JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(8), 336–346 https://doi.org/10.12911/22998993/190349 ISSN 2299–8993, License CC-BY 4.0 Received: 2024.05.29 Accepted: 2024.06.25 Published: 2024.07.01

Transfer of Metals from the Soil to *Medicago sativa* Irrigated with Municipal Landfill Leachate

Lamiaa Belasri¹, Mourad Arabi^{2*}, Safaa Sabri³, Fouzia Hmimid^{1,4}, Samah Ait Benichou¹

- ¹ Health and Environment Laboratory, Department of Biology, Ain Chock Science Faculty, Hassan II University, Casablanca, Morocco
- ² Laboratory for Improvement of Agricultural Production, Biotechnology and Environment (LAPABE)/ Water, Environment and Health Team, Faculty of Science, Mohamed Premier University, PB 717 60000, BV M6, Oujda, Morocco
- ³ Chemistry and Toxicology Unit, Casablanca Regional Laboratory for Analysis and Research, National Office of Food Safety (ONSSA), Morocco
- ⁴ Vegetal Biotechnology, Ecology and Ecosystem Valuation Laboratory, Department of Biology, El Jadida Science Faculty, Chouaib Doukkali University, El Jadida, Morocco
- * Corresponding author's e-mail: m.arabi@ump.ac.ma

ABSTRACT

Morocco faces a growing waste issue due to population growth, economic expansion, and industrialization, leading to environmental concerns, especially regarding leachate. From 1986 to 2022, Casablanca's main landfill produced a total of 800,000 m³ of leachate, which was stored in evaporation ponds, posing significant environmental risks. Our research not only concentrates on traditional chemical analyses for leachate assessments but also emphasizes ecosystem interactions. Phytotoxicity tests assess the impact of contaminants, particularly heavy metals, complementing traditional chemical analyses. Our study investigated the accumulation of these contaminants in the soil and their subsequent transfer to plant tissues. This research aimed to examine the accumulation of heavy metals, including Pb, Cd, and Hg, in soils irrigated with varying leachate concentrations (C0: control; C1: 5%; C2: 7%; C3: 10%; and C4: 15%). The experiments involved the cultivation of *M. sativa* plants under open-field conditions. To assess the transfer of metals from soil to plant tissues, the transfer factor (TF) index was calculated. Our findings revealed that Pb, Cd, and Hg exhibited transfer factor ranges of 0.55–0.93, 0.07 to 0.21, and 0.1 to 0.37, respectively. The accumulation of heavy metals at the different leachate concentrations followed the order Pb > Hg > Cd.

Keywords: Medicago sativa, heavy metals, phytotoxicity test, transfer factors, leachate, landfill.

INTRODUCTION

The rapid advancement of industrial and commercial sectors worldwide has led to a significant surge in both municipal and industrial waste generation, posing substantial challenges for environmental sustainability and public health [Arabi et al., 2020]. In 2014, European Union countries collectively produced over 242 million tons of municipal solid waste (MSW), with a considerable portion of 62 million tons disposed of in landfills [Scarlat et al., 2019]. This escalating trend in waste generation has underscored the urgent need for effective waste management strategies to address the environmental, economic, and social implications associated with the growing waste volume, which is outpacing population growth rates [Renou et al., 2008]. Moreover, in many underdeveloped countries, the prevailing method of solid waste disposal involves traditional landfilling, wherein waste is buried in open areas without due consideration for environmental, topographical and geological factors [Reddy, 2016].

Like many other countries, Morocco is facing a mounting waste management challenge driven by factors such as population growth, economic expansion, and industrialization [Ouigmane et al., 2017]. In recent years, the annual waste production rate in Morocco has reached 5.3 million tons, with urban areas contributing significantly at a rate of 0.76 kg/capita/day, while rural areas account for 1.47 million tons per year at a rate of 0.28 kg/capita/day [SEDD, 2019]. Urban areas produce 5.38 million tons of MSW annually (0.76 kg per capita per day), while rural areas generate 1.47 million tons per year (0.28 kg per capita per day) [Arabi et al., 2024a]. Environmental concerns related to leachate generation from municipal solid waste, characterized by high organic matter content and moisture levels [Arabi et al., 2020], pose significant challenges for waste management in Morocco. Addressing these issues requires comprehensive strategies that prioritize environmental protection, public health, and sustainable resource management.

In Casablanca (Morocco), the Mediouna landfill was the principal disposal site for Casablanca from 1986 to 2022. Handling substantial daily municipal solid waste, often amounting to thousands of tons, this landfill has resulted in significant leachate production [Ghalloudi et al., 2015]. Leachate, which is characterized as a source of nutrients and water, has been utilized as a fertilizer in various contexts [Romero et al., 2013]. The leachate generated from municipal landfills, with its highly viscous and concentrated liquid composition and rich in dissolved organic matter and inorganic compounds such as Ca^{2+} , Mg^{2+} , K^+ , NH_4^+ , Fe, and SO_4^{-2-} , has proven beneficial for plant growth and development [Singh et al., 2017].

A study by Turki and Bouzid [2017] indicated positive growth responses in wheat plants subjected to leachate irrigation, outperforming those in plants irrigated with water alone. Nevertheless, it is crucial to acknowledge that elevated leachate concentrations may induce stress symptoms in plants, potentially attributed to the presence of toxic metals in the irrigation leachate [Singh et al., 2017].

To evaluate the transfer of metals from soil to plant tissues, the transfer factor (TF) index is calculated as the ratio of the metal concentration in plant tissue to that in the soil, with both values expressed in the same units. Higher TF values (≥ 1) suggest increased metal absorption from soil by the plant, rendering it more suitable for phytoremediation. Conversely, lower values indicate a poor response of plants to metal absorption, rendering them potentially safe for human consumption [Rangnekar et al., 2013].

