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Feather's *Passer domesticus* as a non invasive bio-indicator tool of urban heavy metals (*Zn, Pb, Cd*) pollution in Rabat-Salé (Morocco).

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Abstract

The purpose of this study is to evaluate the rate of heavy metals (Zinc, Lead and Cadmium) in industrial and urban areas of Rabat using Feather's Passer domesticus as a non invasive bio-indicator method. The collection was made on 50 house sparrows (Passer domesticus) males and females, which were captured in five different sites of Rabat, (ten specimens by site), according to the road traffic and industrial activities, the results obtained indicate the presence of metals in all the analyzed samples. Cd is above the threshold of contamination (0.1 ppm) for all sites, while the Pb far exceeds the threshold (4 ppm) for two study sites. These results suggest that we have two major sources of contamination by heavy metals: Road traffic, and Industrial activities.

Keywords: Heavy metals, pollution, bio-indicators, feathers, *Passer domesticus*, Rabat.

1. Introduction

Heavy metals are ubiquitous elements, they are natural components of the Earth's crust, these metals are dropped into the environment through the biogeochemical cycle (erosion ,volcanism …) but Man has disturb the natural balance of this cycle by industrialization, mining activities, and increased road traffic. These activities have no impact on the heavy metals volume, nevertheless, they have changed their distribution, their chemical forms and concentrations, by introducing new methods of dispersion [7,10,15]. Some of the metals, at very specific doses are essential for the life of the organisms. They are named trace elements (*which are necessary for biological processes such as iron, zinc, copper...*), and any overdose of these metals has a toxic effect. Other metals are not essential, but extremely toxic for the living organisms such as (Pb, Cd...etc.). Trace metals are highly persistent and are not biodegradable which causes an enormous damage for the ecosystems, and specifically for the living organisms, these metals can be amplified through the food chain from prey to predator, which constitutes a real threat to the wildlife and the human health, even at low doses these metals can be accumulated, for a long period, in the targeted organs like liver, kidneys, and bones …etc. In addition to the industrial activities, road traffic remains a major and chronic source of pollution by heavy metals in the urbanenvironment. Despiteof banning of alkyl lead as an anti-knock in gasoline, several studies have shown that there are other sources of pollution such as motor oil, brake fluid, brake lining, lubricating grease, antifreeze , paints, batteries, tire and ball bearing usury, which release huge amounts of heavy metals specially themajor trio : Zinc , Lead and Cadmium [3,10]. Like the industrialized countries, and aware of the dangers and effects of air pollution on health and the environment, Morocco develops methods suitable methods to ensure better air quality monitoring by governmental and non-governmental institutions and our study is one of those studies.

Direct analyzes of the impact of metallic pollutants emitted by road traffic and industrial activities on the various constituents of the ecosystem are very expensive even if they are essential to provide quantifiable physico-chemical data, but they doesn't allow to know the impact on the living matrix, for this reason it is necessary to install bio-monitoring systems, based on the use of live sentinel organisms in their surrounding environments suspected to be infected with metal emissions, these bio-indicator and sentinel species, especially the most vulnerable ones, constitute an early warning signal to intervene and address ecosystem health problems [9,12,14,19]. Generally birds are very good candidates for monitoring because they are abundant, widely distributed, they live long, are sensitive to atmospheric environmental changes, and are high in the food chain [16]. Several previous studies have suggested pigeons (*Columba livia*) as a biomonitor of pollution in urban environments but there is another species that has not been extensively studied (*a very limited number of studies around the world*) it is the house sparrow (*passer domesticus*) which adapts very well in urban areas with large geographical distribution, the house sparrow is an androphile bird that like to live in human modified environments with an omnivorous diet and high reproductive rate, these characteristics make passer domesticus the ideal candidate for biomonitoring pollution in the cities [3,9,14,18,19]. In this study we chose to use breast feathers as a noninvasive approach to dose the metals, to preserve the birdlife, because the birds excrete elements in the growing feathers on the one hand and, on the other hand the metal concentrations in the feathers may be correlated with those of blood and internal organs [1,4,6].

