days to two soils contaminated by petroleum: (I) landfarming soil, coming from a bioremediation area of contaminated soil from a Petrochemical Complex through landfarming technique and (II) soil of a simulated oil spill in laboratory conditions. The animals were exposed individually in cages containing 1 kg soil with free access to food and water. Control animals were exposed to an artificial uncontaminated soil. At the end of the experiment, animals were anesthetized and blood was collected for hematological profile. The animals exposed to soil landfarming had significant reduction in the number of bands, segmented, eosinophils, monocytes, lymphocytes and increased red cell distribution width (RDW), while animals exposed to simulated soil spillage in laboratory had decreased number of bands, but an increase in the number of lymphocytes and platelets. These changes in hemostasis may indicate an early stage of the development of associated pathologies, while the hematological profile can be used as a crude oil exposure-related marker in wild rodents.

Key words: *Calomys laucha*, hematology, hemostasis, petroleum, WBC differential count

Introduction

Crude oil is a complex mixture of hydrocarbons with a characteristic chemical composition and specific physical properties, depending on the geological and geographical origin (Di Doro et al., 2007). The main constituents of the crude oil are divided into two classes related to their chemical structure; they are aliphatic and aromatic hydrocarbons (Yu, 2005). These hydrocarbons are molecular substances formed by carbon and hydrogen and may contain benzene ring as in the case of aromatic hydrocarbons or just open or closed carbon chains, as in the case of aliphatic hydrocarbons.

Crude oil is currently used as the main energy source, also serving as a base for manufacturing of various products, among which stand out benzines, diesel, gasoline, tar, plastic polymers and even medicaments. These compounds enter the environment starting from stationary or mobile sources and makes up a significant portion of the mixture of contaminants found in groundwater and surface water, coastal areas and the global atmosphere. The drilling, removal, processing, transportation, storage and use of petroleum hydrocarbons involve several operations. During this process, can occur material loss, chemical conversion, and waste discharges and the environment emissions are estimated about 90000000 tons of crude oil (Yu, 2005). Due to discharges, spills and leaks, both aquatic and terrestrial animals may be affected by oil exposure.

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The toxicity of this complex chemical mixture to mammals remains largely unknown (Coppock et al., 1995, Da Slva Júnior et al., 2012). The main concern about oil is carcinogenic potential of some polycyclic aromatic hydrocarbons (PAHs). According to the World Health Organization benzo(a)pyrene and benzo(a)anthracene contained in petroleum are classified as probable human carcinogens, and in small concentrations, induced skin tumors in laboratory rats (*Rattus norvegicus*) (WHO, 2005). Moreover, the effects of these hydrocarbons in other biological parameters have been poorly addressed.

A strategy for the elimination of crude oil constituents in contaminated soil can be through the bioremediation, which uses biological activity for transformation of contaminants into inert substances (Holliger et al., 1997). This technology has been used for presenting low cost and higher efficiency in removing the contaminants compared to physical and chemical techniques (such as soil washing and incineration). It has been used on a commercial scale in treatment of waste and remediation of contaminated sites (Bamforth & Singleton, 2005).

The *landfarming* is a technique *ex situ* bioremediation applied on a large scale. The process consists in the stimulation of heterotrophic microorganisms in the soil surface layer through soil revolving by plowing and harrowing operations in order to aerate and mix the layers with different concentrations of contaminants (Vidali, 2001). Furthermore, there is the addition of correctives, fertilizers and water to optimize the degradation of contaminants present there, transforming them into inert substances such as organic material stabilized, water and CO. However, errors in the landfarming operation and unfavorable environmental conditions to microbial activity as well as physical and chemical characteristics of the contaminants may reduce rates of degradation.

A wild rodent species *Calomys laucha* Olfers 1818 has drawn the attention of the local scientific community and has been used as a biological model for studies mainly by metals contamination; however, studies with organic contaminants are scarce. Thus, the aim of this study was to investigate alterations in hematological profile of wild rodent *C. laucha* exposed to two soils contaminated by petroleum hydrocarbons, one being coming from the process *landfarming* and other soil artificially contaminated.

Materials and Methods

Animals

The animals, *Calomys laucha* males, were from the wild rodents animal house of the Institute of Biological Sciences (ICB-FURG). The animals were maintained under controlled temperature (21°C±3°C) and photoperiod (12h light/12h dark) and fed commercial feed (Bio Base, Bio-Tec, Águas Frias, SC, Brazil) for animals laboratory and water *ad libitum*.

