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Innovative Bioinputs and Their Potential for Bioremediation of Soils Contaminated by Hydrocarbons

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Abstract: The effectiveness of bioremediation with the innovative bio-inputs AOSFA (High-Andean Compost) and NAOLA (Aerobic liquid organic nano-fertilizer) in agricultural soils contaminated by hydrocarbons — specifically dielectric oil, used motor oil, gasoline, diesel, and kerosene — was verified by evaluating physicochemical, biological, and organoleptic soil variables, as well as the characteristics of indicator plants. A direct relationship was found between the metabolism of the bio-inputs by the microbiota and the favorable response in the soil and plant variables. The microbiota of innovative bio-inputs consists of complex microbial communities of fungi, bacteria, actinomycetes, viruses, microfauna, and other organisms. Under natural conditions, these organisms have proven to be potent and resilient, with a multifaceted and versatile capacity to degrade contaminating hydrocarbon molecules through their metabolism and the production of specific substances such as enzymes, vitamins, hormones, proteins, antibiotics, and other as-yet-unknown organic compounds. These processes enable the remediation of contaminated soils and the normal growth of plants. Due to their effectiveness and bioremediation capacity, regardless of the type of contaminant, the high-Andean compost combined with NAOLA treatments, due to their excellent synergy, proved to be the best for soil bioremediation and plant growth.

Key words: bioremediation with organic fertilizers, Andean compost, AOLA and organic nanofertilizers, microbiota, contaminating hydrocarbons

1. Introduction

Soil and crop contamination by hydrocarbons is a global environmental problem caused by various factors, including hydrocarbon transport accidents, the poor condition of gasoline, diesel, and gas pipelines, and several anthropogenic factors. These factors affect large areas of agricultural land, forests, lagoons, and natural grasslands. Conventional physicochemical remediation technologies are costly and have serious limitations. Bioremediation with innovative bio-inputs has emerged as an accessible alternative, gaining significant traction in the context of the environmental crisis due to its successful results in neutralizing and reducing the effects of pollutants. Our research aims to evaluate the bioremediation capacity of the innovative bio-inputs AOSFA (high-Andean compost) and NAOLA (Aerobic liquid organic nano-fertilizer) in the bioremediation of agricultural soils contaminated by hydrocarbon derivatives. We will verify their effects on the physical, chemical, and biological properties of the soils and on the growth of indicator plants.

2. Reference Framework

Hydrocarbons are toxic, hydrophobic, and highly persistent substances that tend to bind and form complexes with soil particles and organic matter, clogging soil pores and creating anaerobic conditions that hinder biodegradation [1]. Hydrocarbon compounds alter soil properties, such as electrical

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conductivity, pH, hydrophobicity, and soil structure, which can lead to changes in the composition of the native soil microbiota, among other negative effects [2]. Gasoline is a complex mixture of hundreds of different hydrocarbons, most of which are saturated and contain 4 to 12 C atoms per molecule, and 22 to 54% of gasoline consists of aromatic hydrocarbons, such as benzene, toluene, ethylbenzene, xylene, which constitute the BTEX compounds, with special significance because they are toxic and water-soluble, with benzene being a known human carcinogen [3].

Many conventional physicochemical decontamination methods developed by contemporary engineering are costly due to the expense of excavating and transporting large quantities of contaminated materials for ex situ treatment. These methods include soil washing, chemical inactivation (using potassium permanganate and/or hydrogen peroxide as chemical oxidants to mineralize non-aqueous contaminants such as oil), incineration, and other conventional methods [4, 5].

Microbial bioremediation is a technique used to treat petroleum hydrocarbon pollution in terrestrial and aquatic ecosystems [6]. Over the last decade, several studies have focused on the biodegradation of petroleum hydrocarbon-derived contaminants. These persistent organic pollutants pose a serious threat to human and environmental health [7]. The biodegradation of these contaminants can be carried out using oleophilic microorganisms, either individually or in combination, for their control.

Among the innovative organic fertilizers with potential for bioremediation are Andean compost and AOLA. Andean compost originates from the recovery of ancestral knowledge from Andean and Mesoamerican peoples, made with local ferments or “chicha” of Andean grains, which, as a fertilizer, enriches the soil with organic matter, beneficial microorganisms, biosubstances, and mineral nutrients. [8, 9].

Recent reports describe novel findings on the multifaceted and versatile microbiota of Andean compost [10]. AOLA (Aerobic Liquid Organic Fertilizer), the predecessor of NAOLA, was created in 2014 by Chilon, E. & Chilon, J. (2015) [11], through an innovative natural microbial nanobiotechnology process, has been tested in several agricultural seasons and at various doses, demonstrating its versatility, its potential as a bioremediator of contaminated soils, and its beneficial effect on plant growth [12].