M. sativa is recognized as the most cultivated forage crop globally [Radović et al., 2009] and plays a vital role in Moroccan agriculture. Cultivated on more than 100,000 hectares, it constitutes approximately 22% of the total acreage dedicated to fodder crops in the country [Bouizgaren et al., 2013]. Renowned for its high productivity, operational flexibility, and quality, alfalfa contributes significantly to 50% of the total fodder units produced. Moreover, rural populations rely on alfalfa as a resilient source of livestock nutrition. Despite its agricultural importance, alfalfa faces challenges, particularly its dependence on water availability, which is a significant limiting factor in arid and semiarid regions, especially in Morocco [Farissi et al., 2013]. The aim of this study was to investigate the levels of heavy metals in *M. sativa* plants and in soil irrigated with various concentrations of leachate, with a specific focus on the transfer of heavy metals from the soil to plants.

MATERIALS AND METHODS

Study area location

Casablanca's main landfill, located in Mediouna from 1986 to 2022, covers 78 hectares, including 60 hectares designated for waste disposal across 15 quarries. Serving as a crucial waste management hub for the region's growing waste volume, it handles approximately 5,000 tons of waste daily, which is transported via the busy main road (P.R.7) linking Casablanca to Marrakech (Figure 1) [Chaouki et al., 2017]. Over its operational years, this landfill generates more than 800,000 cubic meters of leachate, posing significant environmental concerns. With waste accumulation reaching 45 meters, there are risks of collapse and potential explosions due to waste decomposition, endangering worker safety [Smahi et al., 2013]. These challenges underscore the urgent need for sustainable waste management strategies for landfill operations in Casablanca.

Leachate sampling

In this study, leachate samples were collected directly from the intake tube of the untreated leachate pond at the Mediouna landfill site during the year 2022. The collection of leachate samples involved the use of presterilized 5 L capacity polyethylene bottles dedicated to physicochemical



Figure 1. Location of the Mediouna landfill [Chaouki et al., 2017] (modified)

parameter analysis. To ensure the integrity of the samples, the bottles underwent thorough cleaning with the respective leachate before they were filled with the brim. Subsequently, the caps were tightly secured to prevent any gas exchange with the surrounding atmosphere.

For the physicochemical analyses, the leachate was collected in presterilized polyethylene (PET) bottles with a capacity of 5 litters. Thus, to ensure the integrity of the samples, the bottles were thoroughly cleaned and then rinsed with the respective leachate before being filled on board. Subsequently, the plugs were securely fastened to prevent any gas exchange with the surrounding atmosphere. For the analysis of biological oxygen demand (BOD_c), leachate samples were taken in BOD specific bottles with a volume of 300 ml, while for the heavy metal analysis, these leachates were collected in prewashed PET containers of 100 ml and acidified with a few drops of 97.5% concentrated nitric acid on site to avoid precipitation of the metals.

All the samples were quickly transported to the laboratory in coolers at a temperature below 4 °C and then stored in a refrigerator prior to the start of laboratory analysis. The analysis of these leachate samples was conducted within a 48-hour timeframe.

Physicochemical analysis of leachate

The physicochemical parameters of all the leachate samples were analysed according to the methods of Rodier [Rodier, 2009; Rodier et al., 2016]. The parameters analysed included pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), nitrite (NO₂⁻), nitrate (NO₃⁻), ammonium

(NH₄⁺), and orthophosphate (PO₄^{3–}). The pH and electrical conductivity (EC) were measured in accordance with the NF ISO 10390 and NF ISO 11265 standards [Arabi et al., 2024b].

The BOD₅ was determined through a combination of dilution and manometric methods over a 5-day period [Rodier, 1986]. COD analysis was carried out using the potassium dichromate (K₂Cr₂O₇) method according to the HACH method as described by Xiao et al. [2022], where 0.2 mL of the sample and 1.8 mL of distilled water were added to a COD tube. The tube was sealed, agitated, and heated for 2 hours in a COD reactor. After cooling, the samples were homogenized, and the results were read using a DR 2010 reader. For the analysis of biological oxygen demand (BOD₅), a 40 mL leachate sample was taken in a BOD⁵ flask containing a magnetic bar and filled to 160 mL with distilled water. Lithium hydroxide gel was added to the flask capsule.

The flask was placed on a HACH BOD meter in an incubator at 20 °C [Zarei Mahmoudabadi et al., 2021]. After 5 days of incubation, a reading was taken, and the BOD₅ value was corrected with the dilution factor.

Spectrometric methods were employed for the analysis of nitrate (NO_{3}^{-}), ammonium (NH_{4}^{+}), and orthophosphate (PO_{4}^{3-}) to ensure precise results. The ammonium (NH_{4}^{+}) was analysed using the indophenol blue method, involving treatment with sodium hypochlorite and phenol solution, with readings taken at 630 nm using a UV-visible spectrophotometer [Scott et al., 1989]. Orthophosphates (PO_{4}^{3-}) reacted with ammonium molybdate and ascorbic acid to form a blue colouration, measurable at 807 nm, as described by Afkhami and Norooz-Asl [2009]. Nitrates (NO₃⁻) were analysed by colorimetry at 543 nm after reduction to nitrites using a cadmium column treated with copper [Mohale, 2011]. Nitrites (NO₂⁻) were analysed using the sulfanilamide method, where they react with sulfanilamide in an acidic medium to form a diazo compound that reacts with N-naphthylethylene diamine, resulting in a purple complex measurable at 543 nm [Panchagnula, 2018].

