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Terrestrial *isopoda* as Model Organisms in Soil Ecotoxicology

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Abstract:

To better understand how terrestrial *isopods* impact agricultural soil enzymatic activity, methane absorption, and their role in decomposition by consuming and digesting leaf litter, this study examines the function of *isopods* in nutrient cycling, organic matter breakdown, and the safe disposal of diverse wastes. Five locations in the Al-Jadriya region were chosen. Sites 1, 2, and 3 had *isopods,* but sites 4 and 5 were devoid of *isopods* and exclusively populated by earthworms, ground insects, and snails, and their overall density was 2400 ind/m². This study was conducted in five agricultural fields in Baghdad from October 2023 to June 2024.

The results of the current study showed that the presence of *isopods* in sites 1, 2, and 3 had a clear effect in changing the environment, accelerating the processes of recycling nutrients, analyzing organic materials, and getting rid of many cellulosic wastes, as an increase in the effectiveness of soil enzymatic activities was observed: alkaline phosphatase, dehydrogenase, and urease, compared to the sites where the appearance of *isopods* was not recorded.

It also had a clear effect on the numbers of bacteria and fungi, as the numbers of bacteria decreased in the sites inhabited by *isopods* (sites 1, 2, and 3). It also had an impact on the abundance of bacteria and fungi, while only *Bacillus* and *Pseudomonas* species were recorded in sites 1, 2, and 3 (where *isopods* were present). In contrast, high numbers of bacteria were recorded in the soil of sites 4 and 5, where the *isopods* did not appear, represented by the species *Rhizobium*, *Bradyrhizobium*, *Microbacterium*, *Serratia*, *Azotobacter*, *Actinomyces*, *Bacillus*, *Propionibacterium*, *Acetobacter*, and *Methanococcus*.

As for fungi, high numbers were recorded in the sites inhabited by *isopods,* represented by the species *Aspergillus niger*, while the numbers of fungi were moderate in sites not inhabited by *isopods* and included different species such as *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*.

The results of the current study also showed a clear effect of the presence of *isopods* on the abundance of nutrient forms in the soil of the sites they inhabit. High values were recorded for potassium (K) and phosphorus (P), and the sites inhabited by *isopods* also recorded high values of methane (CH_4), ammonium ($N\text{-}NH_4$), nitrate ($N\text{-}NO_3$), and carbon dioxide (CO_2).

Keywords: *Mitigating Emissions, Poultry Farms, Animal Welfare*

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متشابهة الارجل *isopoda* كنموذج في علم السموم البيئية للتربة

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الخلاصة

لفهم تأثير متشابهة الارجل *isopoda* الأرضية على النشاط الإنزيمي للتربة الزراعية، وامتصاص غاز الميثان، ودورها في التحلل عن طريق استهلاك وهضم مخلفات الأوراق، ومعرفة دورها في دورة المغذيات، وتحلل المواد العضوية، والتخلص الآمن من النفايات المتنوعة, تم اختيار خمسة مواقع في منطقة الجادريةتمثلت بالمواقع 1 و2 و3 كانت تتواجد فيها مجموعة متشابهة الارجل isopoda ، بينما كانت المواقع 4 و5 خالية منها، وكانت تسكنها ديدان الأرض والحشرات الأرضية والقواقع فقط، وبلغت كثافتها الإجمالية 2400 بوصة/متر مربع. أجريت هذه الدراسة في خمسة حقول زراعية في بغداد من أكتوبر 2023 إلى يونيو 2024، وأظهرت نتائج الدراسة الحالية أن وجود مجموعة متشابهة الأرجل في المواقع 1 و2 و3 كان له تأثير واضح في تغيير البيئة وتسريع عمليات إعادة تدوير العناصر الغذائية وتحليل المواد العضوية والتخلص من العديد من النفايات السليلوزية، كما لوحظ زيادة في فعالية: الأنشطة الأنزيمية للتربة الفوسفاتيز القلوي، ديهيدروجينيز اليورياز، مقارنة بالمواقع التي لم يسجل فيها ظهور متشابهة الأرجل، كما كان له تأثير واضح على أعداد البكتيريا والفطريات، حيث انخفضت أعداد البكتيريا في المواقع التي تسكنها متشابهة الأرجل(المواقع 1 و2 و3). كما كان له تأثير واضح على وفرة البكتيريا والفطريات اذ تم تسجيل أنواع *Bacillus* و*Pseudomonas* فقط في المواقع 1 و2 و3، وهي المواقع التي تتوجد بها متشابهة الأرجل، بينما تم تسجيل أعداد عالية من البكتيريا في تربة الموقعين 4 و 5، المواقع الخالية من متشابهة الارجل ، اذ تمثلت بالانواع *Rhizobium, Bradyrhizobium, Microbacterium, Serratia, and Azotobacter, Actinomyces, Bacillus, and Propionibacteium) Acetobacter, Methanococcus*. أما بالنسبة للفطريات فقد سجلت أعداد عالية في المواقع التي تسكنها متشابهة الارجل ممثلة بنوع Aspergillus , niger، بينما كانت أعداد الفطريات معتدلة في المواقع غير المأهولة بمجوعة متشابهة لأرجل وشملت أنواع مختلفة من الفطريات مثل *Mucar, Aspergillus, Penicillium, Fusarium, Rhizopus*. كما أظهرت نتائج الدراسة الحالية تأثيرًا واضحًا لوجود متشابهة الأرجل على وفرة العناصر الغذائية (الأشكال) في تربة المواقع التي تسكنهااذ تم تسجيل قيم عالية للبوتاسيوم والبوتاسيوم والفوسفور، كما سجلت المواقع التي يسكنها متماثلات الأرجل أيضًا قيمًا عالية للميثان وCH_4، بالإضافة إلى N-NH_4 وN-NO_3 وCO_2.

