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Effects of long-term heavy metals contamination on soil microbial characteristics in calcareous agricultural lands (Saiss plain, North Morocco)

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Abstract

Soil is a natural resource and support for all economic activities. Its role is particularly crucial in agriculture. However, agro systems are vulnerable to all natural or anthropogenic disturbances such as erosion, organic or inorganic pollution. In this study, conducted in the region of Fez (Morocco), effects of irrigation with contaminated watercourses were investigated on soil heavy metal contents and microbiological characteristics, in comparison with a control soil. The total heavy metal concentrations of contaminated soil samples were 63.4, 201.2, 291.2 and 33.2 mg/kg for Cr, Cu, Zn, and Ni respectively. Quantitative analysis of soil microbial populations showed that certain groups of soil microbes (actinomycetes and fungi) were particularly sensitive to long-term contamination and revealed a strong negative correlation with Cr, Cu and Zn. While a notably higher number of cultivable bacteria was observed in the contaminated soil compared to the control.

1. Introduction

The rapid development of industry and agriculture has resulted in increasing pollution by heavy metals, causing potential threat to ecosystems [1, 2]. Soil may become contaminated with metals from a variety of anthropogenic sources [3] especially those which are irrigated by untreated wastewater. Indeed, the use of contaminated water for irrigation contributes to the accumulation of chemical and biological contaminants in soils and alter the physicochemical and biological properties of soils [4] and disturb the ecological balance [5, 6].

Soil microorganisms play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling [7]. Fungi and bacteria constitute the main components of the soil microbial biomass and serve as very constructive models for studying the harmful effects of metals at the cellular level [3].

Nowadays, there are reports stating that soil microorganisms may adapt to the increased, even toxic heavy metal and other xenobiotics' concentration in soil [8] by developing various mechanisms to resist heavy metal contamination [9]. However, several studies indicated that accumulation of heavy metals in soils exert toxic effects on soil microorganisms [10] and consequently induces disturbances as to the diversity, population size and overall activities of the soil microbial communities [11, 12, 13].

Otherwise, the most evident outcome of the literature search is that the effects on soil microbiota are neglected in the majority of studies on irrigation with wastewater [4].

In this study, we focused on irrigated agricultural soil with contaminated watercourses, in the region of Fez (Morocco) which is a striking example of the contamination pressure. Over the last few decades, the region of Fez experienced extensive industrial development. The waters of Oued Fez are the most affected by effluents from the city of Fez. This organic and inorganic contamination drained by Oued Fez and diverted in Oued Sebou is transferred into the soil through irrigation water. This work aims to evaluate heavy metals

contamination of soil irrigated with Oued Fez water and their effects on major soil microbial groups, in comparison with a control site.

2. Experimental

2.1. Soil sampling and Analysis

Two sites were selected according to a contamination gradient of surface water used for irrigation [14]. They are located on agricultural land irrigated by the waters from the Fez river (contaminated FEZ site) and from the Sebou river upstream (control SAM site). Plant community composition does not present any difference (*Zea mays*) among the sites samples.

For each site, five soil samples from surface horizons (0-10 cm) were collected by using a plastic scoop, stored in clean polyethylene bags for transport to the laboratory at 4°C, disaggregated by hand, air-dried and sieved to 2 mm. It is generally accepted that only elements with a diameter< 2 mm are reactive and the reactivity of elements larger than that can be neglected (NF ISO 11464). For microbial study, disinfected material was used and analyses were assayed in the 24 hours following soil sampling.

2.2. Physicochemical soil characterization

Physicochemical characteristics of the soil sieved at 2 mm were determined, using the following standard analytical procedures: for pH, by electrometry in a soil-solvent suspension (1:2.5) (NF ISO 10390); organic matter content (OM) by pyrolyse at 550 °C during 16 h after predrying at 150 °C (NF EN 12879); carbonate content by the volume of lost gas during dissolution of carbonates by HCl (volumetric calcimetry, NF ISO 10693); Electrical conductivity (Conductivity), by electrometry in a soil-solvent suspension (1:5) (NF ISO 11265). The mineralization for trace elements chemical analysis of soils was conducted on 1g dry sample, after fluoro-nitro-perchloric acid attack following the standard procedure of NF-ISO 14869-1. All reagents used in the preparation of the samples for chemical analyses were of analytical grade. All the relevant material was cleaned prior to digestion with heated aqua regia and distilled water. The total concentrations of elements in all samples were measured by ICP-AES. The results agreed within + or – 5 % of the certified values.