Plant material and growth conditions

This experimental study, undertaken at the Ain Chock Faculty of Science, Hassan II University in Casablanca, delved into open-field conditions, focusing on *M. sativa* L. as the plant material.

The choice of this plant as the focal point of this study aligns with its agricultural importance and adaptability [Jiang et al., 2006]. This versatile plant, belonging to the Fabaceae family, holds a prime position in semiarid regions and has a crucial influence on agricultural systems. The importance of M. sativa L. is underscored by its varied contributions, spanning the diversification of crop rotations, provision of highly nutritious feed for livestock, improvement of soil quality through biological nitrogen fixation, and support for soil and water conservation [Luo et al., 2020].

The soil at the experimental site was specifically selected for its established physicochemical attributes and its positive suitability for agricultural endeavors, as highlighted in a previous study [Ouansafi et al., 2019].

The experimental field was organized using a randomized plot design, where irrigation treatments were applied. Each treatment was replicated twice, resulting in a total of 10 plots dedicated to *M. sativa* plants (5 irrigation treatments \times 2 replicates).

The meticulous arrangement of the experimental plots, characterized by specific dimensions, facilitated a comprehensive study. The plot, subdivided into lanes measuring 1 meter in length and 2 meters in width, served as the testing ground. Within this framework, two lanes subjected to irrigation with potable water were exclusively allocated to the control group. The remaining eight lanes were designated for the application of distinct leachate concentration treatments, namely, C0: control; C1: 5%; C2: 7%; C3: 10%; and C4: 15%. These concentrations were selected based on a prior study covering a wide range from 1% to 100% [Torretta et al., 2016]. Within this range, 5% and 7% were identified as optimal for promoting plant growth, while 15% represented the maximum tolerable concentration. Higher concentrations adversely affected plant health, highlighting the importance of maintaining concentrations within the 5% to 15% range for effective irrigation management.

Sampling conditions and plant growth measurements

At the end of the experiment, *M. sativa* plants were collected at different concentrations, including those in the control group and those subjected to varying leachate concentration treatments (5%, 7%, 10%, and 15%). This sample method included the aerial parts of *M. sativa* plants, including stems, leaves, and any existing fruits. The collected samples were then analysed to determine the impact of varying irrigation treatments on plant growth and physiological reactions. This study meticulously measured both leaf area and plant height, treated them as essential morphological parameters, and employed MESURIM 2 software.

Heavy metal analysis

In accordance with the Association of Analytical Communities (AOAC) international guidelines, leachate and plant samples were mineralized with 1 mL of H_2O_2 and 9 mL of HNO₃ according to the methodology of 'Wastewater' and 'Dried Plant Tissue', respectively, in multiple waves: 150 °C/15 min, 200 °C/15 min. Heavy metals, including cadmium (Cd), lead (Pb), and mercury (Hg), were analysed using flame atomic absorption spectroscopy. Aluminum (Al) and sodium (Na⁺) were analysed by ICP-MS. Each sample was analysed in triplicate [CSG, 1995].

Determination of the transfer factor

The transfer factor was determined by dividing the concentration of heavy metals in vegetables by the concentration of heavy metals in the soil. The heavy metal transfer factor from soil in plants was established using the following equation [Olănescu et al., 2007]:

$$TF = \frac{Concentration (plants)}{Concentration (soil)}$$
(1)

where: *concentration (plant)* and *concentration (soil)* represent the concentrations of heavy metals in plants and soil (mg/kg), respectively.

Statistical analysis

Analysis of variance (ANOVA) for all the measured variables was performed by statistical evaluation with GraphPad Prism software, and the results are presented as the average standard error of the mean (SEM). When p < 0.05, the differences were deemed significant.

RESULTS AND DISCUSSION

Physicochemical analysis of leachate

The results of the physicochemical parameters of the leachate used for irrigation are presented in Table 1. The raw leachate has a hydrogen potential value of 8.2, which aligns perfectly with the requirements for this agricultural activity according to the Moroccan standards, as stated by [DRWP, 2007].

The chemical oxygen demand (COD) at a level of 18037 mg/L and the biochemical oxygen demand (BOD₅) at 2000 mg/L both exceeded the acceptable thresholds set by the Moroccan standards, indicating that chemical pollution was likely attributed to pesticide use in the zone where the leachate was situated (landfill of Mediouna).

The same observation is applicable to BOD_5 , which surpasses the required standards for water intended for irrigation, revealing the presence of discharged organic matter of domestic origin, such as fecal matter [Belghyti et al., 2009]. The

BOD₅/COD ratio, with a value of 0.11, serves as an indicator reflecting the biodegradability of organic matter and the maturation level of leachate. The observed ratio is notably low, falling below 0.4, indicating that the elements in the leachate are only partially biodegradable [Kastali et al., 2022]. The lead (Pb), mercury (Hg) and aluminum (Al) concentrations were 0.22, 0.022 and 21.045 ppm, respectively, for the raw leachate. When the lead concentration remained within the acceptable limit of Pb < 5 ppm, both the aluminum and mercury concentrations surpassed their respective reference limits of Al < 5 ppm and Hg < 0.001 ppm, as per the standards required by the DRWP [2007]. The presence of heavy metals can be attributed to the incorporation of chalcopyrite in batteries and ore processed at smelters, as well as the use of chemicals for photograph processing and Pb-based paints at landfills [Kanmani and Gandhimathi, 2013].

In contrast to the undetectable levels of cadmium (Cd) in the leachate (< 0.02), a markedly high concentration of sodium (Na) was detected.

Statistical tests were employed to assess the significance of differences observed among the dilutions. The results indicated that most parameters did not exhibit significant variations across the dilutions compared to those of the raw leachate, except for COD. Specifically, dilutions of 5%, 7%, 10%, and 15% resulted in highly significant differences in the COD levels compared to those of the raw leachate.

