

Effect of Aloe Vera wastes on physico-chemical properties and microbiological activity in soils

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Abstract—The aim of the present study was to explore the potential for using aloe vera wastes as amendment for soil to improve its fertility.

Soil was exposed to four concentrations of aloin (rich in HAP) for 0, 7, 14 and 28 days. Physico-chemical parameters were analyzed: soil Ph, organic matter (OM), nitrogen, phosphorus, and cation exchange capacity (CEC). The activity of seven enzymes implicated in the C, N and S cycles were measured. Microbial Biomass was determined by the method of substrate induced respiration. BiologEcoPlates (Biolog Inc., Hayward, CA) were used to estimate soil microbial functional diversity.

Our findings suggested a decrease on phosphorus and nitrogen content and an increase on CEC after aloin addition. Also, a decrease on microbial biomass and enzymes activities was observed, except for FDA. EcoPlates results demonstrate a decrease on microbial activities depending on the incubation time. Moreover, our results indicated that bacterial communities of the tested soils have more affinity to consume substrates as Amino acids and polymers.

Our results should be carefully considered in view of the agriculture waste reuse for a sustainable agriculture

Keywords— Aloe vera, aloin, microbial communities, enzymes activities, EcoPlates.

I. INTRODUCTION

The addition of residues as by-products of crop production is a common practice in agriculture. Indeed, previous studies have demonstrated that they support farm productivity, reduce soil degradation, and improve nutrient cycling in the agroecosystem. It has also been reported that crop residues improve soil structure and soil protection by reducing erosion [1,2], and increasing the stock of plant nutrients and soil organic matter content, thus enhancing soil fertility [3,4] and crop yields [5].

Aloe Vera belongs to Liliaceae family is considered as a perennial succulent plant. It produced secondary metabolites with numerous properties such as antibacterial,

anti-inflammatory and antioxidant, [6,7]. Actually, the industry of aloe vera is in continuous expansion in Tunisia generating many wastes which are presenting a real problem. For this reason, aloe vera wastes management constitute an opportunity both to valorize these wastes and in the same time to improve soil fertility. However, information's about the magnitude and the effects caused by aloe vera wastes on soil microbial community, functionalities and diversity is still scarce.

Among toxic substances contained in aloin, polycyclic aromatic hydrocarbons (PAHs) are known by their harmful effect once in the ecosystem; they are one of the most common groups with known or potential toxic properties [8]. PAHs are a substantial threat to ecological function and soil biodiversity [9]. The response of microbial communities to chronic inputs of PAHs [10,11,12] has received little attention compared to the effect of acute contaminations [13,14,15]. Indeed, previous studies demonstrated that PAH change bacterial communities structure and diversity [16,17,18].

Soil microorganisms are measured using the C and N content in the microbial biomass (MBC and MBN). It represents collectively the mass of all soil microorganisms, considered as a single soil organic matter fraction [19]. Among microbial indicators, community-level physiological profiles (CLPP), which are assessed using BiologEcoPlates™, allow for the detection of multiple microbial metabolic activities. The Biolog™ system has been adapted to the investigate the functional diversity of soil microbial communities [20,21]. On the other hand, there is also growing interested in using soil enzymes as potential indicators of soil fertility, since enzyme activities a sensitive to numerous factors such as climate, type of amendment, agricultural techniques, crop type and edaphic properties [22,23,24]. In addition, due to their importance for the soil and their rapid response to soil perturbations, soil enzymes are considered as indicators of soil quality [25,26,27,28]. Indeed, soil enzyme activities such as arylsulphatase, β-glucosidase acid and alkaline

phosphatase, urease, and dehydrogenase are sensitive to the presence of pollutant [29,30,31].

The fast expanding aloe vera industry in Tunisia urgently needs more information on the effect of industrial releases (containing the toxic substance aloin) and their repercussions on the ecosystem. Little is known about the impact of increased aloin reject on soil quality and fertility. The present work was conducted to assess the effect of Aloe vera wastes on microbiological and physico-chemical properties of soils in order to valorize them in sustainable agriculture.

II. MATERIAL AND METHODS

2-1- Experimental protocol

2-1-1 Soil samples

The soils used for this research were collected from an organic farming plot in the region of Chott Mariem. The soils were sampled from the depth of 0-15 cm. The chemical and physical properties of these soils are presented in table 1. Before use, samples were air-dried and crushed to pass a (<2 mm) screen.

2-1-2 Extraction of aloin

Leaves were sampled from the mature plants of Aloe vera (var. *barbadensis*) (age between three and five years). The extraction of aloin was done in three steps (figure 1): First, the leaves were washed with water, then rinds were removed, and finally, yellow exudate (aloe latex) was collected from the leaves after cutting.

2-1-3 Earthworms *Eisenia Andrei*

E. Andrei earthworms [32] were cultured as described in the OECD guidelines [33]. Organisms were selected from a synchronized culture with a homogeneous age structure. Adult worms with clitellum of similar size and weight (400-500 mg) were utilized in the experiments.

2.1-4 Soil contamination and earthworm's exposure

Sampled soils were dried and sieved (<2 mm), then 500 g were placed in polyethylene pots. In this experience, we choose to work on five different concentrations of aloe exudates: C1: 1 %, C2: 5%, C3: 10% and C4: 20% in addition to the control. These concentrations are relative to the weight of the soil. For each concentration, three periods of exposition: 0, 7, 14, and 28 were realized.