2.3. Microbial study

1 g of soil was diluted in 9 ml of TS broth (1 g/l Tryptone, 8.5g NaCl, pH 7.0). Soil suspensions were shaken at 120 rpm for 5 min. Standard serial dilutions followed and 0.1 mL aliquots of dilution were spread on plates. Enumeration of soil microorganisms was performed using the spread plate counting method. Plate Count Agar (PCA) medium was explored for bacterial enumeration, while Actinomycete Isolation Agar (ACT) and Malt agar (pH 5.0) media were used for actinomycetes and fungal enumeration, respectively. Penicillin (25 mg/ml each) was added to Malt agar. The microbial populations were enumerated as colony-forming units (CFU) from a serial dilution of the soil suspensions. The colonies were counted after incubation at 30°C for 48h for bacteria and 5 d for fungi and actinomycetes. The Gram staining method according to Hans Christian Gram (1984).

2.5. Statistical analysis

Results are expressed as mean±SE. The mean values were compared using Mann-Whitney test. Differences were considered statically significant at $P \leq 0.05$.

Data were subjected to Pearson's correlation coefficients, calculated to relate the physicochemical parameters to the microbial parameters using the XLSTAT software.

3. Results

The physicochemical characteristics of the two types of soil samples namely control (SAM) and contaminated one (FEZ), as shown in Table 1, indicated no differences in the pH, carbonates and conductivity between the SAM and FEZ soils. However, significant differences (Mann-Whitney test at $P \leq 0.05$) were observed for Cr, Cu, Zn and organic matter contents, with higher values in FEZ soil. The total concentration of Cr and Ni on the two soil samples did not exceed the permissible limits (64 mg/kgCr, 50mg/kg Ni, 63 mg/kg Cu and 200 mg/kg Zn) established by the SQGE (Canadian soil quality guidelines for the environmental protection) in agricultural soil. These limits were exceeded in the cases of Cu (201.2 mg/kg) and Zn (291.2 mg/kg) for FEZ soil.

Table 1: Physicochemical characteristics and Mean total concentration of heavy metals (mg/kg) of FEZ and SAM soils.

Soil characteristics	Control soil (SAM)	Contaminated Soil (FEZ)
pH	8.4±0.3	8.5±0.2
CaCO3 (%)	35.9±1.5	34.3±4.4
Organic matter (%)	5.9±2.4	9.8±2.5
Conductivity (µS/cm)	69.7±16.3	57.9±10.83
Cr	52.3±4.6	63.4±1.7
Cu	16.2±1.4	201.2±38.5
Zn	44.9±1.6	291.2±47.2
Ni	44.9±10.7	33.2±1.7

The results of quantitative analysis of soil microbial populations are shown in Table 2. There was a large significant difference (Mann-Whitney test at $P \leq 0.05$) in all microbial properties between polluted and unpolluted sites. These counts showed a marked decrease in total number of cultivable actinomycetes and fungi microbial groups with an increase in total aerobic heterotrophic bacterial population for the contaminated soil samples. Gram staining of bacterial cells of different morphotypes showed that bacterial population of the contaminated site is dominated by gram negative strains.

Table 2: Abundance of the analyzed microorganisms in soil samples.

Microorganism	Control soil (SAM)	Contaminated Soil (FEZ)
Aerobic heterotrophic Bacteria(10^5 CFU/g)	1.73±0.25	4.03±0.15
Actinomycetes (CFU/g)	122.00±0.15	10.00±0.10
Fungi (10^4 CFU/g)	3.15±0.03	1.64±0.35

According to the correlation coefficient results (Table 3), a very high positive correlation with a significant probability ($P \leq 0.05$) was observed among bacteria/ Cr, bacteria/ Cu, bacteria/ Zn and bacteria /OM. A high negative correlation ($P \leq 0.01$) was noticed through actinomyctes/Cr, actinomyctes/Cu, actinomycetes/Zn, actinomycetes/OM, fungi/Cr, fungi/Cu, fungi/Zn and fungi/OM. A positive correlation was also recorded among Cr, Cu and Zn at $P < 0.05$.