الكلمات المفتاحية: ***متشابهة الارجل isopoda , انزيمات التربة , سموم بيئة***

Introduction

Soil is a dynamic and complicated system that serves as a home for microbes, plants, animals, and people [1]. Currently, polluted soils represent a critical issue as they may result in the pollution and biomagnification of toxic substances within food webs, potentially impacting human health. Soil contaminants exhibit various fractions based on the kind of contamination and the soil composition. Specific chemical fractions are accessible and may be absorbed by organisms, contingent upon soil physicochemical parameters (e.g., pH, clay content, methane, organic matter content) and the chemical form of the element. Consequently, assessing the overall chemical contents is inadequate for evaluating the ecological risk of polluted soil. Bioassays are effective instruments for determining soil quality by measuring the potential toxicity of pollutants, precisely their bioavailable proportion. Standardized acute and chronic tests have been established in soil ecotoxicology utilizing soil-dwelling invertebrates, such as *isopods.* As macro-detritivores, Terrestrial *isopods,* called woodlice or pill bugs, play a crucial role in decomposition by consuming and digesting leaf litter, distributing microbial spores, and facilitating microbial activity and nutrient cycles. Microbes consumed with food facilitate digestion. *Isopods* can establish symbiotic partnerships with bacteria in their stomach; however, a portion of cellulose digestion appears to be aided by endogenous enzymes (cellulases). Bacterial symbionts in the gut live in the digestive glands, which helps them survive diets low in nutrients. Recycling organic waste begins with the fragmentation of litter, which is the first phase. Woodlice influences microbial activity, which in turn affects the rate of litter breakdown and the availability of nutrients in soil [2]. To enhance nutrient release and soil enzyme activity, *isopods* are essential as biological agents that help organic matter go deeper into the substrate. By changing the rates of substrate decomposition and increasing the substrate surface area, woodlice activity affects soil nutrients like sulfur, nitrogen, and phosphorus [3] [4]. which in turn promotes the mineralization of organic materials from plant cells. Fungivores are able to disintegrate or graze more easily when microbial activities are low, which in turn increases microbial activity [5]. Thus, terrestrial *isopods* indirectly affect the microflora's activities, community structure, and functions [6]. There are several ways in which terrestrial *isopods* might affect the soil microbiome. Among Iraq's saprophagous soil macrofauna, *isopods* are abundant and common. *Isopods* have been extensively studied for their ecological significance, but their effects on soil enzyme activity, methane-cycling processes, and bacterial and fungal populations have received very little attention. Soil dynamics, isopod substrates, and the interplay between the three must be carefully considered.

To better understand how terrestrial *isopods* impact soil enzymatic activity, comparing soil enzyme activities in environments with and without these animals is helpful. The impact of the terrestrial isopod *Porcellionides pruinosus* on agricultural soil enzymatic activity, methane absorption, and the associated interaction system is examined in this study [7], The results will help us understand how *isopods* affect soil enzyme activity. The impact of macroinvertebrates that live below or on the surface on primary greenhouse gasses other than carbon dioxide, particularly methane, is largely unknown. Soil microbes that rely on carbon from methane could be affected by the actions of soil *isopods.* Soil isopod bioturbation activities and their impact on fungal and bacterial communities driven by methane are investigated in this study. scaber was selected as the model organism for proof-of-principle research in order to reduce the confusion caused by these effects. This was due to the wide variability in microbial responses to various macroinvertebrates, as well as their distinct behavioral patterns and interactions with microorganisms in natural communities (for example, grazing, burrowing activity, and fecal deposition) [8]. It is possible that P. scaber improves microbial transportation and distribution, which increases the diversity of soil microbes, changes the makeup of microbial communities, and impacts soil processes in areas where it has recently colonized [9], [10].