At the end of the exposure period, the soil of each pot was homogenized. One part was conserved at 4°C for the determination of enzymatic activities and the functional diversity, and the other part was conserved at ambient temperature for the assessment of microbial biomass and the different physico-chemicals analyses .

2-2- Physico-chemical analyses

Soil pH was measured in soil suspension obtained by shaking 1 g of soil in water (soil/H₂O ratio 1:2.5) for 1 hour and then by using a pH meter (MetrOhm 744). For organic carbon analysis, 25 mg of soil crushed at 250 lm

were decarbonated with hydrochloric acid and then analyzed with a CHN analyzer according ISO 10694 procedure. Organic matter content was calculated by multiplying organic carbon concentration by 1.72 [34]. Mg was determined digestion using the Hossner method [35].

Nitrogen mineralization was determined by measuring the production of mineral N (NH₄⁺ and NO₃⁻) during incubation. measurements were made according to the extraction protocol of Li et al., 2009 and with the use of Spectroquant® kit tests (Merck) according to the supplier's recommendations.

For the measurement of NH₄⁺, 10 g soil sample (dry weight equivalent) was shaken with 50 ml of KCl (2.0 M) for 30 min. Filtration was performed with a PES polyethersulfone filter (0.45) after centrifugation for 15 min at 3500 g. Then the use of kit tests ((Spectroquant®, Merck) Well absorbance (690 nm) was measured with a BioTek EL800 Universal plate reader (Bio-Tek Instruments, Winooski, VT).

For measurement of NO₃⁻, soil samples of 10 g weighed after the 7 day incubation were shaken with 50 ml of CuSO₄ extraction solution (0.01 M) for 30 min. Filtration was performed with a PES polyethersulfone filter (0.45) after centrifugation at 3500 g for 15 min. Then we used the kit (Spectroquant®, Merck) and finally the NO₃⁻ was measured with a spectrophotometer at 493 nm.

The total organic N mineralization was estimated by the sum of the ammonification and nitrification rates.

Phosphorus mineralization was determined by an incubation procedure similar to nitrogen mineralization. The mineralisation of inorganic P was extracted with 0.5M NaHCO₃. Then, we use the kit (Spectroquant®, Merck) and finally the Phosphate was measured with a spectrophotometer at 885 nm.

According to [36]'s method, the cation exchange capacity will be measured with 2.5 g of soil was shaken with 30 ml of BaCl₂ solution (0.1M) for 1h then centrifuged at 3000g for 10min. The supernatant liquid was filtered at 0.45 µm and then used for the determination of the content of sodium, potassium, calcium and magnesium in ICP-AES.

2-3 Microbiological analyses

2-3-1 Microbial Biomass determination by the method of Substrate Induced Respiration (SIR)

Soil respiration was measured in samples at 50% water-holding capacity using the method of [37]. Fresh soil equivalent to 10 g dry soil was weighed into a plastic beaker and supplemented with 10 mg of glucose which corresponded to the amount of glucose required for obtaining a maximum CO₂ flush. The CO₂ production rate was measured hourly during one day, using an automated IR gas analyzer system (490 MicroGC Agilent). Microbial biomass carbon was expressed as µg carbon per g soil [38].

2-3-2 Enzymes activities assay

Arylsulfatase, β -Glucosidase and alkaline-acid phosphatases activity assays were all based on p-nitrophenol release, after cleavage of a synthetic substrate (p-nitrophenyl sulfate and nitrophenylglucopyranoside respectively). Arylsulfatase and β -glucosidase activities were assayed as described by [39]. Alkaline phosphatase and acid phosphatase activities were assayed as described by [40]. Microplate wells were loaded with 50 μ l of a 1:10 soil distilled water solution, 25 μ l phosphate buffer and 50 μ l of the appropriate substrate (71.9 mmol L⁻¹). Microplates were incubated for one hour at 37° C. At the end of the incubation, after added 125 μ l 2% Na₂CO₃ the microplates were centrifuged (14,000g for 5 min) and 50 μ l of the supernatant transferred to a second microplate containing 250 ml 2% Na₂CO₃ to stop the enzymatic reaction. Well absorbance (410 nm) was measured with a BioTek EL800 Universal plate reader (Bio-Tek Instruments, Winooski, VT). The enzyme activity was expressed as the quantity of p-nitrophenol μ g PNP released g⁻¹ soil h⁻¹.

Deshydrogenase activity was assayed using soil (6 g), incubated with triphenyl tetrazolium chloride (3%) for 96 h in the dark. Methanol was added to terminate the enzymatic reaction. The supernatant was filtered and the absorbance was taken at 485 nm [41]. The values were expressed as μ g of triphenyl formazan (TPF) g⁻¹soil h⁻¹.