Parameters	100% raw leachate	5% Dilution	7% Dilution	10% Dilution	15% dilution
pН	8.20 ± 0.26	7.02 ± 0.10	7.00 ± 0.13	7.07 ± 0.10	7.10 ± 0.20
EC (mS/cm)	43.23 ± 2.29	10.54 ± 0.39	15.07 ± 0.21	20.30 ± 0.10	31.00 ± 0.10
DO (mg/L)	0 ± 0	0.78 ± 0.01	0.78 ± 0.02	0.81 ± 0.02	1.00 ± 0.03
NO ₂ -(mg/L)	0.14 ± 0.03	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.20 ± 0.01
NO ₃ -(mg/L)	95.28 ±12.21	7.40 ± 0.29	14.30 ± 0.26	29.00 ± 0.10	41.20 ± 0.90
NH ₄ ⁺ (mg/L)	2014.80 ± 93.61	25.32 ± 0.90	50.3 ± 1.30	80.00 ± 0.86	133.40 ± 0.97
PO ₄ ³⁻ (mg/L)	8.58 ± 4.25	0.40 ± 0.01	0.50 ± 0.01	0.60 ± 0.01	0.90 ± 0.01
COD (mg/L)	18037 ± 8925.20	120.00 ± 1.49	358.50 ± 2.09	580.00 ± 1.87	871.00 ± 13.09
BOD ₅ (mg/L)	2000 ± 311.60	23.00 ± 0.19	25.10 ± 0.20	40.70 ± 0.31	69.68 ± 0.33
BOD ₅ /COD ratio	0.11	0.07	0.07	0.07	0.08
Na⁺ (ppm)	365.00 ± 58.00	8.20 ± 0.01	15.01 ± 0.01	33.70 ± 0.05	41.00 ± 0.02
Pb (ppm)	0.22 ± 0.04	< 0.02	< 0.02	< 0.02	< 0.02
Cd (ppm)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Hg (ppm)	0.02 ± 0.01	< 0.02	< 0.02	< 0.02	< 0.02
AI (ppm)	21.04 ± 4.58	< 0.02	< 0.02	< 0.02	< 0.02

Table 1. Physicochemical characteristics of the leachate used for *M. sativa* irrigation (mean \pm standard deviation).

Note: *Below the detection limit (< 0.02)

Effect of leachate on plant height and leaf growth

Figure 2 shows the effects of different leachate concentrations on the leaf area and stem length of *M. sativa* plants. Indeed, the observed influence of leachate concentration on the morphological characteristics of the plant, particularly stem length and leaf area, aligns with the literature on the effects of metal pollutants on plant growth. Notably, the increase in stem length up to 7% leachate concentration (70.17 cm), followed by a decrease of 10% (60.67 cm) and a significant decrease of 15% (41.33 cm) suggested a complex response to the varying concentrations of pollutants.

Statistical analysis revealed that leaf surface area measurements across different leachate dilutions did not significantly differ from those of the control group (p > 0.05). Conversely, significant differences were observed in the stem length measurements between the control group and the 5%, 7%, and 10% leachate dilution groups, with p values < 0.01 indicating highly significant differences. There was also a significant difference in stem length between the 15% leachate treatment group and the control group, with a p value < 0.01.

According to Breckle [1991], studies have reported an increase in plant biomass in the presence of heavy metals such as Hg and Pb. However, these effects were observed in experiments utilizing low concentrations. This implies that the concentration of pollutants plays a crucial role in determining the nature of the plant's response. In our study, the progressive increase in stem length at leachate concentrations up to 7% may be indicative of an adaptive response or a stimulatory effect at lower leachate concentrations. In contrast, evidence of retarded shoot growth due to the presence of excess Pb in the root environment was found by Seyyedi et al. [1999]. This finding aligns with our observations of a decrease in stem length at a 10% leachate concentration, signifying the potential inhibitory effects of higher metal concentrations on plant development.

The fluctuations in leaf area, showing a positive response at leachate concentrations up to 7% and a subsequent decrease at 10% and 15%, are consistent with the findings of Bożym et al. [2021]. These authors suggested that the combination of heavy metals, especially at higher concentrations, may induce synergistic toxic effects, surpassing the plant's tolerance threshold. This could lead to physiological stress, inhibited cell elongation, and an overall compromise in growth. Our results at 15% leachate agree with this concept, indicating a significant reduction in stem length.

The cumulative toxic effects of heavy metals, including Pb, Hg, and Al, as hypothesized in the study of Bożym et al. [2021], likely contributed to the observed decrease in stem length.

Following the analysis of heavy metal toxicity and its consequences for plant development, notably in terms of Pb, Hg, and Al, it is necessary to investigate the effect of sodium concentration on plant growth.

Sodium is essential for maintaining turgor pressure within plant cells; however, excessive





levels can trigger water stress. Concentrations exceeding 50 mg/L can be toxic to sensitive plants, especially in recirculating irrigation systems. In this study, the initial sodium concentration in the raw leachate was high, but subsequent dilution decreased it. This dilution initially promoted plant growth; nevertheless, a subsequent increase in sodium concentration led to reduced growth. Fortunately, the sodium concentration in the diluted leachate used for irrigation remained below the limit recommended by Brouwer et al. [1985].

Concentrations of heavy metals in soils and plants

Figure 3 reveals varying concentrations of heavy metals, such as Pb, Cd and Hg, in *M. sativa* plants and soil irrigated with different concentrations of leachate (Figure 3).