2. Materials and Methods

Feather samples from the breast of (50 house) sparrows, males and females were captured, pulled and stored in paper envelopes at room temperature [1,4,6], under appropriate license from High Commission for Water and Forests, in five different sites located in the region of Rabat- Salé (ten specimens by site) depending on the density of traffic and industrial activities (Fig 1) **1** kamra , **2** town center (Bab chellah), **3** Agdal ,**4** Complex Oulja (pottery and craft

industry), and **5**th one is a rural site for control (farm of Allal Bahraoui). For hunting sparrows in urban areas we have used the mist nets (Japanese nets), traps with springs, folding nets, and trap cages [3].

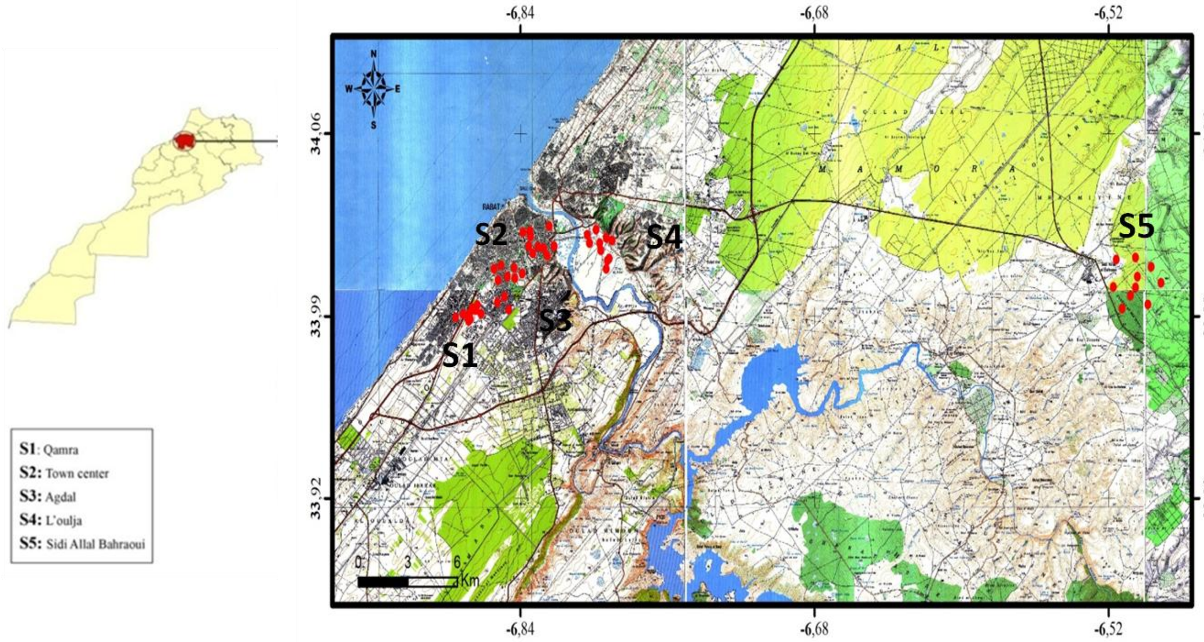


Figure 1:Map of sampling sites.

Before analysis, and to remove external contaminants feathers were washed were washed thoroughly with acetone and deionized water, the washed feathers were put into clean acid-washed glass vials, and oven-dried at 60 C° until constant weights were obtained. Each sample was carefully weighed (0,5)g, then digested by a mixture of nitric and sulfuric acid in a well closed Teflon vessel put in the sand bath at 120° for 4 hours until the solution became clear and no fumes were observed, each sample was diluted to 25 ml with double distilled water, the dosage of Lead (Pb) and cadmium (Cd) was done using the atomic absorption spectrophotometer with graphite furnace (VARIAN GTA 120 AA 240 Z), the background correction was done with Zeeman effect along with a mixture of ($PdCl_2$ and $MgNO_3$) as a matrix modifier. While the dosage of Zn was performed by atomic absorption spectrophotometer (VARIAN AA40 FS) with flame.

Table 1: comparison between Certifiedvalues and measured values using different biological reference materials (Results are expressed in means ± standard deviation µg/g of dry weight).