Soil and water bioremediation is part of the new agricultural science and technology, which supports and establishes that the microbiota and its systemic world are not just part of the soil, but are the soil itself. It takes as its basis the holistic thinking of the ancient Andean and Mesoamerican peoples, and the knowledge of quantum physics, advanced microbiology and 20th century systemic thinking, scientifically demonstrates that the soil is a living entity, which must be cared for and preserved, for the survival and perpetuation of the human species [13].

3. Materials and Methods

3.1 Location of the Experimental Area

This study began in 2014 and was interrupted in 2020 due to the pandemic. It was resumed from 2021 to 2024. The field study was conducted at the Experimental Campuses of the Agronomy programs at UMSA and UACT-UCB, and the final stage at the Soil Biofertility Experimental Center and Laboratory of the Agronomy program at UNIBOL ATK, located in the Indigenous Community of Cuyahuani, Huarina Municipality, Omasuyos Province, La Paz, Bolivia; coordinates 16°13'10.95" S and 68°34'50.64" W, at an altitude of 3848 meters above sea level, and approximately 60 km from the capital city.

Study Method: The research was conducted using the Synergistic Method of local traditional knowledge/scientific knowledge [14]; and with the comparative method, which integrates qualitative paradigms of an explanatory nature, and the quantitative paradigm with observable phenomena, susceptible to measurement and statistical analysis, with the selection and dosage of solid bio-input (compost), and liquid bio-input (NAOLA), which contain a multifaceted and versatile microbiota. Our research approach prioritizes conditions close to the natural environment.

3.2 Methodological Procedure

Preparation of experimental soil: The experimental soil was obtained from the UNIBOL-ATK Agronomy Experimental Campus and presented the following soil characteristics: sandy clay loam texture, pH: 7.3, EC: 2565 µS/cm, total N: 0.26%, available P: 69 p/mg.kg-1, organic matter: 2.3%, CEC: 18.3 Cmol/kg, intercellular Ca: 11 Cmol/kg, intercellular Mg: 1.8 Cmol/kg, intercellular Na: 2.1 Cmol/kg, intercellular K: 3.4 Cmol/kg (Environmental Quality Laboratory data, 2023).

Statistical Design and Data Analysis: Data analysis was performed using a completely randomized multifactorial design with three factors (5x6x1). First factor: contaminated soils: S1AD (soil contaminated with dielectric oil), S2aQ (soil contaminated with used automotive oil), S3GA (soil contaminated with gasoline), S4DIE (soil contaminated with diesel), and S5KE (soil contaminated with kerosene). Second factor: organic fertilizers: A1 (high-dose compost), A2 (medium-dose compost), A3 (high-dose compost + NAOLA), A4 (medium-dose compost + NAOLA), A5 (NAOLA application only), A6 (control, no organic fertilizer). Third factor: plant qualities of the oat (Avena sativa L.) variety Gaviota, as affected by organic fertilizers and bioremediation. The design consisted of 30 treatments and 90 experimental units in pots, evaluated at the end of the 12-month growing season.

Contamination of experimental soil with hydrocarbon derivatives: The experimental soils were contaminated by gasoline, diesel, kerosene, dielectric oil, and used motor oil. These derivatives are considered hazardous pollutants; diesel is identified as highly polluting, and used motor oil as a hazardous pollutant.

Bioremediation with innovative organic fertilizers: The innovative organic fertilizers selected as bioremediators, High-Andean Compost and NAOLA, were applied in experimental doses and modalities to the experimental pots, allowing a six-month incubation period before establishing an indicator culture. High-Andean Compost (AOSFA) is a solid organic fertilizer produced according to a defined protocol [15], and has the following characteristics: pH: 7.8, EC: 3,492 mmhos/cm, Organic matter: 42.3%, %CO: 24.16%, Total N: 1.89%, C/N ratio: 12.7. NAOLA, produced according to its scientific protocol, had a pH of 8.1, EC: 4,010 mmhos/cm, Total N: 16 mg/l, Total P: 20 mgP-PO4/l, and K: 1541 mg/l. (Environmental Quality Laboratory Data, 2023).

Establishment of an indicator oat crop in bioremediated soils: After six months of natural incubation, oat plants (*Avena sativa* L.) of the Gaviota variety were planted as an indicator crop to measure the effect of innovative bioremediating organic fertilizers on contaminated soils. Three seeds were planted per experimental unit, and the plants were monitored throughout their agronomic cycle. The first planting, at three months, was very poor due to phytotoxicity from contaminants. The final planting was carried out after six months of incubation.

Evaluation of bioremediated soils and the oat indicator crop: At the end of the indicator crop's agronomic cycle, the following soil variables were evaluated: bulk density, particle density, porosity, texture, structure, consistency, color, pH, electrical conductivity, organic matter content (as measured by reaction with H_2O_2), odor, and microbial activity (using Pfeiffer chromatography). Plant characteristics were also assessed, including plant height, number of tillers, green weight, dry weight, dry matter percentage, phenotypic changes, and phytotoxicity symptoms caused by contaminants. The research lasted 12 months.