Urease (EC 3.5.1.5) activity was assayed as described by [42,43,44]. Soil (1.0 g) was weighted into screw-top test tube containing 0.5 mL of urea (0.02 M) and 4 mL of borate buffer (0.05 M, pH 10.0). After incubation at 37 °C for 4 h, the reaction was stopped by addition of 3 mL of KCl(2M) and the suspension was mixed for 30 min and centrifuged at 13000 rpm for 5 min. 5 mL of solution containing sodium salicylate, nitroprussate, NaOH (0.3 M) and Na-dichloroisocyanide were added to the 1 ml of the supernatant. Finally, after agitation at 120 rpm in the dark for 30 min, the absorbance was measured at 660 nm and the enzyme activity was expressed as mg N-NH₄⁺g⁻¹ soil h⁻¹.

The total enzymatic activity was measured using the fluorescein diacetate hydrolysis assay (FDA) [45]. Microplate wells were prepared with 100 μ l of samples and were incubated at 37 °C for 2 h with 50 μ l of phosphate buffer Mac Ilvain at PH (7,6 and 8,1) and 25 μ l of 4.8 mM FDA solution. The suspension was centrifuged at 13,000 g at 4 °C for 3 min and 100 μ l of supernatant was taken. The reaction was stopped by adding 100 μ l of acetone. The absorbance was measured at 490 nm and the amount of FDA hydrolyzed was expressed as μ g fluorescein g⁻¹soil h⁻¹.

All measurements were made at ambient soil PH, wells with soil and buffer but without substrate were used as

blanks. The assays were conducted in triplicate thus ensuring the reproducibility of the laboratory analyses.

2-3-3- Functional diversity: BiologEcoplate assay

BiologEcoplates (Biolog Inc., Hayward, CA) were used to estimate soil microbial functional diversity based on utilization of 31 different substrates [46,47,48]

Wet soils (1g) were added in steril condition to 9 ml distilled water 0.85% NaCl and shaken one hour. Then the suspension was centrifuged for 5 min at 1300rpm to remove soil particles. Then the supernatant with bacteria was diluted ten fold in 0,85%NaCl distilled water, and used to inoculate BiologEcoplates with 150 μ l per well. Three replicates per treatment were performed. The plates were incubated at 25 °C in darkness and the absorbance at 570 nm was measured every 24 h for seven days and was used to calculate three factor of the functional diversity indices. Absorbance values were blanked against the control and the first factor the average well color development $AWCD = \sum (C-R)/N$ was calculated where C is color production with each well, R is the absorbance value of the plate's blank well, and N is the number of substrates (ECO plates, N=31), then the second factor is the substrate richness which represents the percentage of positive well (absorbance > 250 nm). The third factor is the substrate evenness where the functional diversity was calculated and classified by six different substrates family according to [49].

2-4 Statistical analyses

Results are presented as mean \pm SD of 3 samples. R software was used for all the statistical analysis in this paper. The normality of the distribution was tested using the Shapiro–Wilk test. For multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data along with Tukey's test..

III. RESULTS**3-1-Effect of aloin on soil physico-chemical properties**

Soil pH was significantly higher in soils amended with C4 in all tested conditions compared to control soil and the other aloin concentrations. Moreover soil pH increased after 7 and 14 days of incubation and decreased after 28 days for all tested concentrations, except for C 1 one (figure 2). No significant difference of organic matter (figure 3) between the different tested concentrations was observed. However, after 28 days, organic matter decreased in all soils, compared to the starting condition. Nitrogen content (table 1) of soil following aloin addition was the highest with the application of C1 and C2 where means were respectively 15,41 \pm 3,34 and 13,51 \pm 1,91 mg/g. After 28 days, nitrogen content decreased in all soils. The most important decrease was observed for C1 (50 %).

Phosphorus content in soil in presence of aloin (table 1) was the highest when C2 was applied. After 28 days of

incubation, phosphorus content decrease in all conditions, except for C3. The most important decrease was noted in soils with C1 where means reached $0,09 \pm 0,03$ mg/g.

Cation exchange capacity (table 1) was initially higher in soils with aloin, in comparison to control one. After 28 days, CEC increases in all soils. The most important increase was observed in the case of C3 and C4 where values reached respectively $18,97 \pm 0,24$ ppm and $18,72 \pm 0,44$ ppm.

3-2-Effect of aloin on soil microbiological activity

3-2-1-Substrate induced respiration

The incubation of soils with aloin resulted on a significant decrease along the incubation time in all the tested conditions, except for C3. The highest microbial biomass was found with the addition of the C4 aloin concentration where the value reached in the first day of incubation $5655,75 \pm 1010,49$ $\mu\text{g carbon g}^{-1}$ soil (Table 2). Moreover, the most important decrease was observed in the case of soil incubated with C4 where microbial biomass reached $37384,1 \pm 544,479$ $\mu\text{g carbon g}^{-1}$ soil after 28 days of incubation (table 2).

3-2-2 Soil enzymes activities

The response of soil enzymes under the effect of aloin incorporation is presented in figure 4. B-glucosidase activity was higher in soils amended with aloin, compared to control soil. The most significant value was observed in the case of C4 initially where the value reached $250,69 \pm 38,16$ PNP $\text{g}^{-1} \text{h}^{-1}$. However, the activity decreased following the incubation time for all the aloin concentration.

The alkaline phosphatase was highest initially with the application of C4 concentration where value was $120,41 \pm 13,25$ PNP $\text{g}^{-1} \text{h}^{-1}$. Along the incubation time, the enzyme activity had the same trend with the application of C1 and C2 where alkaline phosphatase decreased, contrary to C3 and C4 where enzyme activity increased after 28 days of aloin addition.