Reference Material	Metal	Certified values	Measured values in INH Laboratory
11PLO4	Pb	166,3±38,20	173,28±12
	Pb	2,2±0,07	1,9±0,18
S R M 2976	Cd	0,89±0,04	0,82±0,16
	Zn	148±10	137±13

All these experiments have taken placein the accredited laboratory of toxicology in the National Health Institute (Rabat , Morocco) [2,3,9]. The calibration curve was made by the "MSA (Method of Standard Addition), as for external control, the precision and the validity of the obtained results were verified using the reference material: 11PLO4 from the AFSSAPS and S R M 2976 which was provided by the Marine Environment Laboratory of IAEA.

The results of certified and measured values were within the accepted confidence intervals [3]. The percent's recovery obtained was between 86% and 104%, this difference might be acceptable because it did not exceed 15% among the external control laboratories.

3. Statistical analysis

Statistical analysis of data was performed using SPSS-21 and Xlstat 2014 Windows soft ware along with the descriptive statistics, the normality of samples was tested by Shapiro–Wilk Normality test at α=0,01.The maps were developed with Arcgis (10.1). At the risk of error α = 1% the distribution of the samples was not normal since the p-values are much lower than α = 0.01.

Table 2: Shapiro–Wilktest

Variables\Test	P-values
(Zn)	< **0,0001**
(Pb)	< **0,0001**
(Cd)	**0,0022**

3.1. Test of Kruskal–Wallis.

Non- parametric statistics (Kruskal–Wallis) were used because it requires no assumptions about the distribution of the samples, and it has 95% of the power of an ANOVA [5], then we used this test at α=0,05to detect the difference in trace metal between the five locations . P-values are widely smaller than α = 0.05, thus we reject the null hypothesis and conclude that there is a significant difference for each metal among the five studied sites with a significance level (0,05), in other words the sources of pollution by metals are not the same.

Table 3: Kruskal–Wallistest

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of concentrations du Zn is the same across categories of sites.	Independent-Samples Kruskal-Wallis Test	,000	Reject the null hypothesis.
2	The distribution of concentration du pb is the same across categories of sites.	Independent-Samples Kruskal-Wallis Test	,000	Reject the null hypothesis.
3	The distribution of concentration du Cd is the same across categories of sites.	Independent-Samples Kruskal-Wallis Test	,000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is ,05.

H_0: No difference between metal concentrations in those five sites.

H_1: There is a significant difference between the concentrations of each metal in the five studied sites.

3.2. Test of Principal Component Analysis

Analysis of the matrix's data by the multivariate approach allowed exploring the links between the variables (table 4 and **Figure**s 2 and 3) and the trends between samples (**Figure** 4), which make it possible to draw up a spatio-temporal typology of this contamination.

Table 4: Eigen-values of the first 3 factors

	F1	F2	F3
Eigen values	1,69	0,88	0,42
Variability (%)	56,3	29,5	14,1
% Cumulated	56,4	85,9	100,0000

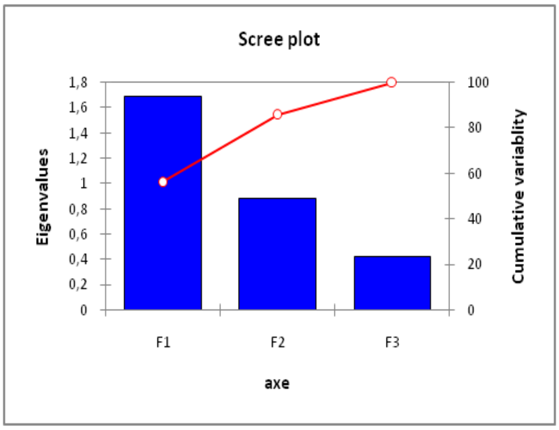


Fig 2 : Eigen-value and cumulative variability.

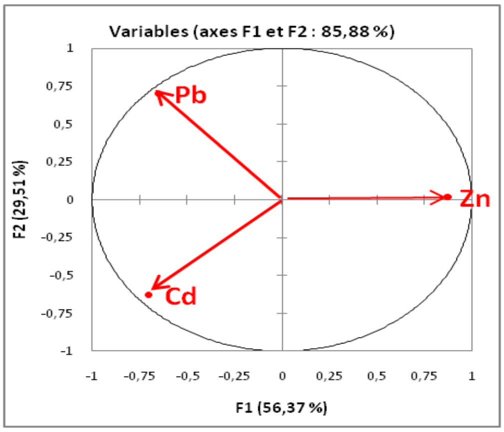


Fig 3 : Circle of correlation.