The impact of terrestrial *isopods* on soil enzymatic activity can be better understood by comparing soil enzyme activities with and without these creatures. Soil enzymatic activity, methane absorption, and the related interaction network were investigated in this study as they pertain to the terrestrial isopod *Porcellionides pruinosus*. The findings will improve the understanding of the impact of *isopods* on enzymatic activity in the soil. There is limited understanding of the impact of (sub-) surface-dwelling macroinvertebrates on non-CO2 primary greenhouse gases, mainly methane. The activities of soil *isopods* may influence soil microorganisms reliant on methane-derived carbon. We shed light on how populations of bacteria and fungi stimulated by methane interacted with the bioturbation of soil *isopods.* To overcome the confounding effects caused by the wide variation in microbial responses to different macroinvertebrates and their unique behavioral patterns and interaction modes with microorganisms in natural communities, a proof-of-principle study using P. scaber was conducted [4].

This study looked at behaviors such as grazing, burrowing activity, and fecal deposition [11], [12]. When P. scaber boosts microbial dispersion and movement, soil microbes may be able to colonize more regions, alter community composition, and expand their range of habitats.

Material and Methods

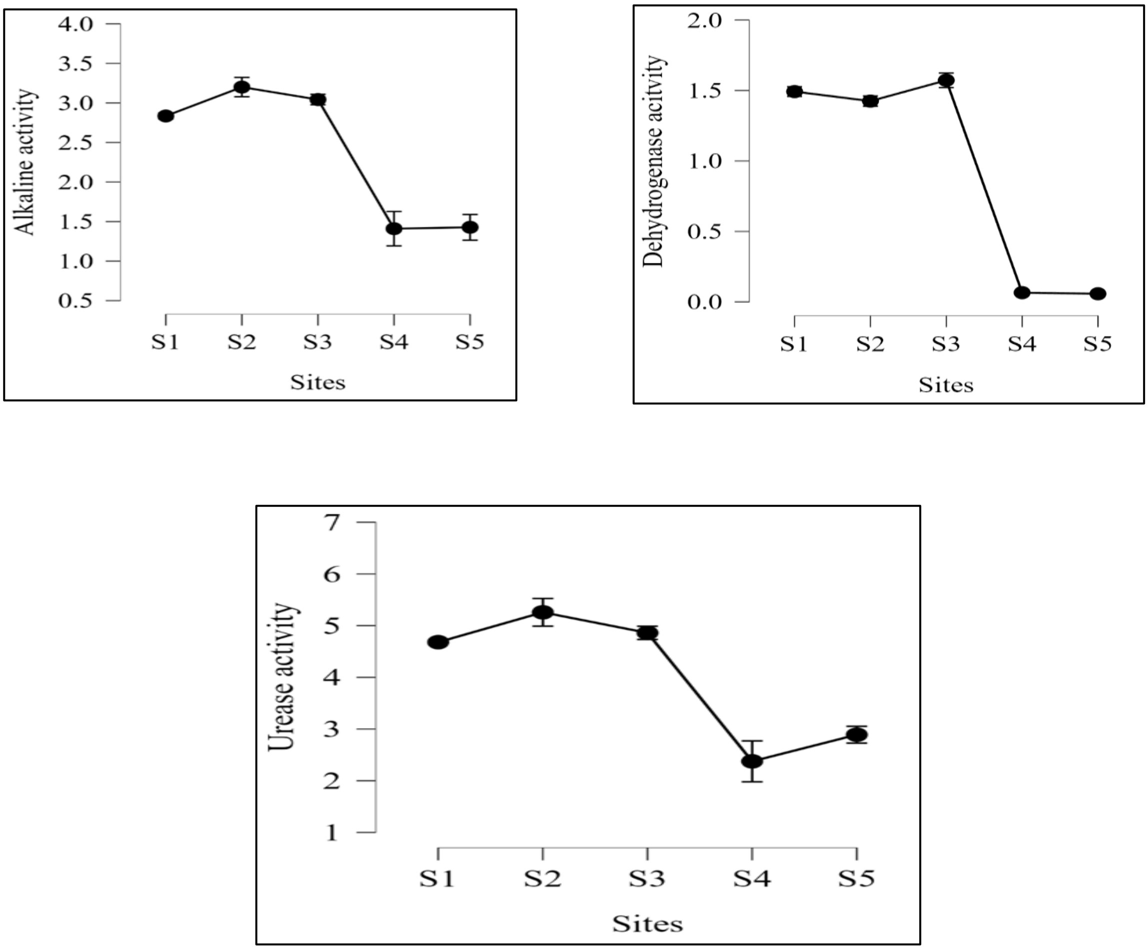
To comprehend the systems, the environment, and the involvement of *isopods* (figure 1) in soil degradation processes. This study examines the function of *isopods* in nutrient cycling, organic matter breakdown, and the safe disposal of diverse wastes. *Isopods* are characterized as environmental cleansers due to their efficient function in decomposing and ingesting various urban and agricultural waste materials. Five locations in the Al-Jadriya region were chosen. Sites 1, 2, and 3 had *isopods* , but sites 4 and 5 were devoid of *isopods* and exclusively populated by earthworms, ground insects, and snails. The *isopods* were classified utilizing the taxonomic key [13], and their overall density was 2400ind\m^2.This study was conducted in five agricultural fields in Baghdad from October 2023 to June 2024. Samples of soil isopoda were collected using a soil core measuring 10 cm in length and 5 cm in diameter. Five independent samples were collected from each research site randomly. Stones and harvested agricultural residues were characteristics that helped identify P. scaber at the sampling location. The headspace methane and carbon dioxide were measured using a gas chromatograph (7890B GC System, Agilent Technologies, Santa Clara, USA) coupled to a pulsed discharge helium ionization detector (PD-HID), with helium as the carrier gas. The rate of methane uptake was determined from the slope (linear regression) of the cumulative methane uptake curve. The soluble ammonium, nitrate, and nitrite concentrations were determined colorimetrically in deionized water (1:5 w/v) following centrifugation and filtration (0.2 μm) of the soil suspension, using an Infinite M plex plate reader (TECAN, Meannedorf, Switzerland). Nitrate and nitrite concentrations were below the detection limit (: [14]. The rate of methane uptake was determined by calculating the slope of the cumulative methane uptake curve after linear regression.The assessment of soil enzymatic activities, particularly Dehydrogenase activity, was conducted using the methodology, where Six grams of soil, 30 mg glucose, 1 ml of 3% 2,3,5-triphenyltetrazoliumchlorid (TTC) solution and 2.5 ml pure water were added. The samples were incubated for 24h at 37oC. The formation of 1, 3, 5 triphenylformazan (TPF) was determined spectrophotometrically at 485 nm and results were expressed as μg TPF g-1 dry sample 24h-1 [15]. Urease activity was assessed using the method, 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7) and 1 ml of 10% urea substrate solution were added to the 1 g sample and the samples were incubated for 1h at 370C. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as μg N g-1 dry sample. [16]. The alkaline phosphatase activity was evaluated using the methodology: 0,25 ml toluene, 4 ml phosphate buffer (pH,8.0) and 1 ml of 0,115 M p-nitrophenyl phosphate (disodium salt hexahydrate) solution were added to the 1 g sample and the samples were incubated for 1h at 370C. The formation of p nitrophenol was determined spectrophotometrically at 410 nm and results were expressed as μg p nitrophenol g-1 dry sample. [17]. The analyzed ecological components comprised phosphorus (P), potassium (K), ammonium nitrogen (N-NH4), nitrate nitrogen (N-NO3), and carbon dioxide (CO2).