4. Results and Discussion

The results of bioremediation with innovative bio-inputs in hydrocarbon-contaminated soils, and on the qualities of oat plants, are as follows.

4.1 Effect of Innovative Bio-Inputs on Physical, Chemical, Organoleptic Variables and Microbial Activity in Bioremediated Soils

(1) Effect of innovative bio-inputs on the physical properties of bioremediated soils

Table 1 presents the physical properties of the bioremediated soils: bulk density, particle density, porosity, and texture.

Table 1 Physical properties of hydrocarbon-contaminated soils with bioremediating organic fertilizers.

Contaminated Agricultural Soils	Innovative Bioinputs	Physical Properties of Soils Bioremediation					
		Apparent Density * (g/cm^3)	Particle Density* (g/cm^3)	Porosity ** (%)	Texture ***	Soil color Munsell ****	
						Dry	Humed
S1 AD Agricultural soil 1 contaminated by Dielectric Oil	A1 Compost high	0.80	1.39	42.19	Fr Ar	10YR 3/2	5YR 2,5/1
	A2 Compost medium	0.92	1.59	42.22	Fr Ar	10YR 4/4	5YR 3/2
	A3 Comp. high+ NAOLA	0.78	1.33	41.61	Fr Li	7,5YR 2,5/2	5YR 2,5/2
	A4 Comp. Med+ NAOLA	0.88	1.59	44.36	Fr Li	7,5YR 2,5/3	5YR2,5/2
	A5 NAOLA	1.20	2.71	55.51	Fr Ar Li	10YR 5/6	10YR 3/4
	A6 Without bioinputs	1.32	2.07	36.49	Fr Ar Ao	10YR 4/6	10YR 3/3
S2 aQ Agricultural soil 2 contaminated by used automotive oil	A1 Compost high	0.92	1.74	46.84	Fr	7,5YR 2,5/2	7,5YR2.5/1
	A2 Compost medium	0.96	1.74	44.82	Fr	10YR 3/3	10YR 2/1
	A3 Comp. high+ NAOLA	0.81	1.37	41.02	Fr Ar	10YR ¾	10YR 3/2
	A4 Comp. Med+ NAOLA	0.89	1.55	42.65	Fr Ar	10YR 3/3	10YR 2/2
	A5 NAOLA	1.14	2.75	58.63	Fr Ar Li	10YR 4/4	10YR 3/2
	A6 Without bioinputs	1.28	2.09	38.59	Fr Ar Ao	10YR 3/3	10YR 3/2
S3 GA Agricultural soil 3 contaminated by gasoline	A1 Compost high	1.04	2.01	46.84	Fr	10YR ¾	10YR 2/1
	A2 Compost medium	1.03	1.89	44.82	Fr Ar Li	10YR 4/6	10YR 2/2
	A3 Comp. high+ NAOLA	0.87	1.54	41.02	Fr Li	10YR 2/2	10YR 2/1
	A4 Comp. Med+ NAOLA	0.98	1.82	42.65	Fr Ar	10YR 4/4	10YR 3/3
	A5 NAOLA	1.21	2.83	57.06	Fr Li	10YR 5/8	10YR 3/4
	A6 Without bioinputs	1.25	2.05	35.59	Fr Ar Ao	10YR 5/4	10YR 5/3
S4 DIE Agricultural soil 4 contaminated by diesel	A1 Compost high	1.10	2.08	47.03	Fr Li	10YR 4/3	10YR 3/2
	A2 Compost medium	0.98	1.77	44.60	Fr Ar	10YR ¾	10YR 2/2
	A3 Comp. high+ NAOLA	0.91	1.55	41.29	Fr Li	10YR 3/6	10YR 2/1
	A4 Comp. Med+ NAOLA	1.02	1.84	44.68	Fr Ar	10YR 4/2	10YR 2/1
	A5 NAOLA	1.16	2.78	58.39	Fr Ar Li	10YR 4/4	10YR 3/2
	A6 Without bioinputs	1.33	2.05	34.92	Fr Ar Ao	10YR 5/4	10YR 3/3
S5 KE Agricultural soil 5 contaminated by kerosene	A1 Compost high	1.07	2,06	47.99	Fr Li	10YR ¾	10YR 2/1
	A2 Compost medium	1.09	1,96	44.46	Fr Li	10YR 3/6	10YR 2/2
	A3 Comp. high+ NAOLA	0.96	1,80	46.57	Fr Ar Li	10YR 3/2	10YR 2/1
	A4 Comp. Med+ NAOLA	1.04	1,96	47.03	Fr Ar	10YR ¾	10YR 2/2
	A5 NAOLA	1.24	2,81	56.00	Fr Ar Li	10YR 4/4	10YR 3/3
	A6 Without bioinputs	1.40	2,28	38.37	Fr Ar Ao	10YR 4/3	10YR 3/2

(*) Apparent and particle densities were determined using the graduated cylinder method.
(**) Porosity was calculated using the formula.
(***) Soil texture was estimated using the feel method.
(****) Color was determined using the USDA Munsell Color Chart.