Soil samples

Environmentally and artificially petroleum contaminated soils were used. The environmentally contaminated soil was coming from the Pólo Petroquímico do Sul, Triunfo Rio Grande do Sul, Brazil arising from an area of strong industrial influence and valuable contribution of hydrocarbons to the environment, where it was subjected to *ex situ* bioremediation technique (landfarming). The PAHs concentration is listed in Table 1. The artificially contaminated soil was obtained from a simulated spillage of crude oil (4% w/w) in an artificial soil (70% sand, 20% clay and vegetable organic matter 10%). The oil used in this simulation was *Hydra* light oil (API of 49.3 and density of 0.778 at 20/4°C).

Experimental design

Approximately 1 kg of soil was placed in the bottom of the cages, and the animals were exposed individually for 14 days without renewal of the soil. The animals were exposed to: (i) environmentally contaminated landfarming soil, (ii) artificially contaminated soil and (iii) control soil (artificial soil uncontaminated). The experimental groups were composed of eight animals. At the end of the experimental period, the animals were anesthetized with halothane and blood taken by cardiac puncture for analysis of hematological profile.

At all times, the experimental procedures were carried out in strict compliance with the Ethics Committee for Animal Use (36/2011) and Guide for the Care and Use of Laboratory Animals by the *Colégio Brasileiro de Experimentação Animal* (COBEA, 1991).

Hematological profile

Heparinized blood was collected and analyzed in an automated manner (ABX MICRO 60) considering the following parameters:

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Table 1. *Chemical analysis of landfarming soil.*

PAHs[1]	Concentration ($\mu g.Kg^{-1}$ dry soil)
Naphthalene	62.6
2-Methyl Naphthalene	71.87
1- Methyl Naphthalene	70.56
2,6-Dimethyl Naphthalene	83.77
1,7- Dimethyl Naphthalene	<1.66
Biphenyl	115.48
Acenaphthylene	5442.47
Acenaphthene	118.56
Fluorene	1056.52
Dibenzothiophene	21.04
Phenanthrene	3422.89
Anthracene	3630.22
Fluoranthene	1540.49
Pyrene	1299.72
Benzo(a)anthracene	2940.08
Chrysene	5348.95
Benzo(b)fluoranthene	930.58
Benzo(k)fluoranthene	205.93
Benzo(e)pyrene	860.07
Benzo(a)pyrene	838.45
Perylene	226.4
Indeno(1,2,3-cd)pyrene	359.68
Dibenzo(a,h)anthracene	188
Benzo(g,h,i)perylene	343.62
Σ Total PAHs	29177.97
Σ 2-3 rings PAHs	14095.99

[1]PAH analysis was performed using gas chromatography coupled to mass spectrometry (GCMS) by injecting a 1 μL aliquot of the extracts, with a split/splitless injector (1:50) and an HP-5 fused silica capillary column (60 m × 0.25 mm × 0.25 μm). Electron impact mass spectra were obtained at 1 keV of ionization energy. Helium was used as the carrier gas at a flow of 1 ml min^{-1}. Temperature was programmed from 120º C to 220º C at 5º C/min, followed by a 10º C/min increasing rate until it reached 280º C. The interface temperature was 280º C.

erythrocytes (RBC, $x10^6$ mm^{-3}), hemoglobin (HGB, dl g^{-1}), hematocrit (HCT, %) red cell distribution width (RDW, %), mean corpuscular volume (MCV, x 10^{-15}), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, %), leukocytes (WBC, x mm^{-3}), myelocytes, metamyelocytes, band cells, neutrophils, eosinophils, basophils, monocytes, lymphocytes, and plasma cells (mm^3) and platelets (Plt, mm^3).

Statistical analysis

Results were expressed as mean ± standard deviation. The parametric data were analyzed by Student's t test and the nonparametric data were analyzed by Mann-Whitney U test. The p value < 0.05 was considered as a critic value for statistical differences.

Results

Exposure to environmentally and artificially petroleum contaminated soil altered the hematological profile of wild rodent *C. laucha* males. The total concentration of leukocytes has increased significantly in animals exposed to soil contaminated by simulated oil spill, while animals exposed to landfarming soil had reduced number of leukocytes in the blood (Figure 1).

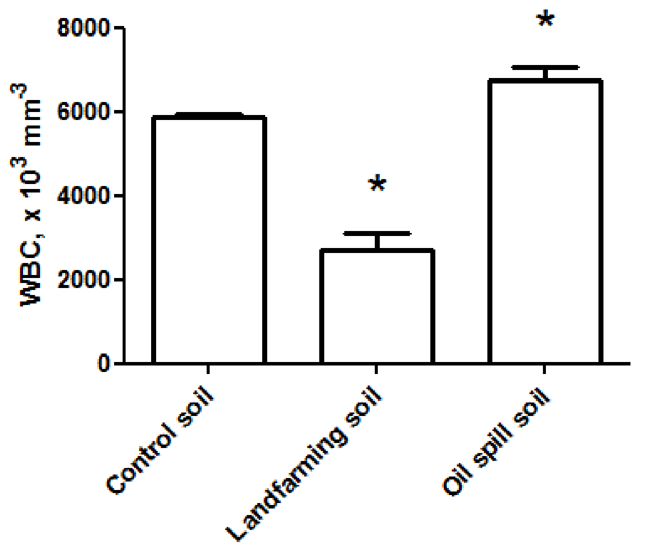

Figure 1. *WBC count in males wild rodent C. laucha exposed to crude oil contaminated soil during 14 days.*