Fungi and bacteria were separated from the soil using the soil dilution plate method outlined by [18], [19]. The Statistical Analysis System (SAS) was employed in the statistical analysis [20] to compare the physical and chemical attributes and to ascertain significant differences using the LSD test. The component percentages were extracted, and the rates, graphs, and standard deviations were computed.

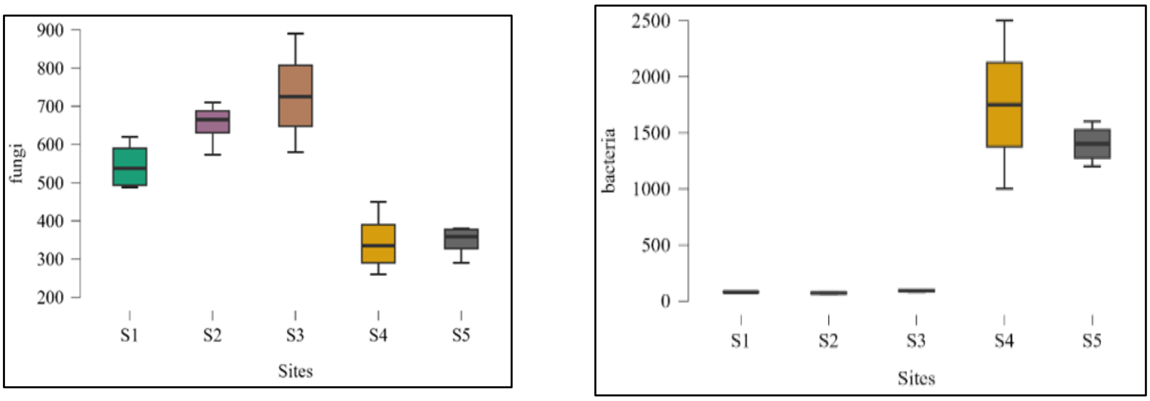
Figurer 1. The external appearance of the adult *isopods* (a-ventral, b- dorsal).