Acid phosphatase activity was higher initially (0 days) with the application of C4 where value was $48,08 \pm 7,00$ PNP $\text{g}^{-1} \text{h}^{-1}$. However, the enzyme activity decreased along the incubation time and reached $12,73 \pm 0,85$ PNP $\text{g}^{-1} \text{h}^{-1}$ after 28 days of aloin incorporation. The same trend was observed for C3 despite an increase observed after 7 days. For C1 and C2, an increase was observed on acid phosphatase activity after 28 days of aloin addition.

The activity of arylsulphatase was highest initially with application of C4 where value was $148,97 \pm 32,54$ $\mu\text{mol PNP.g}^{-1} \text{dry soil}^{-1} \text{h}^{-1}$. However, the enzymatic activity decrease with the incubation time. The same trend was observed in soils amended with all aloin concentrations.

Urease activity was the highest initially in the case of the application of C3 where value was $0,005 \pm 0,0001$ μg

$\text{NH}_4\text{-g}^{-1} \text{h}^{-1}$. However, the maximum of urease activity was observed with C4 after 7 days of incubation where value reached $0,006 \pm 0,0003$ $\mu\text{g NH}_4\text{-g}^{-1} \text{h}^{-1}$. Moreover, urease activity increased along the incubation time and reached the maximum after 28 days of aloin addition for all the concentrations applied except C4 one.

The dehydrogenase activity was the highest initially in the case of C2 and C3 concentration where values were respectively $2,179 \pm 0,05$ TPF $\text{g}^{-1} \text{h}^{-1}$ and $2,242 \pm 0,014$ TPF $\text{g}^{-1} \text{h}^{-1}$. However, the maximum of the enzyme activity was noted with C4 after 7 days of incubation where mean reached $3,124 \pm 0,39$ TPF $\text{g}^{-1} \text{h}^{-1}$. Moreover, dehydrogenase activity decreased along the incubation for all the concentrations.

The FDA activity was more important with the increase of the aloin concentration. However, the enzymatic activity decreased within the incubation time. This decrease was noted especially with C1 where the enzymatic activity reached $7,84 \pm 0,51$ $\mu\text{g de TPF/g sol/d}$.

3-3 Functional diversity: (Biolog)

The metabolic activity determined as AWCD (table 3) was the maximal at the starting point in soils amended with C3 and C4 aloin concentrations where values were respectively $1,52 \pm 0,10$ and $1,61 \pm 0,06$. However, AWCD significantly decreased along the incubation time.

Regarding substrate consumption, ecoplates results (fig 5) suggested that there is no significant effect of the exposure period while the dose of aloin significantly affected microbial activity in soils. Moreover, amines and amides consumption increased as the dose of aloin increased, which is not the case for carboxylic acids. In general, the microbial communities of the tested soils have more affinity to consume Amino acids substrates than polymers and various other compounds.

IV. DISCUSSION

The aim of the present work was to assess firstly the impact of aloe vera wastes on physico-chemical properties of soils and second how these wastes affect soil microbiological activities. Indeed, Aloe vera wastes can be used as amendment in soil to improve its fertility. However, these wastes are rich in natural PAHs such as the aloin A and B. Indeed, PAHs are resistant to be degraded tend to accumulate in the soil and potentially threaten soil ecology and more in general human health through food chain [50]. So, environmental impact of aloe vera wastes must be assessed to avoid soil disturbance. Moreover, the impact of HAP pollution on both soil enzymes activities and bacterial functional diversity is poorly documented. It is of ecological interest to determine which functional and metabolic capabilities are selected for in a bacterial community under drastic selective pressure by different pollutants.

For this purpose, soils were exposed to different aloin concentrations. Firstly, their effects on physico-chemical properties of soil were determined and secondly, their impact on soil microbiological activities was assessed. The physicochemical properties were obviously modified after 28 d of exposure to Aloe vera waste. Soil pH had significantly increased with aloin concentration and this was probably due to the basic character of this industrial waste.

Furthermore, a slight decrease in OM amount after 28 d was recorded but there is no significant effect between treatments. As reported by several studies OM is one of the most important factors affecting hydrocarbons distribution in soils [51,52]. Thus, this decrease must be a result of the higher metabolization of those organic wastes which can be absorbed by organic matter along experimentation as proved by [53].

On the other hand, the CEC values increased with exposure period in all the experiments, and this could be related to the adsorption of the organic molecules to the clay particles of experimented soils which can increase the exchange capacity of soils and this was proved by [54,55]. Moreover, a slight decrease was also observed for nitrogen and phosphorus mineralization. This was in concordance with the work of [56].

Our findings suggested that aloin increases microbial biomass in soils in a dose dependant manner. This demonstrates that the addition of aloin to soils can act as significant sources of carbon for microbial growth and activity. Our results are consistent with previous studies indicating that the PAHs induce microbial biomass in soils [57]. Besides being a pollutant with potential toxicity, HAPs are also a carbon source that could support bacterial growth [58]. The number of benzene rings determines a PAH's ability to stimulate enzymatic activity. Organic compounds containing three or four rings constitute a rich source of energy and carbon for microorganisms, whereas compounds containing a higher number of rings are toxic, mutagenic, and carcinogenic [59,60]. However, along the time incubation, microbial biomass decreased. This can be explained by the fact that carbon sources which were provided by the HAPs decreased along the incubation time. Other authors found that depending on its concentration, phenanthrene could decrease the bacterial biomass [61] or activity [62].