In *Apparent Density* (g/cm^3), highly significant statistical differences were detected for the interactions, with a coefficient of variation of 4.02%, indicating data reliability. Duncan's test found that the interaction S1ADxA3 (Soil 1 contaminated with dielectric oil X high-dose compost + NAOLA) was superior, with a Dap of 0.78 g/cm^3; the interaction S5KExA6 (Soil 5 contaminated with kerosene, without the application of organic fertilizers) was least superior, with a Dap of 1.40 g/cm^3. The beneficial effect of the innovative bio-inputs was verified, compared to the control treatments, which ranked last due to not receiving organic fertilizers.

In *Particle Density* (g/cm^3), highly significant statistical differences were also found for the interactions, with a coefficient of variation of 6.91%, confirming data reliability. Duncan's test indicates the superiority of the S1ADxA3 interaction (Soil 1 contaminated with dielectric oil x high-dose compost + NAOLA) with a Dr of 1.33 g/cm^3; the S3GAxA5 interaction (Soil 3 contaminated with gasoline x NAOLA) ranked last, with a Dr of 2.83 g/cm^3. NAOLA exhibited the highest values, possibly due to its being a liquid organic fertilizer and the short duration of its application.

In terms of *porosity,* no statistically significant differences were detected between interactions, with a coefficient of variation of 4.65%, indicating the reliability of the data. However, numerical differences were observed, and based on the specific cases, the highest porosity corresponds to the interaction S2aQxA5 (Soil contaminated with used automotive oil x NAOLA) with 58.63% porosity; followed by S4DIExA5 (Soil contaminated with diesel x NAOLA) with 58.39% porosity; then S3GAxA5 (Soil contaminated with gasoline x NAOLA) with 57.06% porosity. The effect of NAOLA is likely due to its influence on the predominance of micropores. The lowest porosity corresponds to the control soil S4DIExA6 (Soil contaminated with diesel, without the application of organic fertilizers), with only 34.92% porosity, which limited microbiological activity and plant growth.

Regarding *texture*, in our case, the laboratory reported a sandy clay loam (Fr Ar Ao) texture for the experimental soil. After 12 months of research, the effect of the organic treatments on soil texture was evaluated. Using the "touch method", it was found that the bioremediated soils showed a slight variation in their texture without drastic textural changes, and only the contaminated control soils without organic fertilizers maintained their initial textural class. The qualitative evaluation by touch established that the innovative organic fertilizers Compost and NAOLA, in the various treatments, acted as indirect textural modifiers, with a greater influence on other properties of the contaminated soils under study.

Soil color was determined using the Munsell color chart [16]. The initial soil without contaminants presented a color of 10YR 5/4 (dry) and 10YR 4/2 (wet), and the innovative organic fertilizers, used in bioremediation, presented varied natural colors, the moderately dark high-Andean Compost a color of 2.5YR 2.5/2 when dry and 2.5YR 2.5/1 when wet, and the NAOLA in its liquid state at 20% dilution 2.5Y 5/4; the interactions presented a color when dry from 7.5YR2.5/2 to 10YR 2/2, and when wet from 5YR 2.5/1 to 10YR 5/3; The colors (Munsell) of control soils contaminated with hydrocarbons, when dry, varied between 10YR 3/3 and 10YR 5/4, and when moist, from 10YR 3/2 to 10YR 4/3. Innovative organic fertilizers, when incorporated into the soil, tend to change the soil color.

The *soil structure* was characterized using the qualitative field method [17], with criteria of shape, size, and degree or clarity (resistance). Aggregates from treatments with Andean compost and compost+ NAOLA presented aggregates in the form of subangular blocks (Bs), ranging in size from fine (f) to medium (m), and exhibiting a degree or clarity (resistance) of weak (1) to moderate (2). The influence of organic fertilizers on soil aggregates was verified through mucilaginous substances produced by the soil microbiota, which facilitate the formation of stable aggregates. The NAOLA treatments showed a unique and slow influence, with aggregates in the form of subangular blocks (Bs), varying in size from fine (f) to coarse (g), and a degree of resistance ranging from moderate (2) to strong (3). The contaminated soils showed no changes, with aggregates forming subangular blocks (Bs), fine in size (f), and in all cases exhibiting a strong degree (3), which is related to their original clay loam texture.

Soil consistency expresses the degree of cohesion and adhesion of its components, with a certain resistance to breakage or deformation. The consistency of soils was evaluated in dry and moist states [17], and most of the treatments with high-Andean compost and compost + NAOLA improved their consistency, becoming softer and more friable. NAOLA had less impact on consistency, possibly due to its liquid nature and the limited time available. The contaminated control soils exhibited a hard and firm consistency without changes.