Results and Discussion

This study emphasizes the relationships between *isopods* and soil enzyme activity, underscoring the necessity for more research on the ecological roles of soil fauna as well as highlighting the relationships between *isopods* and soil enzyme activity, underscoring the necessity for more research on the environmental roles of soil fauna, Soil Enzymatic Activities(figure2): Alkaline Phosphatase, Dehydrogenase Urease**,** In areas devoid of *isopods,*



Figurer 2. Soil enzymatic activities in study area.



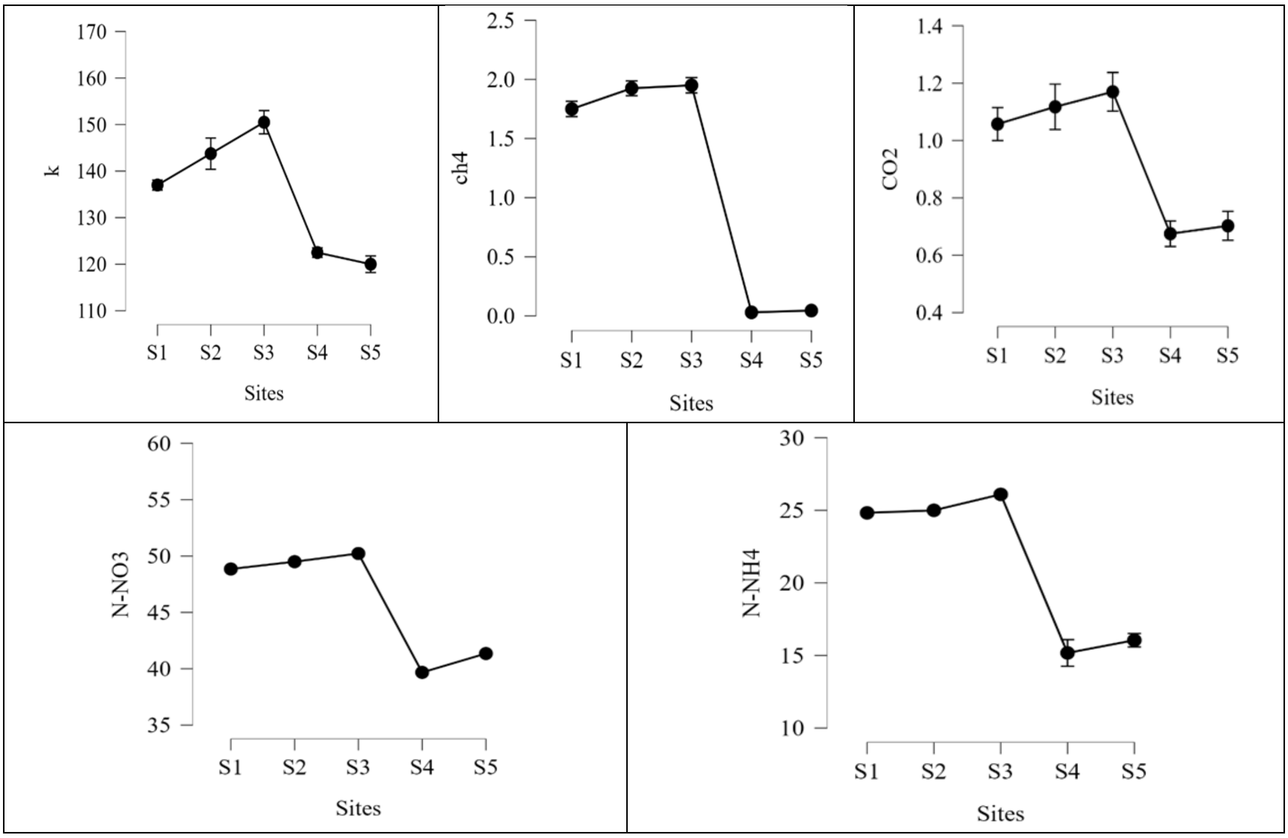
Figurer 3. The abundance of the bacterial and fungal communities in soil of study area

The findings demonstrate that the *isopods* could accelerate litter decomposition and improve soil dehydrogenase, urease and alkaline phosphatase activities in soil, so the study showed that isopod can significantly ($P < 0.05$) promote enzymatic activities compared, that the dehydrogenase activity of the soils increases when isopoda presence in soil in sites (1,2,3) which