4. Discussion

Our study permitted to show that the effects of irrigation by contaminated surface water on physicochemical characteristics of soils (pH, carbonates and salinity) are insignificant. The Cr, Cu and Zn metal and OM accumulation is the main change between the contaminated and control soils. Consequently, because both soil samples (contaminated and control) have almost similar properties concerning pH, salinity, it is reasonable to assume that any changes in the microbial properties of the contaminated soil sample can be attributed to the effects of Cr, Cu and Zn metal contamination and the presence of OM. The soil samples under study showed alkaline pH (8.5), which can contribute to reducing the harmful effects of heavy metal contamination by precipitation. Soil pH is often found to have the largest effect owing to its strong effects on solubility and speciation of metals both in soil as a whole and particularly in the soil solution [3].

Table 3: Matrice of correlation (Pearson (n)) between microbial populations and heavy metals in soil samples.

Variables	Aerobic heterotrophic bacteria	Actinomycetes	Fungi
Cr	0.9181 (0.0013)*	-0.8767 (0.0043)*	-0.8426 (0.0086)*
Cu	0.9663 (<0.0001)*	-0.9685 (<0.0001)*	-0.9611 (0.0001)*
Zn	0.9738 (<0.0001)*	-0.9724 (<0.0001)*	-0.9692 (<0.0001)*
Ni	-0.2649 (0.5260)	0.3561 (0.3866)	0.3726 (0.3633)
OM	0.7733 (0.0244)*	-0.7377 (0.0367)*	-0.7138 (0.0467)*

Each value represents r with the p-value in parenthesis. Correlations were carried out based on five subsamples for each site (n = 25). Significance was corrected for multiple comparisons with Pearson's correlation coefficients α=0.05. Significant correlations marked with '*'.

Results showed the positive effect of wastewater irrigation on the total bacterial population. This effect may be attibuted to the fact that irrigation waters loaded with heavy metals are also rich in organic matter that can be a source of nutrients for bacteria and therefore promote their proliferation. Metals can also facilitate secondary metabolism in microorganisms [15, 16]. Nevertheless, the stimulation of the soil bacterial abundance may have negative impacts on soil properties [4]. The bacterial growth stimulated by irrigation with wastewater led to the formation of biofilms, with the concomitant clogging of the pore spaces between particles, with implications in the soil hydraulic conductivity [17].

The effect of heavy metals on the number of culturable microorganisms remains unclarified, as findings differ between studies. We showed that among bacteria, gram negative bacteria appear to be more tolerant than gram-positive ones which is in accordance with previous statement [18, 19]. The predominance of gram-negative bacteria in contaminated site is probably due to their higher level of intrinsic metal resistance than majority of the gram-positive bacteria. The basis of this difference might be due to the differences in the chemical composition of cell wall of gram-negative and gram positive bacteria.

Our results also support the idea that soil microorganisms play an important role in monitoring the possible impact of heavy metal contamination. We demonstrated that changes in soil conditions due to heavy metals (Cr, Cu and Zn) contamination have a large negative effect on actinomycetes and fungi populations of soil. Soil actinomycetes are the filamentous gram positive bacteria representing several morphological types. This group of soil bacteria is considered to play very important role in maintaining soil properties although, they are poor competit or than other soil bacteria [20]. Soil fungi form three functional groups: decomposers, mutualists and pathogens. Fungi, along with bacteria, are important decomposers of hard to digest organic matter.

In this study, as evidenced by the results of the microbial counting, the number of cfu of fungi and actinomycetes was reduced in the contaminated site, indicating that the actinomycetes and the fungi groups had lower tolerance to heavy metals (Cr, Cu and Zn) than aerobic heterotrophic bacteria. However, it has often been stated that fungi are more tolerant to heavy metals than bacteria [21, 22, 23]. In fact, further studies have shown that the ability of microbes to tolerate a definite level of heavy metals under natural conditions might be different owing to the complex nature of the soil environment. Compared to the soils studied in the previously cited works that are usually very acidic, with low organic matter content, but our soils are alkaline and rich in organic matter.

Otherwise, the results of this study conducted under field conditions in an area that had been continuously irrigated by contaminated water courses with heavy metals at low concentrations differ from many other studies. Indeed, most studies of heavy metals have been carried out with soil samples incubated under laboratory conditions where a high concentration of heavy metals is added to soil on a single occasion or under field conditions where studies were carried out in highly contaminated areas [24-28].

Conclusion

Here we report the finding that heavy metals contamination in alkaline soils of the region of Fez, have a negative effect on actinomycetes and fungi soil populations. However, we also find the positive effect of this contamination on the total aerobicheterotrophic bacterial population. Our results differ from several other studies and emphasized that the effect of heavy metals on microbial population of the soil is dependent on several factors related to soil environment and soil physicochemical characteristics.

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