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Using statistical analyses as a key for accurate metal phytoremediation assessment: a case study

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ABSTRACT: Phytoremediation projects are frequently assessed upon the measured metal concentrations basis with regard to standards. Since these standards. Therefore, any to these references .Whereas data generated from samples must necessarily be statistically interpretated with regard to standards like the permissible limits. This study aimed to assess statistical significance of measured metal concentrations in four urbain plants analysis contribution to estimating metal phytoremediation capabilities from seewage of an invasive and characteristic flora in Daloa, Côte d'Ivoire. To do that, a tropical climatic dry season sampling of wastewater, sediments, and four plants (Oenothera biennis L., Eichhornia crassipes (Mart) Solms, Alternanthera philoxeroides (Mart.) Griseb, and Phalaris arundinacea L.) was conducted from december 2017 to february 2018. Samples were microwave assisted acid digested according to Huang and Schulte (1985) for multi-element analysis by ICP-MS. Data normality was Shapiro-Wilk's checked for means comparison under t-student test. That test was used to check the significance of the measured plant metal contents with regard to the permissible limits of FAO/WHO (1996). Results obtained revealed that P. arundinacea, E. crassipes and A. philoxeroides globally bioaccumulated Cd, As, Cu, Cr, Zn, Hg, Ni, Pb and Mn ; with a restricted bioaccumulation activity toward As, Hg, Pb and Zn for O. biennis from the study metals. These metal concentrations were found to be below the permissible limits in absolute values. Hyperaccumulation was observed only in E. crassipes for Hg and Zn ; A. philoxeroides for Hg ; and P. arundinacea for As and Hg. However, t-student tests showed that the overwhelming measured plant metal contents were above the permissible limits. For instance, the O. biennis measured As content was 0.060 mg/Kg. That absolute value of As concentration is lower than 0.43 mg/Kg, the FAO/WHO (1996) permissible limit for that metal. A t-student test of means comparison displayed a p-value = 0.0058 (0.58%). It means that the null hypothesis (µ0) pointing out that O. biennis As content lower than the permissible limit must be rejected at 0.58% risk of error. This finding outlines an eventual misappreciation of those plant heavy metals phytoremediation capabilities wether the measured concentrations were only considerated. Therefore, that invasive macroflora can be used as a biotechnological tool for wastewater treatment in Daloa, and everywhere else where it can be found around the drainage systems.

Keywords: Statistical analyses, phytoremediation, metal pollution, wastewater, biotechnology, Daloa

INTRODUCTION

Organic and metal pollution phytoremediation is an eco-friend and accessible biotechnology for developing countries (Chen et al. 2019). Even though this is passively implemented, technology remains the main lever for urban pollution management in Africa (Odoh et al., 2019). That ongoing challenge to these countries leads to frequent metal pollution characterizations (Akpetou et al., 2020). Thus, metal pollution effects on human health are routinely studied (Kalonda et al. 2017; Yapi et al. 2019; Rai et al. 2019); unlike the incidence in the ecosystems. That could result from certain phytoremediation expansions in recent years regardless of utilization challenges. Indeed, the shores of wastewater drainage systems are increasingly colonized by some spontaneous and specific flora to these environments. These plants' bloom highlights a metal-driven environmental selection (Chen et al., 2019). Thus, such flora could constitute some biotechnological models and a critical organic and metal pollution factor in human or animal use. That is the context in which four macroplants (*Oenothera biennis L., Eichhornia crassipes (Mart.) Solms, Alternanthera philoxeroides (Mart.) Griseb, and Phalaris arundinacea L.)* metal phytoremediation capability was monitored in situ along with the drainage systems during a dry climatic season in Daloa. This flora pattern was also observed elsewhere in cities (Kowarik and Lippe, 2018). The study plants in Daloa have no proven human food value.