Has reached in each of them 1.49,1.42and 1.57 While the dehydrogenase activity was low in the soil where it was not present isopoda , as it reached in each of them 0.0655 and 0.057, The soil enzyme activities Urease and Alkaline Phosphatase and increased in the presence isopoda sites (1,2,3)as it reached in each of them: Urease was recorded 4.68 , 5.25 ,4.86, so Alkaline Phosphatase was recorded 1.49 ,1.42 ,1.57, While low values were recorded in the locations where it did not appear isopoda(4.5 sites) , as they reached in each of them Urease was recorded 2.37 ,2.89 and 0.0.655 and Alkaline Phosphatase was recorded low values were reached to 0.065 in site 4 and reched to 0.0575 in site 5. Their presence significantly affected the abundance of bacteria and fungi (figure 3). The bacterial count decreased in the regions inhabited by *isopods* (sites 1, 2, and 3), The results of the study showed that the presence of the isopoda reduced the number of bacteria, which reached 979 , 39.85 and 91 in sites 1 ,2 and 3, while the numbers were higher in sites 4 and 5, reaching 1750 and 1400 ,While the result differed in comparison to the number of fungi, as the values were higher in sites (1 ,2 and 3) which reached of each it 545.75 and 653.25 and lower number in sites 4 and 5 which recorded 345 and 346.75 .

Furthermore, it affected the bacterial quality, as only the genera *Bacillus* and *Pseudomonas* were recorded. In contrast, higher bacterial counts were observed in sites 4 and 5 soils, where *isopods* were absent. The identified genera included *Rhizobium, Bradyrhizobium, Microbacterium, Serratia, Azotobacter, Actinomyces, Bacillus,* and *Propionibacterium*,*Acetobacter* and *Methanococcus* are two distinct genera of microorganisms. Fungal populations were significantly higher in areas inhabited by *isopods*, primarily consisting of *Aspergillus niger*. At the same time, fungi were observed in non-isopod sites, which included various fungal species in soil samples from locations (4 and 5.) Mucar, Aspergillus, Penicillium, Fusarium, Rhizopus, the current study results showed an apparent effect of *isopods* ' presence on the abundance of nutrient elements (figureures4) in the soil of the sites they inhabit. High values were recorded for both k and p, and the sites occupied by *isopods* also recorded high values of methane (CH_4), N-NH_4, N-NO_3, and CO_2. According to the results, *isopods* affect the soil enzyme activity in several ways. There are two ways in which *isopods* aid in the breakdown of litter: directly and indirectly [21]. In contrast to regions devoid of isopoda, the present study shows that enzymatic activities are considerably ($P < 0.05$) enhanced by isopod treatment. There are mainly two explanations for this event. *Isopods* have the unique ability to consume garbage and break it down into smaller pieces. In addition, they have the ability to indirectly affect the amount, make-up, and activity of soil microbial communities [21], [22]. Soil microbes may get a lot of energy and nutrients from the waste products macro-detritivores produce when they consume much litter [23], [24]. *Isopods* significantly affect soil enzymatic activity, according to the results. The effects were most noticeable during the present studies' incubation periods when microorganisms are predicted to produce most enzymes.

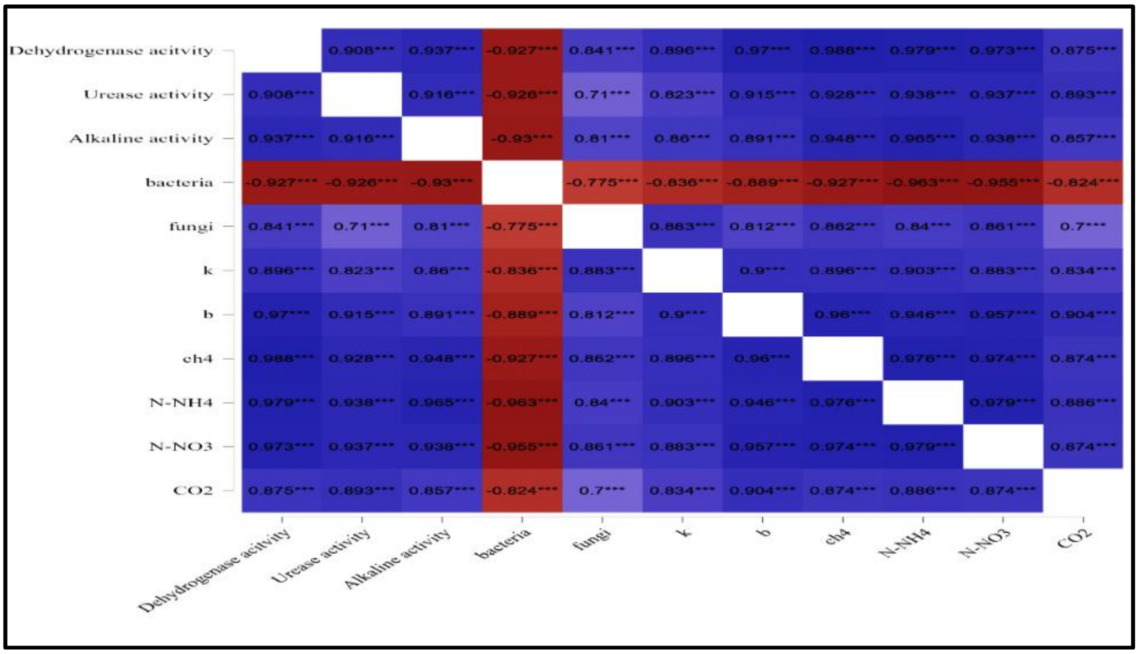
The study's subject was the impact of *isopods* on soil's enzymatic activities [25]. In vitro experiments determined how the presence of the terrestrial isopod Philoscia muscorum affected the incubation-related enzyme activity. Sources of nutrition obtained from wheat straw were created in the lab. Soil activities of dehydrogenase, urease, alkaline phosphatase, and arylsulfatase were tested after 28 days of incubation in soil treated with varying concentrations of *isopods* and wheat straw. Isopod abundance was found to have increased significantly.