In the differential counting, animals exposed to environmentally contaminated soil showed a decrease in the number of rods, segmented cells, eosinophils, monocytes and lymphocytes. On the other hand, animals exposed to artificially oil contaminated soil had decreased only the number of rods and a significant increase in the number of lymphocytes (Table 2).

Exposure to crude oil contaminated soil did not cause alterations in the parameters of the erythrocyte lineage in *C. laucha*, except for the increased red cell distribution width (RDW) in those animals exposed to landfarming soil (Figure 2, Table 3). On the other hand, exposure to artificially contaminated soil affected the number of platelets in order to increase the concentration of this parameter in the blood (Figure 3).

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Table 2. *WBC differential count in males wild rodent C. laucha exposed to crude oil contaminated soil during 14 days.*

Soil samples	Band cells	Segmented cells	Eosinophils	Monocytes	Lymphocytes
Control	57.1±3.6	820.4±164.9	863.9±164.91	330.5±32.7	3796.9±174.2
Landfarming	13.0[1]±5.0	210.2[1]±29.3	163.1[1]±35.2	156.9[1]±27.0	2163.1[1]±306.4
Oil spill	0[1]	595.0±83.9	438.9±55.2	442.6±43.6	5123.6[1]±261.1

[1] Statistical difference in relation to control.

Table 3. *Red cell parameters in males wild rodent C. laucha exposed to crude oil contaminated soil during 14 days.*

Soil samples	Hematocrit	HGB	VCM	HCM	CHCM	RDW
Control	36.9±3.4	12.1±1.0	62.2±1.6	20.6±0.5	33.1±0.4	16.4±0.8
Landfarming	37.7±2.9	12.5±0.9	61.6±0.9	20.6±0.5	33.2±0.4	18.4[1]±0.7
Oil spill	40.2±2.1	13.1±0.6	65.7±1.8	21.4±0.5	32.6±0.4	17.3±0.7

[1] Statistical difference in relation to control.

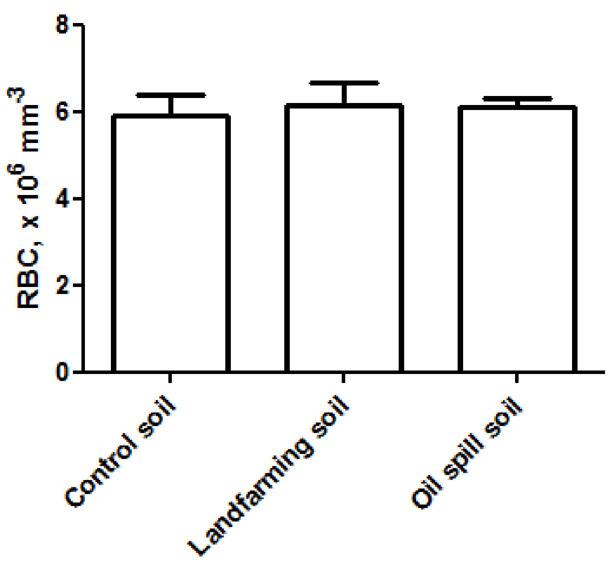

Figure 2. *RBC count in males wild rodent C. laucha exposed to crude oil contaminated soil during 14 days.*

Discussion

Although the mutagenic and carcinogenic effects of some petroleum components are well established, the toxic effects such as hematological, physiological and biochemical changes are controversial. Several studies have been devoted to investigating the effects of crude oil and its components on the hematological parameters in birds. However, the responses have been inconsistent and sometimes even contradictory (Newman et al., 2000, Akporhuarho, 2011). These variations may be related to species-specific responses and the exposure time.

In rodents, exposure to crude oil seems to have little or no effect on the hematological parameters. Khan et al. (2002) investigated the effects of ingestion of low concentrations of crude oil and have not found alterations in hematological profile of male Sprague-Dawley rats used in the experiment. A study using the same experimental model investigated the effect of oral ingestion of crude oil in concentrations exceeding the previous study and show hematological evidence of dehydration and leucopenia (Parker et al, 1986).