However, periodic clearances are used for livestock feed, rice, and other crop cultivation in the wetlands. Therefore, the plant's lifetime assimilated chemicals are inevitably released into the environment or potentially transmitted to foodwebs (McGrath and Zhao, 2015). As a result, urban wastewaters in Daloa are generally metal-polluted (N'zakilizou, 2016). This work aimed to assess the level of essential and non-essential metals in spontaneous and characteristic flora boarding the sewage drainage systems in Daloa as proof of metals removal capability from wastewaters. Statistical analyses were run on the measured plant metal contents to accurately assess the significance of the permissible limits of FAO/WHO (1996). This work highlights the pivotal role of statistical analyses in scientific environmental pollution characterizations. Decision-making toward ecosystems and urban mediums segmentation could be facilitated, and the urgency of mechanisms development for the plants accumulated heavy metals elimination or recovery.

METHODOLOGY

Background

The city of Daloa is located in west of Côte d'Ivoire at 6.8877N and 6.4485W. The average rainfall range between 1100-1250 mm/year (Prici, 2016). Rainfall deficits lead to regular droughts favourable to the weak variation in wastewater flows. These unconventional discharges into streets, drainage systems and other faulty or non-existent equipment reach about 90% of the daily produced wastewater volumes in the cities of Côte d'Ivoire (Kouamé et al., 2014). These effluents result from a transitional economy between agriculture and industry (Chauhan et al., 2015; N'zakilizou, 2016, Prici, 2016). Household, agricultural, industrial, artisanal and commercial activities coexist to shape the physicochemical composition, production regime, and discharge of solid and liquid wastes with no prior treatment (Huang et al., 2017). Wastewater drainage systems are severely affected by mainly microbiological and metallic pollutants in Daloa (Sarker et al., 2015; N'zakilizou, 2016; Abo and Bakayoko, 2018). At the same time, several techniques, such as phytoremediation, can be implemented for these effluents' ecological treatment (Kumar and Saravanan, 2017). This is the context in which this work was done to raise interest for statistical analyses during the implementation of such technologies for urban pollution management projects in Daloa and other cities.

Biological material analysis collected at the study stations

Four invasive plants (*O. biennis, E. crassipes, A. philoxeroides and P. arundinacea*), inferred to the urban drainage systems in Daloa were monthly sampled from December 2017 to march 2018. The sewage drainage system crosses the city of Daloa from North to South, from the Gbokora to Abattoir towns. *O. biennis*, a native North American plant (Rostański, 2010), is ubiquitous in wastewater drainage systems in Daloa. *E. crassipes* spongy petioles lead it to be unsinkable. The polluted wastewaters remain an optimal growth substrate that allows a weekly doubling *E. crassipes* population (Su et al. 2018). *E. crassipes* is mainly found in the wetlands of Kennedy town in Daloa. The small and robust *A. philoxeroides* stems are hollowed out to allow good buoyancy once the water (Julien et al. 1992). Native to southern Brazil, this plant has shown peak growth in summer in various phytogeographic regions (Wang et al., 2018; Chatterjee and Dewanji, 2012). Concerning *P. arundinacea*, this uncommonly studied plant mainly settles on the banks of rivers and streams, where water slightly flows, more or less deeper, with varied physicochemical characteristics (Wei et al. 2008). The favorable attributes of our study stations (Gbokora, Kennedy, Commerce and Abattoir) to the four plants' growth are confined in Table 1.

Table 1 : Characteristics and location of the study stations for this work.

Study station	Geographic position	Urbanization (rural/anthropic)
Lake of Gbokora	6°54'30 N 6°27'27 W	Peri-urban rural area (under anthropic threat)
Wetland of Kennedy town	6°53'28 N 6°27'54 W	Industrial area (anthropic)
Wetland of Commerce town	6°52'48 N 6°26'45 W	Urban area (anthropic)
Wetland of Abattoir town	6°52'38 N 6°25'55 W	Urban area (anthropic)

Plants chemical analysis involved roots, stems and leaves, which were dried, crushed, and sieved for fine powder collection for heavy metal contents determination; namely As, Cd, Cr, Cu, Hg, Mn, Ni, Pb et Zn. Two (02) mg of each respective fraction were blended into a 6 mg sub-sample for microwave mineralization, according to Huang and Schulte (1985). These plant masses were microwave digested at 60-80°C for 120 minutes with 2.0 mL HCl, 7.0 mL HNO3 and 0.5 mL concentrated hydrogen peroxide. The final volume of the digestates were finally taken to 50 mL by dilution with demineralized water. ICP-MSICP-MS determined these sub-samples metals contents determined these sub-samples metals contents after settling overnight. Data generated on the plant contents in As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn were statistically analyzed using ADE4 and R (Thioulouse et al., 1997; Chesneau, 2018). The aim was to assert that these metal concentrations were higher than the permissible values of FAO/WHO (1996). The relevant plant exhibited a proven phytoremediation activity for heavy metals (Tangahu et al., 2011). And the returned p-value represented the risk of misappreciation of the plant phytoremediation activity.