(2) Effect of Innovative Bio-inputs on Chemical and Organoleptic Properties in Bioremediated Soils

Table 2 presents the chemical properties of pH, electrical conductivity, and organic matter, as well as the organoleptic property of odor.

Table 2 Chemical and organoleptic properties of bioremediated soils with innovative organic fertilizers.

Contaminated Agricultural Soils	Innovative Bioinputs	Chemical and Organoleptic Properties of Bioremediation Soils				
		pH	Electrical conductiv	organic matter *		Odor **
				Reaction H_2O_2	Level O.M.	
S1 AD Agricultural soil 1 contaminated by Dielectric Oil	A1 Compost high	7.53	2.57	4 (very high)	> 4% OM	1 (Low odor)
	A2 Compost medium	7.61	1.07	3 (high reaction)	2-4% OM	1 (Low odor)
	A3 Comp. high + NAOLA	7.58	2.06	3 (high reaction)	2-4% OM	0 (absent)
	A4 Comp. Med + NAOLA	7.93	1.96	3 (high reaction)	2-4% OM	1 (Low odor)
	A5 NAOLA	7.08	0.48	2 (average reaction)	1-2% OM	1 (Low odor)
	A6 Without bioinputs	7.20	0.75	1 (very low reaction)	< 1% OM	2 (moderate)
S2 aQ Agricultural soil 2 contaminated by used automotive oil	A1 Compost high	7.25	1.89	4 (Very high)	> 4% OM	0 (absent)
	A2 Compost medium	7.55	3.18	3 (high reaction)	2-4% OM	1 (Low odor)
	A3 Comp. high + NAOLA	7.40	1.94	4 (very high)	> 4% OM	1 (Low odor)
	A4 Comp. Med + NAOLA	7.86	1.47	4 (very high)	> 4% OM	0 (absent)
	A5 NAOLA	7.02	0.51	2 (average reaction)	1-2% OM	1 (Low odor)
	A6 Without bioinputs	7.57	0.63	1 (very low reaction)	< 1% OM	2 (moderate)
S3 GA Agricultural soil 3 contaminated by gasoline	A1 Compost high	7.67	2.54	4 (very high)	> 4% OM	0 (absent)
	A2 Compost medium	7.45	3.18	4 (very high)	> 4% OM	0 (absent)
	A3 Comp. high + NAOLA	7.21	3.92	4 (very high)	> 4% OM	0 (absent)
	A4 Comp. Med + NAOLA	7.34	3.38	3 (high reaction)	2-4% OM	0 (absent)
	A5 NAOLA	7.15	0.48	2 (average reaction)	1-2% OM	0 (absent)
	A6 Without bioinputs	6.40	1.82	0 (no reaction)	< 0.5-0%OM	2 (moderate)
S4 DIE Agricultural soil 4 contaminated by diesel	A1 Compost high	7.87	2.87	4 (very high)	> 4% OM	0 (absent)
	A2 Compost medium	7.79	2.24	3 (high reaction)	2-4% MO	0 (absent)
	A3 Comp. high + NAOLA	7.70	2.82	4 (very high)	> 4% OM	0 (absent)
	A4 Comp. Med + NAOLA	7.90	2.54	3 (high reaction)	2-4% OM	1 (Low odor)
	A5 NAOLA	7.08	0.75	2 (average reaction)	1-2% OM	0 (absent)
	A6 Without bioinputs	7.20	0.74	1 (very low reaction)	<1% MO	3 (strong)
S5 KE Agricultural soil 5 contaminated by kerosene	A1 Compost high	7.83	2.86	3 (high reaction)	2-4% OM	0 (absent)
	A2 Compost medium	7.53	2.70	3 (high reaction)	2-4% OM	0 (absent)
	A3 Comp. high + NAOLA	7.74	2.90	3 (high reaction)	2-4% OM	0 (absent)
	A4 Comp. Med + NAOLA	7.56	2.37	3 (high reaction)	2-4% OM	0 (absent)
	A5 NAOLA	7.10	0.48	2 (average reaction)	1-2% OM	1 (Low odor)
	A6 Without bioinputs	6.75	0.66	1 (very low reaction)	< 1% OM	2 (moderate)

(*) Organic matter, qualitative evaluation by reaction to H_2O_2, following grading 4: Very high reaction, 3: High reaction, 2: Average reaction, 1: Low reaction, 0: No visible reaction. (**) Organoleptic property Odor was evaluated with the human sense of smell, with the following scale: 4: very strong odor, 3: strong odor, 2: moderate odor, 1: weak odor, 0: no odor.