Pb concentrations in plants and soil exhibit a progressive increase, which is correlated with higher leachate concentrations. Similarly, the Cd and Hg concentrations exhibited comparable trends in response to leachate amendments, while the soil concentrations of Cd and Hg moderately increased. The concentrations of cadmium (Cd) in plants ranged from 0.001 ppm to 0.08 ppm, whereas in soil, they varied between 0.013 ppm and 0.38 ppm. The recommended permissible limit for Cd in plants set by the WHO [2015] is 0.02 ppm. Additionally, the maximum allowable level of Cd in soil, according to MEF [2007], is 0.8 mg/kg. For lead (Pb), the concentrations in plants ranged from 0.19 ppm to 6.56 ppm, while in soil, they varied from 0.32 ppm to 7.05 ppm. The recommended permissible limit for Pb in plants by the WHO [2015] is 2 mg/kg, whereas the limit for lead in soil is 85 mg/kg MEF [2007]. The mercury concentrations in the plants ranged

from 0.001 ppm to 0.03 ppm, and in the soil, they varied between 0.006 ppm and 0.079 ppm. The Hg concentrations in both plants and soil remained below the limit for soils intended for agricultural activities, which is Hg < 0.5 ppm, as outlined by MEF [2007] and the WHO [2015].

The results of the statistical analysis revealed no significant differences in the Cd and Hg concentrations between the various leachate dilutions in both the plants and soils. However, the concentration of Pb showed varying levels of significance across the dilutions. For Pb, a marginally significant difference was observed at the 7% leachate dilution, indicating a potential but weak impact on Pb concentrations. A significant difference was noted at the 10% dilution, suggesting a more pronounced effect on Pb levels in both plants and soils. Notably, the 15% leachate dilution had a highly significant effect on the Pb concentration, indicating a substantial impact on the accumulation of lead in both plants and soils. These results therefore provide a good understanding of the complexity of heavy metal uptake and the need for proper management and regular surveillance of the environment in the case of any effects.

Transfer factors of heavy metals from soil to plants

The transfer factor (TF) of heavy metals from soil to vegetables provides important insights into the complex mechanisms of metal absorption and accumulation by plants. This pivotal parameter for assessing metal transfer dynamics serves as an indicator of heavy metal concentrations in vegetables relative to the soil [Rangnekar et al., 2013]. A higher TF implies a greater concentration of heavy metals in vegetables, indicating either poor soil retention or effective metal absorption by



Figure 3. Concentrations of heavy metals in *M. sativa* plants and soil. (a) Concentration of lead (Pb); (b) concentration of cadmium (Cd); (c) concentration of mercury (Hg) *: p < 0.05 (marginally significant); **: p < 0.01 (significant); ***p < 0.001 (highly significant)

plants. A lower TF, on the other hand, indicates that heavy metals are less concentrated in vegetables than in soil, indicating high metal sorption to soil colloids [Mirecki et al., 2015].

The analysis of the transfer factors (TFs) of Pb, Cd, and Hg from agricultural soil to *M. sa-tiva* plants under varying leachate treatments revealed insightful trends (Table 2). TF ratios >1 signify plant accumulation of elements, ratios near 1 suggest minimal influence, and ratios <1 indicate element exclusion by plants, offering crucial insights into the intricate soil–vegetable metal transfer dynamics [Olowoyo et al., 2010]. Despite the observed lead accumulation, the TF ratio for Pb was less than 1, indicating that *M. sativa* plants did not exhibit lead uptake, possibly due to plant-specific absorption and translocation mechanisms [Olowoyo et al., 2010].

The TF for Pb exhibited a consistent increase with increasing leachate concentration, reaching its peak at 15% with a value of 0.930, signifying significant lead uptake. In contrast, the TF for Cd fluctuated across treatments, ranging from 0.076 to 0.215, indicating varying cadmium accumulation levels. The TF for Hg displayed an increasing trend with leachate concentration, with the highest value of 0.379 at 15%, suggesting substantial mercury uptake. These findings emphasize the impact of leachate concentrations on metal transfer to M. sativa plants and highlight the plant's selective exclusion of lead despite the observed accumulation, shedding light on the complex mechanisms governing metal uptake in plant systems.

Statistical analyses were performed to assess the significance of the observed differences in transfer factors. The results suggest a uniform soil-to-plant transfer efficiency for these metals, as the transfer factors did not differ noticeably between leachate dilutions for Cd and Hg. However, the transfer factor showed a marginally significant difference in plants irrigated with 15% leachate dilution for Pb. These

 Table 2. Transfer factors (TFs) of Pb, Cd and Hg from agricultural soil to *M. sativa* plants

Treatments	Pb	Cd	Hg	
0% (Control)	0.593	0.076	0.100	
5%	0.554	0.083	0.166	
7%	0.691	0.215	0.142	
10%	0.885	0.207	0.196	
15%	0.930	0.210	0.379	

results may indicate a minor effect of leachate dilution on the transfer of lead from the soil to plants at the dilution level used.

The study of Rusu et al. (2005) aligns with results on lead transfer factors, which fall within a similar range of values (0.55–0.93), where a ratio less than 1 signifies lead exclusion by *M. sativa* plants. For cadmium (Cd) and mercury (Hg), the TF ranged from 0.07 to 0.21 and from 0.1 to 0.37, respectively, suggesting a lower transfer efficiency than that of lead. The results of Jafarian-Dehkordi and Alehashem [2013] support these findings, highlighting the high transfer factor for lead and notable concentrations of heavy metals in their samples.

Soil pH is a critical factor that significantly influences the transfer of metals within plant cells, as emphasized by Griffith et al. [2001]. High alkalinity, as observed in soils with a measured pH ranging between 7.49 and 7.89, plays a crucial role in stabilizing metals in the soil matrix, effectively reducing leaching effects [Zhang et al., 2018]. This mechanism leads to lower metal concentrations in the soil solution, thereby limiting the absorption of metals by plants and their translocation into crop tissues [Ouansafi et al., 2019].