To do that, data normality was tested under a t-student test at a 95% confidence level. A null hypothesis (Ho) and the complementary (H1) were issued as follows:
Ho : $\mu \leq \mu 0$
H1 : $\mu > \mu 0$
Ho (plant metal content below the permissible limit of FAO/WHO (1996)), H1 (plant metal content higher than the permissible limit of FAO/WHO (1996)), μ (the measured plant metal content in this work), μ0 (permissible metal limit of FAO/WHO (1996)). Shapiro-Wilk's test was conducted on data to determine p-values at a 5% confidence level. A plant-proven phytoremediation activity was assigned when measured metal content μ was higher than the permissible limit μ0 at 5% threshold. The p-value, therefore, expressed misappreciation risk (Ho rejection) in the following ranges (Chesneau, 2018):

- □ Significant Ho rejection if p-value □]0.01 ; 0.05], indicated by *,
- □ Very significant Ho rejection if p-value □]0.001 ; 0.01], indicated by **,
- □ Highly significant Ho rejection if p-value < 0.001, indicated by ***,
- □ No Ho rejection if p-value > 0.05.

RESULTS

Correlation between wastewater, sediment and plant-heavy metal contents
Correlation between wastewater, sediment and plant-heavy metal contents was run toward two factorial plans F1 and F2. Those factorial plans expressed roughly 77,4% of the information modeling the metal pollution-phytoremédiation process (Figures 1 and 2).

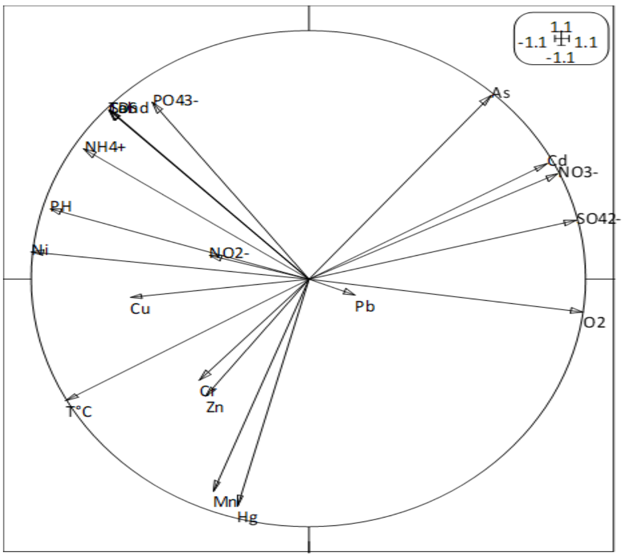

Fig 1 : Factorial design of physicochemical parameters and wastewater at the study stations.