The measurement of enzymatic activities was used to evaluate soil fertility [63]. Soil enzymatic activities are considered to be important soil biological activities influenced by contamination occurring in the soil ecosystem. Our studies indicated that enzymes activities were enhanced by the addition of the aloin. The chemical composition of aloe vera contains anthraquinone and PAHs which can be sources of energy to bacterial

communities that enable them to produce enzymes in soils. These results are consistent with those of many studies on the effects of HAPs on soil enzymes activities [64,65,66].

The urease activity is based on hydrolysis of urea to carbon dioxide and ammonium and it originates mainly from microorganisms, plants and animals [67,68,69,70]. An increase in urease activity levels in soils treated with aloin was observed in our study. Similar results of the stimulating effect of PAHs were reported by [65,71]. The use of hydrocarbons as substrates for microbial growth which use them as a source of carbon and energy is well documented.

Arylsulphatase is produced by bacteria and fungi to limit sulphur, [72], this enzyme catalyzes the hydrolysis of sulphate esters in the soil [73]. Its activity in soil is correlated with microbial biomass and with the rate of immobilization of sulfur [74,75]. This can explain the fact of the increase in arylsulphatase activity found in our results as a consequence of the increase in microbial biomass observed.

β -Glucosidase produces glucose, an important C energy source for microbes in the soil [76], by hydrolyzing the dimers of glucose produced by cellulolytic microorganisms. This enzyme represents a good soil quality indicator and can inform about the capacity of the soil to stabilize organic matter [77,78]. In fact, for soils with aloin only there has been a significant increase which can be a result of the activation of this enzyme with the addition of aloin.

Several researchers reported that acid and alkaline phosphatase activities were therefore considered as a good indicator of soil fertility and play a fundamental role in the soil system [79,80]. Aloin enhanced these two enzymes activity. In fact, the variation of these enzymes depends and are correlated with the phosphate [81] and soil organic matter content [82,83], so contrary to other activities, the results of phosphatase cannot support to discriminate samples, given that this enzyme is both an intra- and extracellular and the extracellular part is not very sensitive to variations in environmental conditions to affect microorganisms [84].

The dehydrogenase activity is an indicator of biological activity in soils [85], it only exists in living microbes and represents active viable and intact cells [86]. This enzyme acts by oxidizing soil organic matter by acting on the transfer of protons and electrons. Therefore the enzyme participates in the process of respiration of the microorganisms which depends on the conditions and properties of the soil [87,88]. Also, a decrease in the enzyme activity was observed along the incubation time. This was also observed in the work of [89] who noted that typically, addition of PAHs reduced dehydrogenase activity initially, with activity subsequently recovering to

control levels. The higher levels of dehydrogenase activity observed in fluoranthene amended soil may reflect that degradation of this PAH was proceeding rapidly. This would suggest, in the longer term, that PAH amendment, whether of 3-, 4- or 5-ring, did not have a toxic effect overall. This does not preclude specific toxic effects on individual microbial populations which might affect degradation rates.

FDA (fluorescein diacetate) hydrolysis represents a total indicator of soil microbial activity; it has the property to measure the activities of proteases, lipases and esterases [90,91]. Our results showed an increase on this enzyme activity with aoin incubation. However, it decreased along the incubation time.

Using community-level physiological profiles (BiologEcoplates™), we observed that aloe vera exudates had an impact on the community physiology. Indeed, a decrease in metabolic activity was observed with the application of C3 and C4 and increased with the small concentrations. This is in concordance with the work of [56]. Moreover, [92] demonstrated that PAH amendment had a profound effect on functional catabolic bacterial community in sandy pea soil. Moreover, soils tested have more affinity to consume substrates of the categories of amino acids, polymers and various compounds. These observations suggest that the presence of HAPs modified the range of substrates and degradation efficiency. This observation was also noted in the work of [56].

V. CONCLUSION

This work provided clues about a possible positive effect of aloe vera wastes incorporation in agriculture soils despite the known toxic effects of major constituents such as aoin A and B. Indeed, it increased microbiological activities in soils even if this effect does not last in time.

Amendment of Aloe vera residues to soil can be an interesting way to valorize these specific wastes and may promote sustainable agriculture in regions where aloe vera production is the main activity.