The *soil pH* analysis revealed highly significant statistical differences for the interactions, with a coefficient of variation of 3.37%, indicating very reliable data. According to Duncan's multiple range test, the highest-performing treatment corresponded to the S1ADxA4 interaction (Soil 1 contaminated with dielectric oil x medium-dose compost + NAOLA) with a pH of 7.93; however, the lowest-performing treatment was S3GAxA6 (Soil 3 contaminated with gasoline, without the application of organic fertilizers) with a pH of 6.40. It is worth noting that variations in soil pH can also significantly modify biological activity [18]. In our case, the microbiota of the innovative bio-inputs regulates and improves pH values, neutralizes acidity through bio-liming with Bacillus subtilis bacteria, and regulates alkalinity with acidic organic substances [19].

Electrical conductivity showed highly significant statistical differences, with a coefficient of variation of 15.66% based on reliable data. According to Duncan's multiple range test, the S3GAxA3 interaction (soil 3 contaminated by gasoline x high-dose compost + NAOLA) was superior, with an EC of 3.92 mmhos/cm. The lowest EC values, each at 0.48 mmhos/cm, corresponded to treatments S5KExA5 (soil 5 contaminated by kerosene x NAOLA), S1ADxA5 (soil 1 contaminated by dielectric oil x NAOLA), and S3GAxA5 (soil 3 contaminated by gasoline x NAOLA), highlighting the role of NAOLA in salt neutralization, as evidenced by its reduction.

The *organic matter* content of the bioremediated soils was evaluated by reaction with hydrogen peroxide (H_2O_2), applying the reaction scale and correlating it with the percentage of organic matter content. In our case, it was verified that the treatments with high-Andean compost showed Grade 4: Very high reaction with H_2O_2, with more than 4% organic matter. The same trend was observed in the interactions. In the case of soils with NAOLA, the reaction with (H_2O_2) was lower, with Grade 2: medium reaction, with 1 to 2% organic matter; but in the case of the contaminated control soils, they showed Grade 1: low reaction to Grade 0: absence of reaction, which establishes a content of less than 1% organic matter.

Regarding the *organoleptic property of odor*, it was evaluated using the human sense of smell, and in most of the soils bioremediated with innovative bio-inputs, the pungent odor of hydrocarbons disappeared or decreased significantly. After 12 months, level 0 (no odor) and level 1 (low odor) predominated. However, the contaminated control soils without organic fertilizers presented level 3 (strong odor) and level 2 (moderate odor). The role of the innovative bio-inputs was verified; through the action of their microbiota, they degraded hydrocarbons, inducing the loss of their pungent odor and replacing it with a pleasant, fresh earth smell.

(3) Effect of Innovative Bio-inputs on Microbial Activity in Bioremediated Soils

The *Pfeiffer chromatographic* method allowed for the evaluation of the biological activity of bioremediated soils, identifying three groups: The first group, characterized by good effectiveness and quality, includes treatments with high-Andean compost and the interactions of contaminated soils with high-Andean compost + NAOLA. Their chromatograms show intense microbiological activity and the capacity to overcome adverse conditions and degrade contaminants, with good organic and mineral integration in the bioremediated soils. The second group, characterized by fair effectiveness and quality, corresponds to treatments with NAOLA. These treatments exhibit a moderate effect on some soil properties, being surpassed by the compost and the compost + NAOLA treatments. Their chromatograms indicate only fair microbial activity, and the correlation of field and laboratory data confirms their fair quality. The third group, characterized by low effectiveness and quality, consists of the control treatments of contaminated soils. Their chromatograms indicate deficient biological activity, and the soils failed to recover during 12 months of research. However, during this period, there were indications of incipient degradation of the contaminants, possibly due to the action of native soil microorganisms that survived the adverse contaminating conditions.

4.2 Effect of Innovative Organic Fertilizers on the Qualities of Oat (Avena sativa L.) Indicator Plants

Table 3 shows the agronomic variables — plant height, number of tillers, green weight, dry weight, and percentage of dry matter — of oat indicator plants.

Table 3 Plant height, number of tillers, green weight, dry weight, and % dry matter.