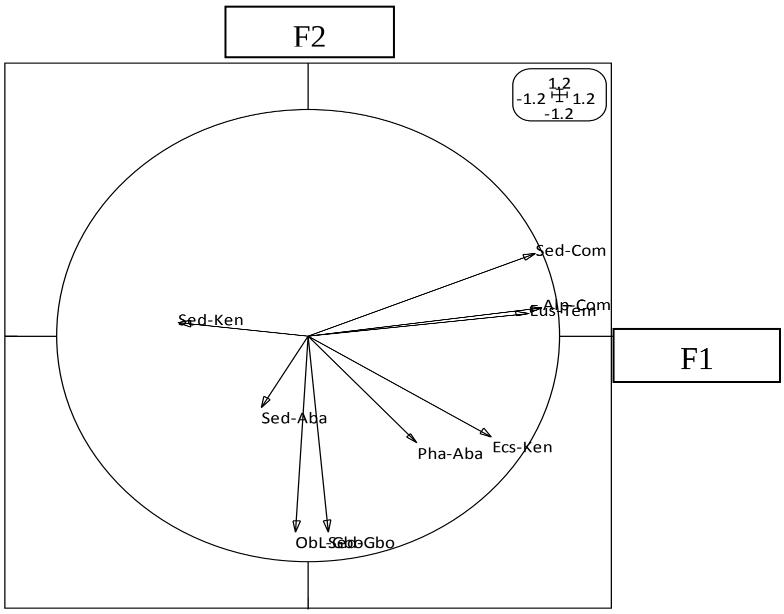

Fig 2 : Factorial design of sediments, and plants at the study stations.

Both Figures 1 and 2 read together to show a clear correlation between plants, sediments and Physico-chemical parameters. Specifically, nitrogen (NH4+, NO2-) and phosphates (PO43-) were actively utilized in plants for growth (negative correlations). As for heavy metals, Cd, As and Pb were negatively correlated to the physical and chemical parameters, except for nitrates (NO_3-) and sulfates (SO_4^{2-}). Therefore, the plant potential phytoremediation activities had to be assessed considering these parameter variations.

Plant study metal contents
O. enotherabiennis L. heavy metals phytoremediation activity
O. biennis exhibited bioaccumulation activity for As, Hg, Pb and Zn. The average metal contents in milligrams were measured to be 0.06 ; 0.011 ; 1.16 et 0.2, respectively. The returned p-values for means comparison to FAO/WHO (1996) permissible limits

were higher than 5% confidence threshold, ranking nearly 100% (1). Only As exhibited a p-value below 5 % (0,0058) as displayed in Table 2.

Table 2: *O. biennis* average heavy metal contents comparison with the FAO/WHO (1996) permissible limits.

Plant	***O. biennis***								
Heavy metal	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Average heavy metal contents	0.060	0	0	0	0.011	0	0	1.16	0,2
Standard deviation	0.06	0	0	0	0.004	0	0	0.048	0.033
Interval comprising measurements	]0 ; 0.12[	0	0	0	]0.007 ; 0.015[	0	0	]1.11 ; 1.21[	]1.17 ; 2.23[
FAO/OMS (1996) permissible limits	0.43	0.02	1.3	10	0.03	10	10	2	0.6
p-value (Shapiro-Wilk's 5%)	0.0058**	1	1	1	0.9987	1	1	0.4619	0.683
p-value (t-test 95%)	0.8413	-	-	-	0.1152	-	-	0.4619	0.999

2.2.2. Eichhornia crassipes (Mart.) Solms heavy metals phytoremediation activity

E. crassipes bioaccumulated every study heavy metal in more or less elevated proportions (Table 3). Average measured concentrations have distributed on both sides of FAO/WHO (1996) standards. Thus, only Hg (***) and Zn (**) displayed p-values lower than 5% (0,0012). As for the other metals (As, Cr, Cu, Mn, Ni and Pb), p-values were higher than 5% (≈ 1).

Table 3 : *E. crassipes* average heavy metal contents comparison with the FAO/WHO (1996) permissible limits.

Plant	*E. Crassipes*								
Heavy metal	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Average heavy metal contents	0.133	0.0025	0.0025	0.505	0.778	1.16	1.304	0.148	0.845
Standard deviation	0.024	0.0005	0.0005	0.056	0.058	0.06	0.063	0.021	0.071
Interval comprising measurements	]1.089 ; 1.561[	]0.002 ; 0.003[	]0.002 ; 0.003[	]0.449 ; 0.561[	]0.719 ; 0.835[	]1.124 ; 1.196[	]1.24 ; 1.367[	]0.127 ; 0.168[	]0.774 ; 0.916[
FAO/WHO (1996) permissible limits	0.43	0.02	1.3	10	0.03	10	10	2	0.6
p-value (t-test 95%)	0.22	0.0012	0.0012	0.759	0.722	0.405	0.687	0.572	0.842
p-value (Shapiro-Wilk's 5%)	0.999	0.997	1	1	0.00063***	1	1	0.991	0.003**

Alternanthera philoxeroides (Mart.) Griseb heavy metals phytoremediation activity

As and Hg concentrations were measured to be higher than the permissible limits of FAO/WHO (1996), unlikely to Cd, Cr, Cu, Mn, Ni, Pb, and Zn in E. philoxeroides. On the other hand, this group's average metal contents comparison generated p-values of about 1, unlikely to As and Hg with p-values below 0.05 (Table 4).