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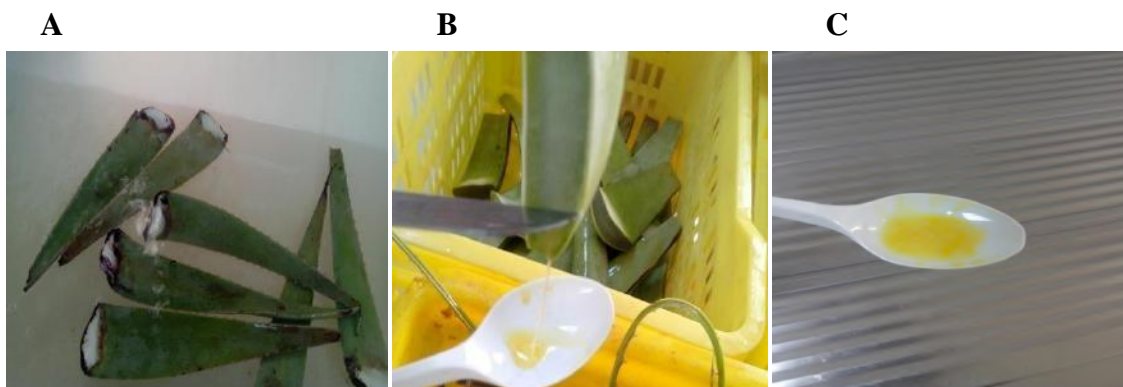


Fig.1: Aloi Extraction .A : Leaves of Aloe veracutted, B : Yellow exudate is collected , C : Aloi

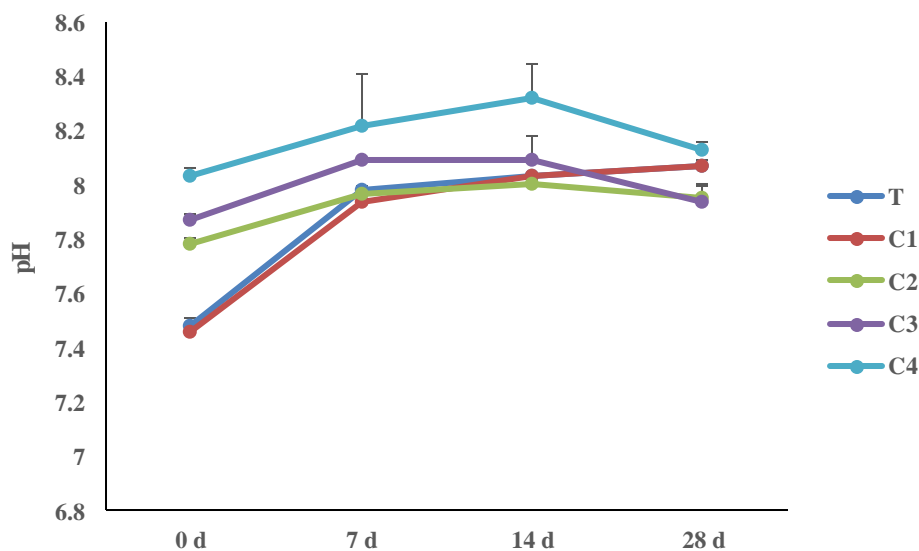


Fig.2: Changes in soil pH after 0, 7, 14 28 and 60 days of incubation with different concentrations of aloi

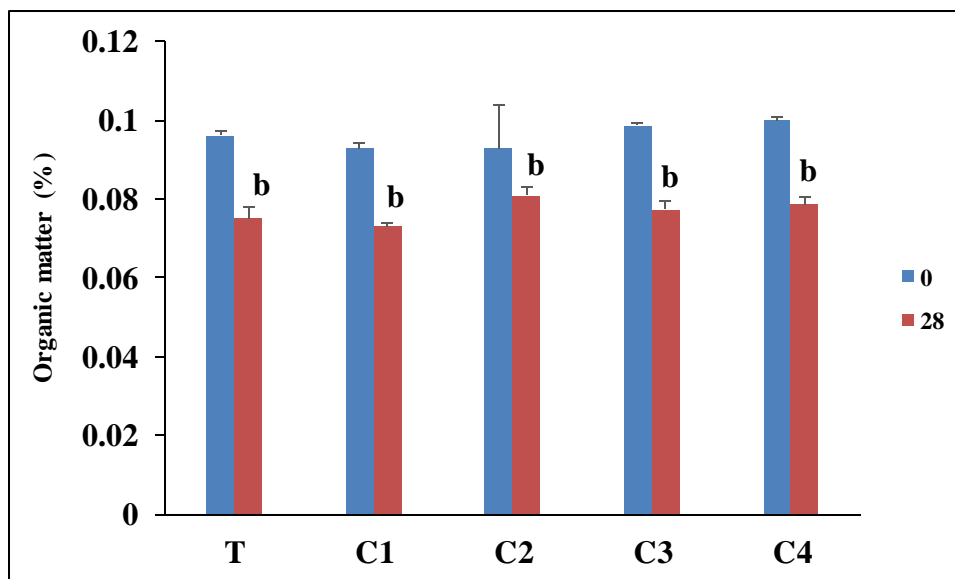


Fig.3: Changes in organic matter (%) after 28 days of incubation with different concentrations of aloi. were analyzed by ANOVA + Tukey's post test. a: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. b: Statistically significant differences (n= 10; P<0.01) in comparison with soils exposed to the same condition for 0 d.