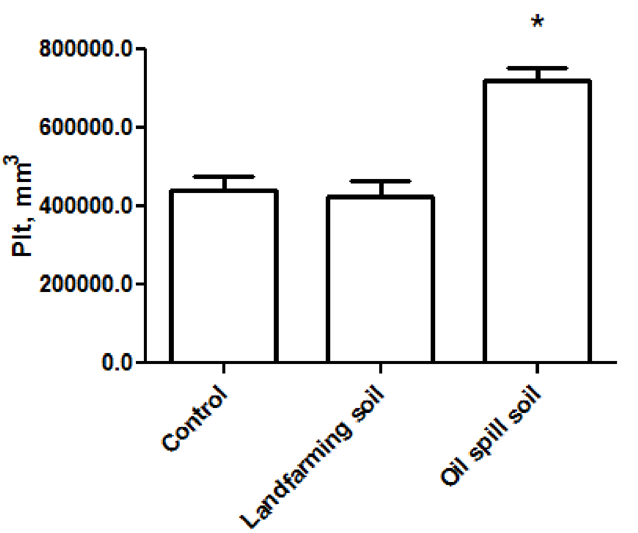

Figure 3. *Platelets count in males wild rodent C. laucha exposed to crude oil contaminated soil during 14 days.*

Another study using wild rodents as *in situ* models evaluated the risk of soils associated with petrochemical area in the remediation process and it showed that the hematological parameters were little changed in the animals collected in the treatment area, except only for the platelet count increased (Rafferty et al., 2001)..

In this study, the exposure of wild rodent *C. laucha* to contaminated soils by petroleum resulted in changes in the hematological profile, especially if we consider the differential leukocyte count. In those animals exposed to soil contaminated by petroleum and bioremediation process (landfarming), the acute exposure increased the RDW and reduced the number of total leukocytes, the number of rods, segmented cells, eosinophils, monocytes and lymphocytes. On the other hand, exposure to artificially petroleum contaminated soil caused an increase in platelet counts, total leukocytes and lymphocytes, but a decrease in the number of rods.

Alterations in hematological profile may help in early diagnosis of health problems. Disturbances in the RDW, known as anisocytosis may indicate preclinical signs of some diseases, including: ineffective erythropoiesis, inflammation, kidney disorders (Förhécz et al., 2009), leukemia (Miyaishi et al., 2000), some types of anemia (England & Down, 1974) and coronary heart disease (Zalawadiya et al., 2010).

Similarly, alterations in immune cells may be related to the action of toxic agents and provide health hazards. These cells are responsible for defense against aggressor agents and constitute the innate immune system that acts in the early inflammatory response such as phagocytic cells (neutrophils and monocytes), cytotoxic (eosinophils), inflammatory mediators-releasing cell (basophils), and antigen-presenting cells (monocyte/macrophage). The lymphocytes constitute the acquired immune system and they are responsible for cytotoxic activity and antibody production. The decrease of defense cells may be related to cytotoxicity or decreased cell proliferation even in bone marrow (local production of these cells) (Evans, 2009). The detection of monocytopenia, basopenia and eosinopenia is due to the low concentration of these cells in the bloodstream. This situation is found in conditions of severe bone marrow toxicity, while the incidence of neutropenia is mediated by immune mechanisms, such as opsonization of cells and consequent attack by macrophages (Evans, 2009).

The increase in the number of lymphocytes such as seen in the context of exposure to simulated contaminated soil may represent an advanced stage of inflammation or macrocytic anemia (red cell volume increase, MCV). Prolonged conditions of excitation and stress also contribute to the lymphocytosis (Evans, 2009).

Finally, changes in the number of platelets (thrombocytes) can also reveal disturbances in organism hemostasis and indicate hazardous conditions to which the organisms are exposed. The excessive increase in the number of platelets (thrombocytosis) is usually a transient event and may be related to stress, spleen problems, reverse effects of thrombopenia, inflammation, iron deficiency, some types of anemia, among other causes. Also, overproduction of platelets could be related to stimulation of megakaryocytes in the bone marrow (Evans, 2009).

The amendments in hematological profile due to acute exposure to *C. laucha* males to petroleum contaminated soils highlight the effects of toxic components of crude oil on the health of wild rodents. On the basis of our results, the hypothesis of a weak relationship between crude oil exposure and hematological alterations in rodents described in the literature can be refuted based on the refinement of hematological analysis, incorporating parameters such as RDW and WBC differential count. Further investigations should be encouraged in order to elucidate the action mechanisms of the toxic components of petroleum in the peripheral blood and bone marrow.

Conclusion

Exposure to soil contaminated by crude oil caused changes in the hematological profile of exposed animals. The studied species, *Calomys laucha* proved to be a model sufficiently sensitive to detect changes in hemostasis resulting from exposure to environmental contaminants.

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