The study of Griffith et al. [2001] further illustrated the impact of soil pH on metal accumulation, demonstrating heightened metal levels in vegetation thriving on alkaline, anthropogenic soil compared to natural soil conditions. Additionally, Kukier et al. [2004] highlighted the increase in nickel concentrations in Alyssum plant shoots with increasing soil pH, indicating a direct correlation between soil pH and metal uptake. Moreover, Harter [1983] underscores the intricate relationship between soil pH and the adsorption of lead, copper, zinc, and nickel in soils, with enhanced retention observed at pH levels above 7.0 to 7.5. Singh et al. [1995] contributed to this discussion by showing a decrease in cadmium concentration in plant species as soil pH increases. Furthermore, the combination of immobilizing agents such as lime, gypsum, and guano has been proven to be highly effective in reducing the phytoavailability of heavy metal(loid)s, as evidenced by Kim et al. [2019].

These collective findings emphasize the pivotal role of soil pH in mediating the transfer of metals within plant cells. Overall, the observed trend in the accumulation capacity of heavy metals (Pb > Hg > Cd) aligns with the broader understanding of how soil pH influences metal uptake and distribution in

plant systems. The interplay between soil pH and metal transfer dynamics underscores the complexity of plant–soil interactions and highlights the importance of considering soil pH as a key determinant in assessing metal bioavailability and plant uptake.

CONCLUSIONS

In this work, our investigation into the effects of leachate on plant height and leaf growth under irrigation at varying concentrations revealed noteworthy trends. Both leaf area and plant height demonstrated a positive response up to a 7% leachate concentration, followed by a subsequent decline at higher leachate concentrations (10% and 15%). This nuanced response underscores the significance of considering optimal leachate concentrations to foster plant growth while mitigating potential adverse effects. An essential finding is that despite the presence of certain elements (Cd, Pb, and Hg) in both the leachate and soil due to human activities, the accumulation of contaminants in M. sativa plants remains relatively low. This finding is particularly reassuring for the use of M. sativa as a feed crop in landfill surrounding soils. The transfer factor (TF) analysis further supported this conclusion, indicating a decreasing order of Pb > Hg > Cd. This implies that the transfer of these elements from soil to plant tissues follows a pattern that minimizes the potential risk of contamination in cultivated feed crops. In summary, while metal contamination in soil is a global concern, our study provides insights into the specific dynamics of leachate effects on plant growth and the subsequent accumulation of contaminants. These findings reassure the suitability of M. sativa as a feed crop, emphasizing the importance of careful consideration of leachate concentrations to optimize growth and mitigate potential risks. Nevertheless, there is still a need to improve leachate treatment, especially in landfills built in large urban areas, to protect surrounding plant-soil-water ecosystems.

Acknowledgements

The authors would like to thank the Regional Laboratory of Analysis and Research in Casablanca (LRARC) for their assistance in the analysis of metals. The authors are grateful to the editorial board of the 4th edition of scientific manifestation "the Oriental Days for Environment (JOE4)", through which this paper was examined and improved.

REFERENCES

- Afkhami, A., Norooz-Asl, R., 2009. Cloud point extraction for the spectrophotometric determination of phosphorus (V) in water samples. Journal of Hazardous Materials, 167(1–3), 752–755. https:// doi.org/10.1016/j.jhazmat.2009.01.039
- Arabi, M., Mechkirrou, L., El Malki, M., Alaoui, K., Chaieb, A., Maaroufi, F., Karmich, S., 2024a. Overview of Ecological Dynamics in Morocco– Biodiversity, Water Scarcity, Climate Change, Anthropogenic Pressures, and Energy Resources– Navigating Towards Ecosolutions and Sustainable Development. In E3S Web of Conferences - EDP Sciences, 527, 01001. https://doi.org/10.1051/ e3sconf/202452701001
- Arabi, M., Chaïeb, A., Khattach, D., Hritta, D., Gamgami, O., 2024b. Impact assessment of the effects of the former uncontrolled landfill of Sidi Yahya Oujda (Morocco) on groundwater using physicochemical, geological and electrical triple approaches. In E3S Web of Conferences - EDP Sciences, 527. 02018. https://doi.org/10.1051/e3sconf/202452702018
- Arabi, M., Sbaa, M., Vanclooster, M., Darmous, A., 2020. Impact of the municipal solid waste typology on leachate flow under semiarid climate – a case study. Journal of Ecological Engineering, 21(6), 94-101. https://doi. org/10.12911/22998993/123250
- Belghyti, D., El Guamri, Y., Ztit, G., Ouahidi, M., Joti, M., Harchrass, A., Amghar, H., Bouchouata, O., El Kharrim, K., Bounouira, H., 2009. Caractérisation physico-chimique des eaux usées d'abattoir en vue de la mise en œuvre d'un traitement adéquat: cas de Kénitra au Maroc. Afrique Science: Revue Internationale des Sciences et Technologie, 5(2). https://doi.org/10.4314/afsci.v5i2.61730
- Bouizgaren, A., Farissi, M., Ghoulam, C., Kallida, R., Faghire, M., Barakate, M., Al Feddy, M. N., 2013. Assessment of summer drought tolerance variability in Mediterranean alfalfa (*Medicago sativa* L.) cultivars under Moroccan fields conditions. Archives of Agronomy and Soil Science, 59(1), 147-160.
- Bożym, M., Król, A., Mizerna, K., 2021. Leachate and contact test with *Lepidium sativum* L. to assess the phytotoxicity of waste. International Journal of Environmental Science and Technology, 18, 1975– 1990. https://doi.org/10.1007/s13762-020-02980-x
- Breckle, C.W., 1991. Growth under Heavy Metals, in: Y. Waisel, A. Eshel and U. Kafkafi, Plant Roots: The Hidden Half, (Marcel Dekker, New York 1991).
- Brouwer, C., Goffeau, A., Heibloem, M., 1985. Introduction to irrigationa. Irrigation Water Management. Training manual (FAO), 1.
- 10. CSG, 1995. Collaborative Study Guidelines. J. AOAC Int. 78(5), 143A–161A.