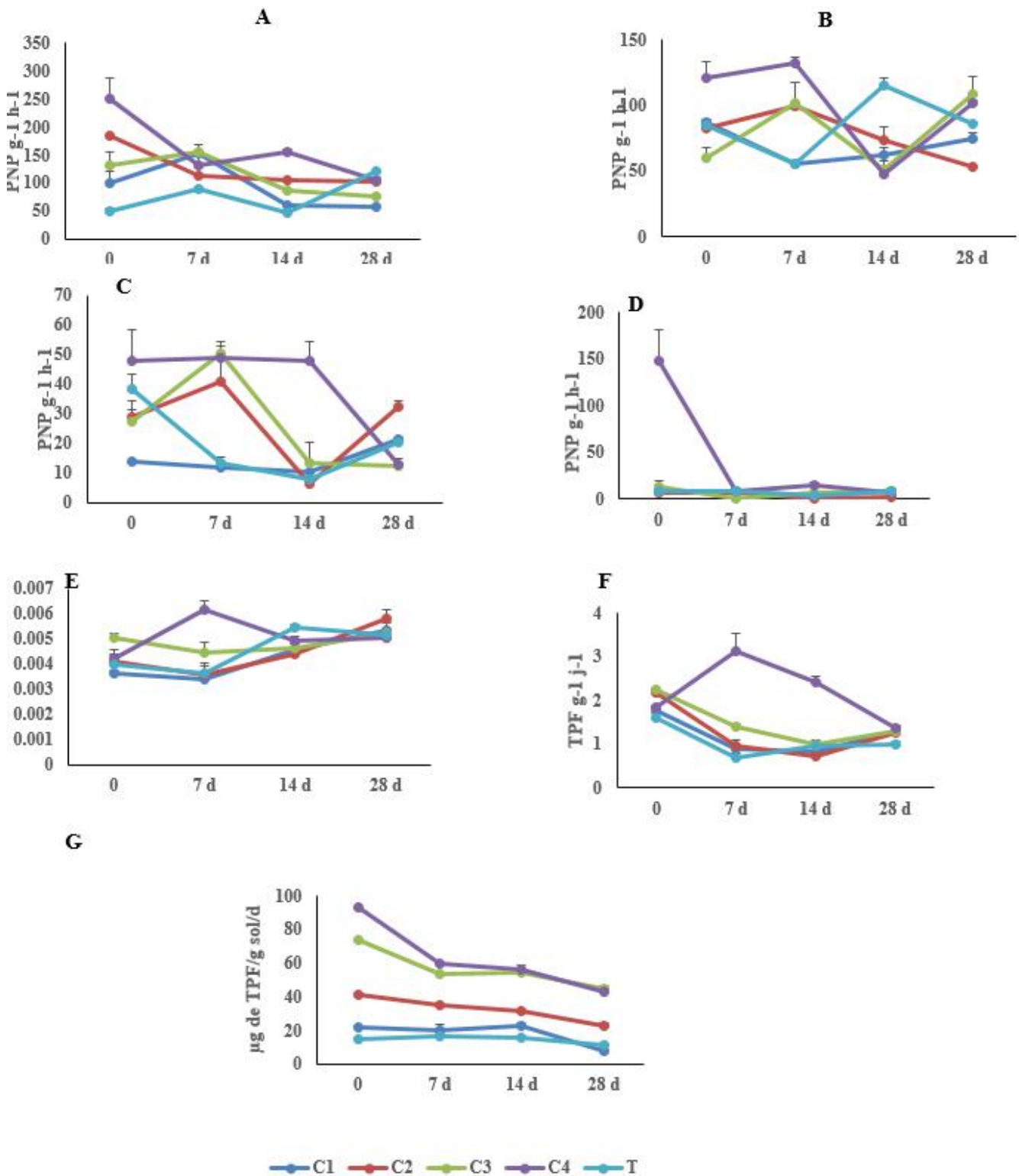


Fig.4: Enzymes activities in soils with different aloin concentrations : A : β -glucosidase , B : Acid phosphatase, C : Basic phosphatase, D : urease, E : Arylsulphatase, F : Deshydrogenase, G : FDA.