Figurer 4. The essential elements in soil of study area

Isopoda exhibits omnivorous feeding behavior, consuming grasses, leaf detritus, carrion, and, on occasion, young seedlings (Holonutrition), They are among the most soil-affine organisms, capable of decomposing and ingesting the remnants of deceased organisms and waste (Maricon). Additionally, they disperse soil contents [26]. Thereby enhancing nutrient recycling processes, analyzing organic materials, and safely eliminating various wastes. *Isopods* are characterized as environmental cleansers owing to their efficacy in dissolving and devouring various urban and agricultural pollutants [27], as they utilize natural processes such as biodegradation to facilitate environmental remediation [28]. Isopoda serves as significant bioindicators in locations polluted with heavy metals due to their capacity to absorb iron (Fe), zinc (Zn), copper (Cu), and other poisons and insecticides [29]. Considering the aforementioned summary regarding the significance of analogous feet and the escalating issue of waste, particularly cellulosic waste prevalent in various environments—given that cellulose is the most ubiquitous element in nature [30] alongside the utilization of conventional treatment methods such as incineration or open disposal, which lead to detrimental health, economic, and aesthetic repercussions that adversely impact human development [31].

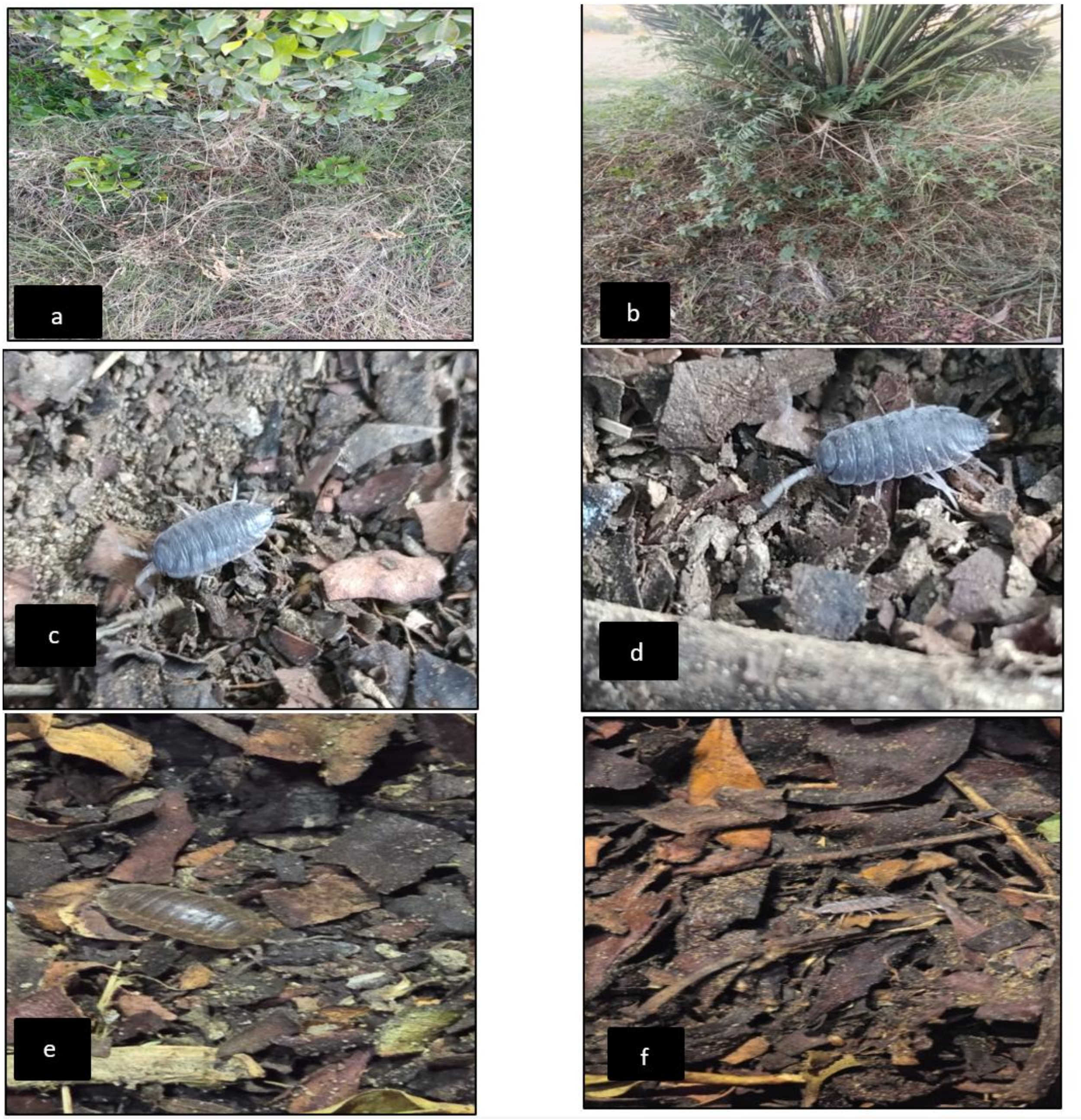
Consequently, it was essential to identify alternate environmentally sound waste treatment techniques, fostering a clean environment that aligns with climate and ecological conservation efforts and promotes environmental sustainability. Consequently, bioremediation emerged as a technology capable of treating and eliminating waste [32] across several ecological spheres. The United Nations Environment Program (UNEP) characterized bioremediation practitioners as green career professionals due to their significant contribution to environmental preservation [33]. The findings of the present research demonstrated the efficacy of *isopods* in expediting decomposition processes and the reintegration of beneficial nutrients into the soil. The present study's findings indicate this use in waste treatment methods, particularly those involving cellulose.