Table 4 : Comparison of *A. philoxeroides* average heavy metal contents with the FAO/WHO (1996) permissible limits.

Plant	*A. philoxeroides*								
Heavy metal	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Average heavy metal contents	0.0612	0.01	0.0025	0.0025	0.205	0.605	3.203	0.068	0.078
Standard deviation	0.078	0.004	0.0005	0.0005	0.042	0.058	0.319	0.021	0.005
Interval comprising measurements	]0.534 ; 0.69[	]0.006 ; 0.014[	]0.002 ; 0.003[	]0.002 ; 0.003[	]0.162 ; 0.247[	]0.547 ; 0,.663[	]2.883 ; 3.521[	]0.047 ; 0.088[	]0.547 ; 0.663[
FAO/WHO (1996) permissible limits	0.43	0.02	1.3	10	0.03	10	10	2	0.6
p-value (t-test 95%)	0.908	0.683	0.001	0.001	0.899	0.908	0.8702	0.572	0.001
p-value (Shapiro-Wilk's 5%)	0.009**	0.992	1	1	0.0018**	1	1	1	1

Phalaris arundinacea L. heavy metals phytoremediation activity

P. arundinacea average metal contents were lower in absolute values than the FAO/WHO (1996) standards, except As. And the respective p-values were nearly about 1. Only As showed a p-value (0,0007) lower than 5% (table 5).

Table 5: *P. arundinacea* average heavy metal contents comparison with the FAO/WHO (1996) permissible limits.

Plant	*P. arundinacea*								
Heavy metal	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Average heavy metal contents	0.653	0.011	0.085	0.005	0.031	0.58	1.738	0.998	0.575
Standard deviation	0.038	0.004	0.029	0.0039	0.005	0.207	0.078	0.148	0.126
Interval comprising measurements	]0.614 ; 0.691[	]0.007 ; 0.015[	]0.056 ;0.114[	]0.0011 ; 0.0089[	]0.026 ; 0.036[	]0.373 ; 0.787[	]1.659 ; 1.815[	]0.848 ; 1.146[	]0.449 ; 0.7[
FAO/WHO (1996) permissible limits	0.43	0.02	1.3	10	0.03	10	10	2	0.6
p-value (t-test 95%)	0.712	0.845	0.962	0.024	0.85	0.622	0.339	0.377	0.406
p-value (Shapiro-Wilk's 5%)	0.0007 ***	0.99	1	1	0.394	1	1	0.999	0.641

DISCUSSION

A positive correlation between the (Hg, Mn) couple and sediments at the Gbokora and Abattoir stations exhibited metal-contaminated water discharges. The respective upstream and downstream positions of these stations confirm wastewater drainage from the artisanal minings in the catchment of the tributary wetlands to rivers and anthropogenic activities, particularly tanneries in the city of Daloa (Garcia-Salgado et al., 2012; Chauhan et al., 2015; Abo and Bakayoko, 2018). However, organics were weakly mineralized due to volumes, especially at Commerce station, where intensive putrefactions occurred, unlikely at the Kennedy station (negative correlation). At that station nutrients, such as NH4+ et PO43-, were mainly produced from organic mineralization and set to bioavailability to these invasive plant's growth along with the wastewater drainage systems in Daloa (Chen et al., 2019). On the other hand, Cu, Cr, Zn, Hg, and Mn showed adverse correlation with NO3, SO42-, O2, Cd, and As bioavailability to plants. This highlights the competition of As, Cd with essential elements such as Cu, Cr, Zn, and Mn as shown in plankton by Xu and Morel (2013). Thus, Hg, As and Cd were shown to drive a shift in essential elements uptake in the study plants. O. biennis growth displayed a significant positive correlation with heavy metal concentrations in the decreasing order of Mn, Hg, Zn, Cr, and Cu. Specifically, O. biennis bioaccumulated As, Pb, and Zn only, contrary to Remon et al. (2009), who did not notice any competitive exclusion among these heavy metals. That metal exclusion by O. *biennis* is mainly possible through a rhizofiltration process (Tangahu et al., 2011). However, it can not be asserted that *O. biennis* heavy metal contents were lower than the FAO/WHO (1996) permissible limits regarding the high p-values of 5%. This null hypothesis (H0) can even be rejected very significantly with a 5,8% risk level for As.