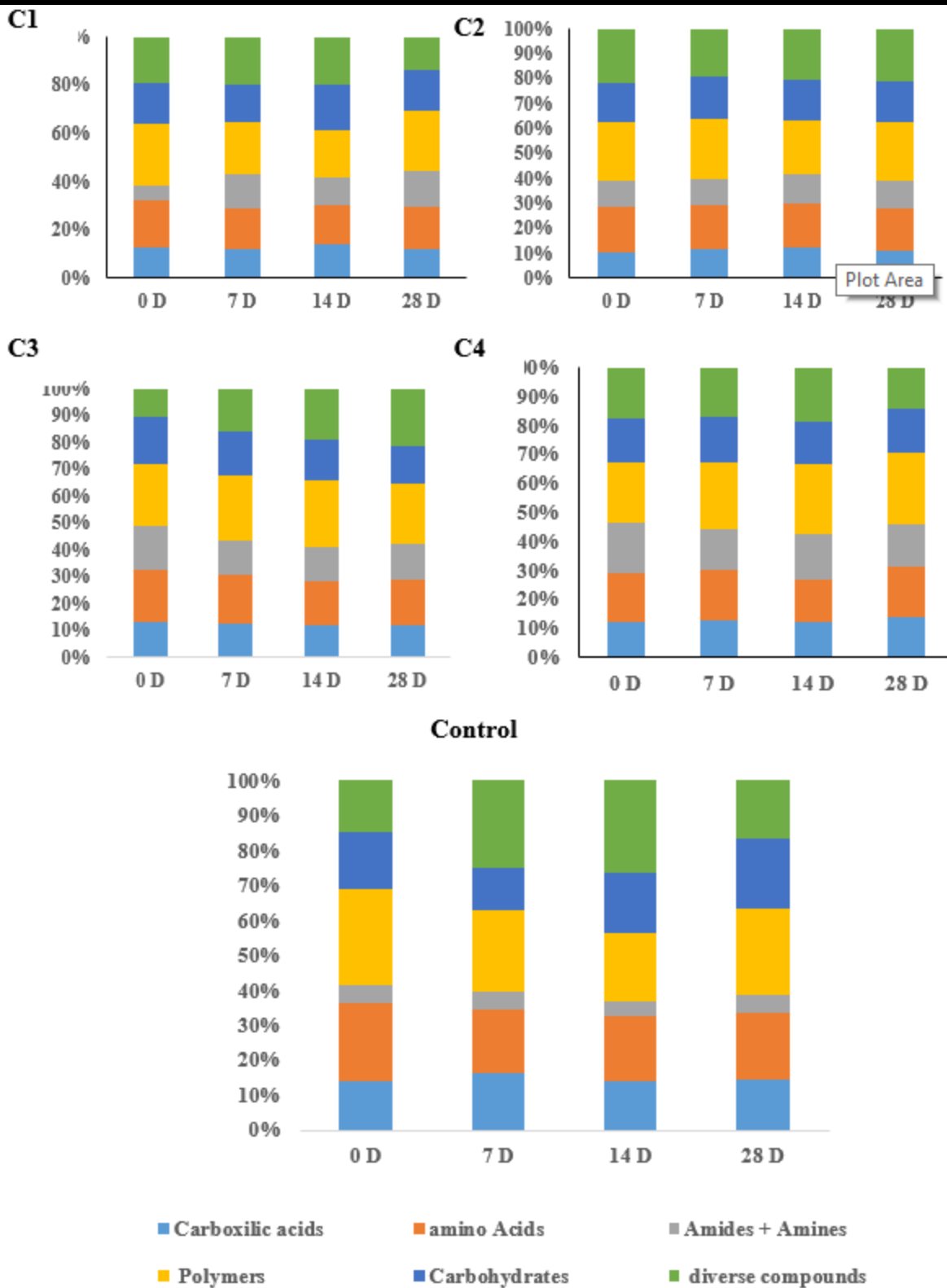


Fig.5: Ecoplates substrates utilization pattern in soils with different aloin concentrations after 0, 7, 14 and 28 days of incubation

Table 1. Effect of Aloe vera wastes on Nitrogen content, phosphorus content and CEC. #: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. *: Statistically significant differences (n= 10; P<0.01) in comparison with soils exposed to the same condition for 0 d.

		Nitrogen (mg/g)	Phosphorus	CEC
Control	0 days	12,19±2,53	7,33±0,28	9,74±2,44
	28 days	7,66*±0,39	5,60*±0,72	13,67*±1,95
C1	0 days	15,41± 3,34	6,40±0,78	13,54±1,09
	28 days	7,27*±1,17	0,09*±0,03	13,79±1,25
C2	0 days	13,51* ±1,91	8,80±0,89	13,43±1,36
	28 days	8,07±0,35	6,71*±0,68	17,91*±3,58
C3	0 days	11,50±2,92	6,73±1,35	13,49±0,28
	28 days	8,54*±2,21	16,47*±0,79	18,97*±0,24
C4	0 days	3,47#±0,73	6,76±1,24	13,60±0,54
	28 days	4,86*±1,09	2,86*±1,89	18,72*±0,44

Table 2. Substrate induced respiration pattern in soils incubated with aloin in mg CO₂.g⁻¹.h⁻¹. *: Statistically significant differences (n= 10; P<0.01) in comparison with control condition.

Condition	0	7 d	14 d	28 d
T	43337,00± 791,44	38941,9*± 459,21	34201,3*±2593,49	41361,3±2962,69
C1	47635,69± 9013,11	39725,8*±1091,06	37842,3*±685,06	45256,2± 1409,06
C2	47635,69± 9013,11	45213,5±2878,84	41065,4±1075,6	48398,1*±3257,83
C3	28959,83*± 975,77	48340,7±3396,91	46137,1±887,24	38631,9*±1675,2
C4	52655,75* ± 1010,49	52436,75*±1204,45	38327,5*±1525,56	37384,1*±544,479

Table 3. Evolution of AWCD in soils with aloin after 0, 7, 14 and 28 days of incubation. . *: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. *

Condition	0	7 d	14 d	28 d
T	0,755 ±0,24	0,670± 0,12	0,707±0,15	0,854± 0,25
C1	0,820± 0,17	1,281 *± 0,23	1,150*± 0,27	1,022*± 0,14
C2	1,20* ± 0,05	1,184* ± 0,14	1,242*±0,17	1,144* ± 0,22
C3	1,520* ±0,10	1,218* ± 0,04	1,242*±0,07	1,230* ± 0,19
C4	1,617 *±0,06	1,264*±0,04	1,276*± 0,06	1,259*± 0,22