Contaminated Agricultural Soils	Innovative Bioinputs	Agronomic Variables of Indicator Plants				
		Floor height (cm)	Number of tillers/plant	Green weight (g/plant)	Dry weight (g/plant)*	Dry matter** (%)
S1 AD Agricultural soil 1 contaminated by Dielectric Oil	A1 Compost high	113.33	3.67	19.82	3.61	18.15
	A2 Compost medium	99.33	4.33	24.42	4.50	18.57
	A3 Comp. high + NAOLA	97.00	6.33	32.38	7.60	23.09
	A4 Comp. Med + NAOLA	117.67	3.33	26.67	5.03	18.40
	A5 NAOLA	71.00	2.00	12.42	2.48	19.97
	A6 Without bioinputs	40.33	1.33	3.28	0.50	15.42
S2 aQ Agricultural soil 2 contaminated by used automotive oil	A1 Compost high	118.33	3.00	20.35	3.51	17.20
	A2 Compost medium	97.67	3.00	21.81	3.84	17.80
	A3 Comp. high + NAOLA	111.67	5.00	37.70	7.16	18.87
	A4 Comp. Med + NAOLA	126.00	5.33	42.33	8.37	19.42
	A5 NAOLA	77.00	2.33	18.63	3.82	20.75
	A6 Without bioinputs	38.32	1.33	5.61	0.88	15.66
S3 GA Agricultural soil 3 contaminated by gasoline	A1 Compost high	81.00	4.00	22.76	4.74	20.85
	A2 Compost medium	75.00	2.33	27.71	6.15	21.46
	A3 Comp. high + NAOLA	82.00	5.33	32.33	7.42	23.17
	A4 Comp. Med + NAOLA	81.00	3.33	24.41	5.43	22.22
	A5 NAOLA	78.00	2.67	12.06	2.98	24.07
	A6 Without bioinputs	41.33	1.33	10.46	1.66	16.42
S4 DIE Agricultural soil 4 contaminated by diesel	A1 Compost high	89.00	6.33	34.41	7.87	22.82
	A2 Compost medium	85.00	6.00	33.56	7.05	21.18
	A3 Comp. high + NAOLA	98.67	5.33	22.13	5.67	25.64
	A4 Comp. Med + NAOLA	95.33	4.00	19.25	4.70	24.38
	A5 NAOLA	74.67	2.67	12.58	2.32	18.46
	A6 Without bioinputs	37.00	1.00	5.87	0.81	13.82
S5 KE Agricultural soil 5 contaminated by kerosene	A1 Compost high	96.50	4.67	24.93	5.45	21.85
	A2 Compost medium	81.67	4.33	19.85	4.04	20.32
	A3 Comp. high + NAOLA	78.21	5.00	29.53	7.11	23.97
	A4 Comp. Med + NAOLA	74,67	4.67	28.23	6.26	22.18
	A5 NAOLA	72,00	2.33	18.82	3.39	18.11
	A6 Without bioinputs	39.67	1.33	4.70	0.72	15.11

(*) Dry weight of plant, dried in a muffle furnace at 70°C for 48 hours.
(**) Percentage of dry matter was calculated using a formula.

Plant height (cm) showed highly significant statistical differences, with a coefficient of variation of 12.36%, verifying the reliability of the data. According to Duncan's multiple range test, the interaction S2aQxA4 (Soil 2 contaminated by automotive oil x medium-dose compost + NAOLA) was superior, with the greatest plant height of 126 cm. The lowest was S1ADxA6 (Soil 1 contaminated by dielectric oil, without the application of organic fertilizers) with a height of only 37 cm/plant.

The number of tillers per plant did not show statistical significance between interactions, but there were numerical differences. The highest numbers were observed in the interactions S4DIExA1 (Soil 4 contaminated by diesel x high-dose compost) and S1ADxA3 (Soil 1 contaminated by dielectric oil x high-dose compost + NAOLA), both with 6.33 tillers/plant. Finally, treatment S4DIExA6 (Soil 4 contaminated with diesel, without the application of organic fertilizers) yielded only 1 tiller/plant.

In terms of green weight yield (g/plant), highly significant statistical differences were detected, with a coefficient of variation of 30%. According to Duncan's test, the interaction S2aQxA4 (Soil 2 contaminated with automotive oil x medium dose compost + NAOLA) yielded the highest result at 42.33 g/plant, followed by treatment S1ADxA6 (Soil 1 contaminated with dielectric oil, without the application of organic fertilizers), with only 3.28 g/plant. Dry weight (g/plant) also showed highly significant statistical differences, with a coefficient of variation of 25.40%. According to Duncan's test, the interaction S2aQxA4 (Soil 2 contaminated with used automotive oil x medium dose compost + NAOLA) yielded the highest result at 8.37 g/plant. The lowest-ranking treatment was S1ADxA6 (Soil 1 contaminated with dielectric oil, without the application of organic fertilizers), with a dry weight of only 0.50 g/plant.

Dry matter content (%) did not show statistically significant differences; the coefficient of variation was 12.85%. However, numerical differences were observed. The highest-ranking treatment was S4DiexA3 (Soil 4 contaminated with diesel x high-dose compost + NAOLA) with 25.64% dry matter, while the lowest-ranking treatment was S5KExA6 (Soil 4 contaminated with kerosene, without the application of organic fertilizers) with only 15.11% dry matter. There is limited information on the characteristics of indicator plants in bioremediated soils; our research contributes to the understanding of the effect of innovative bio-inputs on contaminated soils. It was found that high-Andean compost, in conjunction with NAOLA, are the best alternatives for the bioremediation of agricultural soils contaminated with hydrocarbons.