- Chaouki, Z., El Mrabet, I., Khalil, F., Ijjaali, M., Rafqah, S., Anouar, S., Nawdali, M., Valdes,
- H., Zaitan, H., 2017. Use of coagulation-flocculation process for the treatment of the landfill leachates of Casablanca city (Morocco). Journal of Materials and Environmental Sciences, 8(8), 2781–2791.
- DRWP, 2007. Dirctorate of Research and Water Planning, Water Quality Standards for Water Irrigation (State Secretariat at the Ministry of Energy, Mines, Water and Environment, Rabat, Morocco).
- 14. Farissi, M., Bouizgaren, A., Faghire, M., Bargaz, A., Ghoulam, C., 2013. Agrophysiological and biochemical properties associated with adaptation of *Medicago sativa* populations to water deficit. Turkish Journal of Botany, 37(6), 1166–1175. https://doi. org/10.3906/bot-1211-16
- 15. Ghalloudi, J., Zahour, G., Talbi, M., 2015. Évaluation de la première expérience de gestion déléguée des déchets ménagers à Casablanca, Maroc. European Scientific Journal, 11(2), 237–263.
- 16. Griffith, M., Super, K., Lynch, W.C., Fishman, B.E., 2001. Accumulation of metals in vegetation from an alkaline artificial soil. Journal of Environmental Science and Health, Part A, 36, 49–61. https://doi. org/10.1081/ESE-100000471
- Harter, R.D., 1983. Effect of soil pH on adsorption of lead, copper, zinc, and nickel. Soil Science Society of America Journal, 47, 47–51. https://doi.org/10.2136/ SSSAJ1983.03615995004700010009X
- Jafarian-Dehkordi, A., Alehashem, M., 2013. Heavy metal contamination of vegetables in Isfahan, Iran. Research in pharmaceutical sciences, 8(1), 51.
- Jiang, H.M., Jiang, J.P., Jia, Y., Li, F.M., Xu, J.Z., 2006. Soil carbon pool and effects of soil fertility in seeded alfalfa fields on the semiarid Loess Plateau in China. Soil Biology and Biochemistry, 38(8), 2350– 2358. https://doi.org/10.1016/j.soilbio.2006.02.008
- 20. Kanmani, S., Gandhimathi, R., 2013. Assessment of heavy metal contamination in soil due to leachate migration from an open dumping site. Applied water science, 3, 193–205. https://doi.org/10.1007/ s13201-012-0072-z
- 21. Kastali, M., Mouhir, L., Madinzi, A., Taleb, A., Anouzla, A., Souabi, S., 2022. Reducing Pollution of Stabilized Landfill Leachate by Mixing of Coagulants and Flocculants: A Comparative Study. IntechOpen. http://dx.doi.org/10.5772/ intechopen.97253
- 22. Kukier, U., Peters, C., Chaney, R.L., Angle, J.S., Roseberg, R.J., 2004. The effect of pH on metal accumulation in two Alyssum species. Journal of environmental quality, 33(6), 2090–102. https://doi. org/10.2134/JEQ2004.2090
- Kim, H., Lee, M., Kim, H.S., Kim, K., 2019. Exploration of heavy metal(loid)s immobilizing agents

available for agricultural lands and their combination effects. Korean Journal of Soil Science and Fertilizer. https://doi.org/10.7745/kjssf.2019.52.3.297

- 24. Luo, Y.Z., Li, G., Yan, G., Liu, H., Turner, N.C., 2020. Morphological features and biomass partitioning of lucerne plants (*Medicago sativa* L.) subjected to water stress. Agronomy, 10(3), 322. https:// doi.org/10.3390/agronomy10030322
- 25. MEF, 2007. Ministry of the Environment Finland : Government decree on the assessment of soil contamination and remediation needs, Finland.
- Mirecki, N., Agic, R., Sunic, L., Milenkovic, L., Ilic, Z.S., 2015. Transfer factor as indicator of heavy metals content in plants. Fresenius Environmental Bulletin, 24(11c), 4212–4219.
- Mohale, L.R., 2011. Impact of cyanobacterial toxins on water quality and supply. Doctoral dissertation, University of the Free State Bloemfontein.
- 28. Olănescu, G., Gament, E., Dumitru, M., 2007. Fitoextracția solurilor poluate Cu metafile grele. Lucrări Ștințifice Facultatea de Agricultură București, seria A, I, 359.
- Olowoyo, J.O., Van Heerden, E., Fischer, J.L., Baker, C., 2010. Trace metals in soil and leaves of Jacaranda mimosifolia in Tshwane area, South Africa. Atmospheric Environment, 44(14), 1826–1830.
- 30. Ouansafi, S., Abdelilah, F., Kabine, M., Maaghloud, H., Bellali, F., Bouqdaoui, K.E., 2019. The effects of soil proprieties on the yield and the growth of tomato plants and fruits irrigated by treated wastewater. AIMS Agriculture & Food, 4(4), 921–938. https://doi.org/10.3934/agrfood.2019.4.921
- 31. Ouigmane, A., Boudouch, O., Hasib, A., Berkani, M., Aadraoui, M., Dhairi, E., 2017. The size effect in the distribution of combustible components in the municipal solid waste produced in the summer time. Case of the City of Beni Mellal-Morocco. Journal of Materials and Environmental Science, 8(8), 2729–2737.
- Panchagnula, S., 2018. Spectrophotometric analysis of water for nitrate and nitrite nitrogen. International journal of research and analytical reviews, 5, 226–230.
- 33. Radović, J., Sokolović, D., Marković, J.J.B.A.H., 2009. Alfalfa-most important perennial forage legume in animal husbandry. Biotechnology in Animal Husbandry, 25(5–6–1), 465–475. https://doi. org/10.2298/bah0906465r
- 34. Rangnekar, S.S., Sahu, S.K., Pandit, G.G., Gaikwad, V.B., 2013. Study of uptake of Pb and Cd by three nutritionally important Indian vegetables grown in artificially contaminated soils of Mumbai, India. International Research Journal of Environmental Sciences, 2(1), 1–5.
- Reddy, P.J., 2016. Energy recovery from municipal solid waste by thermal conversion technologies, 474.