Concerning *E. crassipes* and *A. philoxeroides* growth, there was a significantly positive correlation with Pb and Cd, as well. These plants growth could positively influence wastewater oxygenation. *E. crassipes* and *A. philoxeroides* proved to be more permeable to the surrounding heavy metals. Cd, As, Cr, Cu, Hg, Mn, Ni, Pb, and Zn bioaccumulation in ranges higher than the permissible limits was observed, with a particular affinity for the (Hg/Zn) and (As/Zn) couples, respectively. The p-values allowed highly significant rejection of *E. crassipes* and *A. Philoxeroides* heavy metal contents lower than the permissible limits (Ghaderian et al., 2012). Indeed, Ogundele et al. (2015) used only these measured metal concentrations for low risks assertion of contaminated plants. At the same time, pigmentary stresses occurred quite rapidly in A. philoxeroides above 10 mM Cd (Vajravel and Saravanan, 2013). But, rainfall deficits favourable to a warm climate and continuous wastewater discharges without prior treatments support *A. philoxeroides* growth and multiplication (Rane et al., 2015; Wang et al., 2018). Those two invasive plants use various mechanisms such as phytoaccumulation in leaves to mitigate the effects of metals on growth (Opaluwa et al., 2012; Yang et al., 2014; Jeevanantham et al., 2019; Salehi et al., 2020). As of Phalaris arundinacea, a particular affinity for As in absolute concentration value, higher than FAO/WHO (1996), was shown. The p-value (0,0007) allowed assertion in a highly significant way that P. *arundinacea* hyperaccumulated the study metals, particularly As, in addition to Cd (Cristaldi et al., 2017). The average contents in As of *P. arundinacea* reached 150 times the permissible limits. Cd, Pb, and Zn were accumulated in P. arundinacea in concentrations ranging 55-95% of the permissible limits of FAO/WHO (1996). The relatively low metal concentrations other than As could be attributed to the competition between metals for the *P. arundinacea* trace element requirements. That could result in either of the different metallophytes phytoremediation mechanisms used to resist and adapt to the wastewater metal stress in Daloa (Jeevanantham et al., 2019). These plants adaptation activities are vital since a major metal fraction input to the environment results from traffic and mechanics in Daloa, as outlined by Chauhan et al. (2015).

CONCLUSION

The phytoremediation activity study confirmed the *P. arundinacea, E. crassipes* and A. *philoxeroides* phytoaccumulative character for Cd, As, Cu, Cr, Zn, Hg, Ni, Pb, and Mn. However, *P. arundinacea* is specific to As and Hg hyperaccumulation, while *E. crassipes* and *A. philoxeroides* were shown to be specific to Hg and Zn. This metallophytes group activity has differed from *O. biennis* which has effectively accumulated As, Pb, and Zn only. Indeed, *O. biennis* would have particular capabilities in survival mechanisms such as phytodegradation and/or rhizofiltration that allow differentiation of the heavy metals in forms or compositions. This phytoremediation activity accurate assessment was carried out using statistical analyses. That made it possible to determine whether or not the measured heavy metal concentrations were below the permissible limits. Furthermore, factors affecting plant metal uptakes, such as correlation, interaction or competition, and plant phytoremediation mechanisms, were considered. Therefore, the invasive plants along the wastewater drainage systems in Daloa can be used in biotechnology for environmental remediation, being aware of their appropriate destruction at the end of an experiment.

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CONFLICT OF INTEREST:

The authors confirm no conflict of interest.

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