(1) Effect of Innovative Organic Fertilizers on Phytotoxicity

Phytotoxicity was evident in all five soils contaminated with hydrocarbons, affecting and delaying the germination of oat seeds in the first sowing, carried out three months after incubation, with a germination rate of less than 10%. Therefore, oat seeds were sown again six months later. In this regard, Zucconi et al. (1981) [20] indicate that a germination rate of less than 50% indicates strong toxicity. Other indicators of phytotoxicity were the delayed growth of plants cultivated in contaminated soils, and the visual signs of discoloration and wilting in the control plants, with a noticeable decrease in plant quality caused by the phytotoxins. Finally, to verify phytotoxicity, plants from bioremediated soils were compared with plants from contaminated soils. It was observed that oat plants from bioremediation treatments with high-Andean compost + NAOLA, and NAOLA alone, showed normal growth and development, verifying that the innovative bio-inputs reduced and neutralized the phytotoxicity of hydrocarbon contaminants, with a beneficial effect on plant physiology.

4.3 Role of the Microbiota of Innovative Bio-inputs in Soil Bioremediation and Plant Qualities

The bioremediation of hydrocarbon-contaminated soils with the innovative bio-inputs High-Andean compost and NAOLA is a beneficial, essentially aerobic process. Our hypothesis is that the microbiota of these innovative bio-inputs, being multifaceted and versatile, disintegrates the contaminating hydrocarbon molecules, catalyzing key reactions in the breaking of benzene rings. This is achieved through its capacity to metabolize and biosynthesize substances such as enzymes, hormones, vitamins, amino acids, proteins, antibiotics, humic and fulvic organic acids, mucilaginous compounds, and other unknown substances, which facilitate soil cleanup and enhance plant qualities. In this regard [21] found that our high-Andean compost CA-TB3g, in addition to restoring the fertility of agricultural soils, has a potential capacity for the bioremediation of soils contaminated by gasoline; another bioremediation trial of soils contaminated by dielectric oil found that the high-Andean compost was the one that brought the best benefits, compared to earthworm humus and sheep manure [22]; it was also found that some bacteria from the high-Andean compost and AOLA synthesize molecules similar to the phytohormones abscisic acid, gibberellic acid, indoleacetic acid and others that modulate and regulate the processes of plant development [23]. Given the certainty of the collaborative and "communal" work of the microbiota in high-Andean compost and NAOLA, some researchers support our position, noting that bacteria and fungi coexisting in the same population work together to degrade contaminants, and that the microbial consortium has greater potential than individual cultures [24]. It is also indicated that metagenomics analyzes genetic material directly from the environment, overcoming the limitations of culturing microbes in sophisticated laboratories [25]. Our results contradict those that maintain that recalcitrant compounds present in contaminated soils are difficult to remove due to their adsorption to soil particles and organic matter surfaces, as well as their physical entrapment in micropores [26].

4.4. Perspective on an Industrial Bioremediation Complex

The design of an industrial complex for the bioremediation of hydrocarbon-contaminated soils prioritizes the use of microbiota in innovative bio-inputs. For both in situ and ex situ operations, alternative energy sources such as solar radiation, wind, temperature, ice, and others are prioritized to power solar panels, wind turbines for groundwater extraction, cooling chambers, and energy for processing and quality control laboratories. Above all, the complex includes an open field for the natural laboratory for bio-input production, microorganism collection and preservation, warehouses and storage facilities, the bioremediation area, and the spraying and bio-irrigation system [27].

5. Conclusions and Recommendations

The research on bioremediation with the innovative bio-inputs High-Andean compost and NAOLA, in agricultural soils contaminated by hydrocarbon derivatives, verified that the innovative bio-inputs were effective in the bioremediation of contaminated soils and in the growth of indicator plants. A direct relationship was found between the action and metabolism of the microbiota of the innovative bio-inputs and the favorable response of the soil's physicochemical, biological, and organoleptic variables, as well as the qualities of the plants. The microbiota of innovative bio-inputs consists of complex microbial communities of fungi, bacteria, actinomycetes, viruses, microfauna, and other organisms. Under adverse conditions, these organisms demonstrated their potency and resilience, as well as their multifaceted and versatile capacity to degrade contaminating hydrocarbon molecules. This is achieved through the metabolism of specific substances such as enzymes, vitamins, hormones, proteins, antibiotics, and other as-yet-unknown organic compounds, which enable the cleanup of contaminated soils and the normal growth of plants. Due to their effectiveness and bioremediation capacity, regardless of the type of contaminant, the high-Andean compost combined with NAOLA (Aerobic liquid organic nano-fertilizer) treatments proved to be the most effective for soil bioremediation and plant growth, thanks to their excellent complementarity. Further research on bioremediation with innovative bio-inputs in soils and water contaminated by petroleum hydrocarbons, pesticides, and agrochemicals is recommended. Additionally, the development of industrial bioremediation plants is advised.

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Disclaimer (Artificial Intelligence)

The authors declare that NO generative AI technology, such as long Languaje Models (ChatGPT), Copilot, etc., nor text, table, and figure generators, were used during the writing of this manuscript.

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