Boca Raton: CRC press. https://doi.org/10.1201/ b21307

- 36. Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., 2008. Landfill leachate treatment: Review and opportunity. Journal of Hazardous Materials, 150(3), 468–493. https://doi.org/10.1016/j. jhazmat.2007.09.077
- Rodier, J., 2009. L'analyse de l'eau (9^{ème} Edition. Dunod, Paris, 2009).
- 38. Rodier, J., Legube, B., Merlet, N., 2016. L'analyse de l'eau-10e éd. Dunod.
- 39. Rodier, J., 1986. Water analysis: natural waters, waste water and sea water. 7th edition, Dunod, Paris (1986).
- 40. Romero, C., Ramos, P., Costa, C., Márquez, M.C., 2013. Raw and digested municipal waste compost leachate as potential fertilizer: comparison with a commercial fertilizer. Journal of Cleaner Production, 59, 73–78. https://doi.org/10.1016/j. jclepro.2013.06.044
- 41. Rusu, M.C., Mărghitaș, M., Mihăiescu, T., 2005. Tratat de agrochimie. CERES.
- 42. Scarlat, N., Fahl, F., Dallemand, J.F., 2019. Status and opportunities for energy recovery from municipal solid waste in Europe. Waste. Biomass and Valorization, 10(2019), 2425–2444. https://doi. org/10.1007/s12649–018–0297–7
- 43. Scott, T.J., Mitchell, M.J., Santos, A., Destaffen, P., 1989. Comparison of two methods for measuring ammonium in solution samples. Communications in soil science and plant analysis, 20(11–12), 1131– 1144. https://doi.org/10.1080/00103629009368141
- 44. SEDD, 2019. Stratégie Nationale de Réduction et de Valorization des Déchets. Report (In French). Environment Ministry, Morocco.
- 45. Seyyedi, M., Timko, M.P., Sundqvist, C., 1999. Protochlorophyllide, NADPH-protochlorophyllide oxidoreductase, and chlorophyll formation in the lip1 mutant of pea. Physiologia Plantarum, 106(3), 344–354. https://doi. org/10.1034/j.1399-3054.1999.106313.x
- 46. Singh, B.R., Narwal, R.P., Jeng, A.S., Almås, Å.R., 1995. Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. Communications in Soil Science

and Plant Analysis, 26, 2123–2142. https://doi. org/10.1080/00103629509369434

- 47. Singh, S., Raju, N.J., RamaKrishna, C., 2017. Assessment of the effect of landfill leachate irrigation of different doses on wheat plant growth and harvest index: A laboratory simulation study. Environmental Nanotechnology, Monitoring & Management, 8, 150–156. https://doi.org/10.1016/j.enmm.2017.07.005
- 48. Smahi, D., Fekri, A., El Hammoumi, O., 2013. Environmental impact of Casablanca landfill on groundwater quality, Morocco. Journal of Geosciences, 4, 202–211. https://doi.org/10.4236/ ijg.2013.41017
- 49. Torretta, V., Ferronato, N., Katsoyiannis, I.A., Tolkou, A.K., Airoldi, M., 2016. Novel and conventional technologies for landfill leachates treatment: A review. Sustainability, 9(1), 9. https://doi. org/10.3390/su9010009
- 50. Turki, N., Bouzid, J., 2017. Effects of landfill leachate application on crops growth and properties of a Mediterranean sandy soil. Journal of Pollution Effects & Control, 5(186), 1000186. https://doi. org/10.4176/2375-4397.1000186
- WHO, 2015. World Health Organization : Guidelines for Drinking Water Quality; Final Task Group Meeting, (Geneva, Switzerland, 2015).
- 52. Xiao, H., Yan, W., Zhao, Z., Tang, Y., Li, Y., Yang, Q., Luo, S., Jiang, B., 2022. Chlorate induced false reduction in chemical oxygen demand (COD) based on standard dichromate method: Countermeasure and mechanism. Water research, 221, 118732. https://doi.org/10.1016/j.watres.2022.118732
- 53. Mahmoudabadi, T.Z., Ehrampoush, M.H., Talebi, P., Fouladi-Fard, R., Eslami, H., 2021. Comparison of poly ferric chloride and poly titanium tetrachloride in coagulation and flocculation process for paper and cardboard wastewater treatment. Environmental Science and Pollution Research, 28, 27262– 27272. https://doi.org/0.1007/s11356-021-12675-6
- 54. Zhang, H., Ma, G., Sun, L., Li, H., 2018. Effect of alkaline material on phytotoxicity and bioavailability of Cu, Cd, Pb and Zn in stabilized sewage sludge. Environmental Technology, 39, 2168–2177. https:// doi.org/10.1080/09593330.2017.1351496