Figurer 5. Pearson's Correlations of the impact of isopod, on the soil enzyme activities, bacteria fungi and nutrient contents in study area.

Moreover, the present study concurs with the idea that the presence of *isopods* significantly contributes to the elevation of methane gas and its evident impact on soil organisms.As "soil bioengineers," the (sub-) surface-dwelling *isopods* Porcellio scaber (woodlice) change the edaphic features of their habitat and impact the mineralization of carbon and nitrogen, leading to emissions of greenhouse gases. Soil *isopods* have not yet been found to have any effect on methane-cycling processes. We examined the impact of P. scaber, a model macroinvertebrate used in microcosm research, on agricultural soil methane absorption and the associated interaction network. Together, co-occurrence network analysis and stable isotope probing (SIP) using 13C-methane allowed us to directly link activity to the methane-oxidizing population (fungi and bacteria) involved in the trophic relationship. P. scaber significantly improved methane absorption, changed the soil's nutritional status and was associated with more complex interactions among bacteria and fungus, in contrast to microcosms lacking the isopod. Specifically, the quantity of fungi in the soil infected with P. scaber increased dramatically while 13C was transferred from methanotrophs to the fungus. This points to the fact that, in the food chain, both bacteria and the fungus population used substrates produced by methane. The findings shed light on the role of P. scaber in regulating methanotrophic activity, which has consequences for the dynamic between fungal and bacterial communities [34]. It seemed clear from the relationship that the presence of *isopods* has a clear effect on accelerating the decomposition of plant leaves, and thus a clear increase in soil enzymes and the basic elements studied, and a reduction in the numbers of bacteria and fungi.figure (5).

As The results of the current study showed that the presence of *isopods* had a clear effect on the decomposition of loose leaves, if sites 1,2 and 3 appeared to be the color of the soil and the leaves were black, while sites 4 and 5, where *isopods* were not present, had green soil and yellow leaves (figure 6).



Figurer 6. The presence of *isopods* had a clear effect on the decomposition of leaves, and the color of the soil (a,b sites 4,5 without isopoda ,c,d,e,f soil presences isopoda)

Conclusion

The results of the current study showed that the presence of *isopods* had a clear effect on the decomposition of loose leaves, if sites 1,2 and 3 appeared so a clear effect of the presence of *isopods* on the abundance of nutrients (forms) in the soil of the sites they inhabit. High values were recorded for potassium, K, and phosphorus, and the sites inhabited by *isopods* also recorded high values of methane, CH_4, as well as N-NH_4, N-NO_3, and CO_2,]. It seemed clear from the relationship that the presence of *isopods* has a clear effect on accelerating the decomposition of plant leaves, and thus a clear increase in soil enzymes and the basic elements studied, and a reduction in the numbers of bacteria and fungi.

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