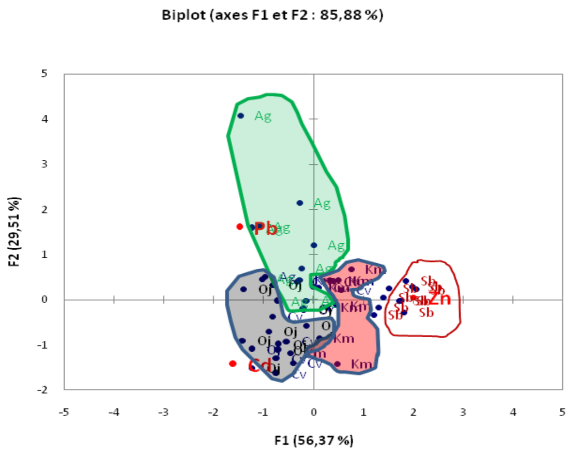


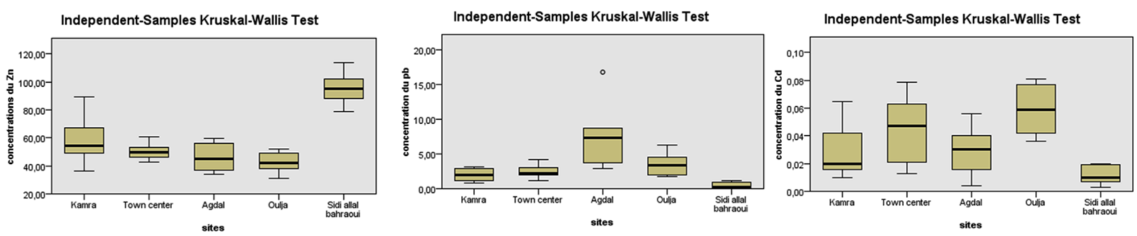
Fig 4: Factorial map ($F_1 \times F_2$).
(*Kamra= Km ; Centre ville= CV ; Agdal= Ag ; l'Oulja= Oj et Sidi Allal Bahraoui = SB*)

The projection of the sites on the factorial map (F1 ×F2) reveals the distinction of four groups, the site of Sidi Allal Bahraoui (SB) tends towards the Zn which can be explained by the use of phosphate rich in Zn, the site of Agdal (Ag) is rather affected by Pb contamination, the Oulja industrial complex (Oj) is affected by the two contaminants Pb and Cd due to industry activities (*galena and painting ceramics*). The sites of Kamra (Km) and the Center of the city (Cv) are located in the center of the factorial map, which means that in these locations, feathers are contaminated by the three metals and this contamination is mainly linked to road traffic pollution.

4. Results and discussion

The Zn concentration is highest on the Sidi Allal Bahraoui farm with a range of (78.70 to 114.10 µg/g), this may be related to a diet rich in Zinc or to external contaminants (fertilizers), Zn is an essential element that plays important

roles in different metabolic reactions, originates both from natural and from anthropogenic sources, it's necessary for the development and growth of feathers. Zn plays a protective role against oxidative DNA damage it is an antagonist of Cd and Pb [18]. The highest Cd values are found in the Oulja and the minimum value was found in the control site (A. Bahraoui), Cadmium is toxic a non-essential element, with deleterious effects for birds on egg formation, testicular damage, oviduct malfunctioning and kidney damage higher concentrations of Cd may also affect the metabolic processes through replacement of essential elements at the active sites of biologically important molecules. Cadmium is known as a teratogen and carcinogen, and a probable mutagen [1, 5, 8]. Cadmium causes lethal behavioral effects at lower concentrations than lead, a slow growth rate, known levels may cause adverse effects in birds range from 0.1 ppm to 2 ppm according to (Eisler, 1985) [5,8]. All values found in this study are below the threshold of 0.1 ppm considered to be a sign of Cd contamination. The maximum Cd value is found in Oulja with an average of 0.059 ± 0.016 µg / g at (p = 0.0001)



Total N	50
Test Statistic	28,498
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	,000

1. The test statistic is adjusted for ties.

Total N	50
Test Statistic	34,708
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	,000

1. The test statistic is adjusted for ties.

Total N	50
Test Statistic	25,060
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	,000

1. The test statistic is adjusted for ties.

Figure 5:Box plots metal contents in the feathers.

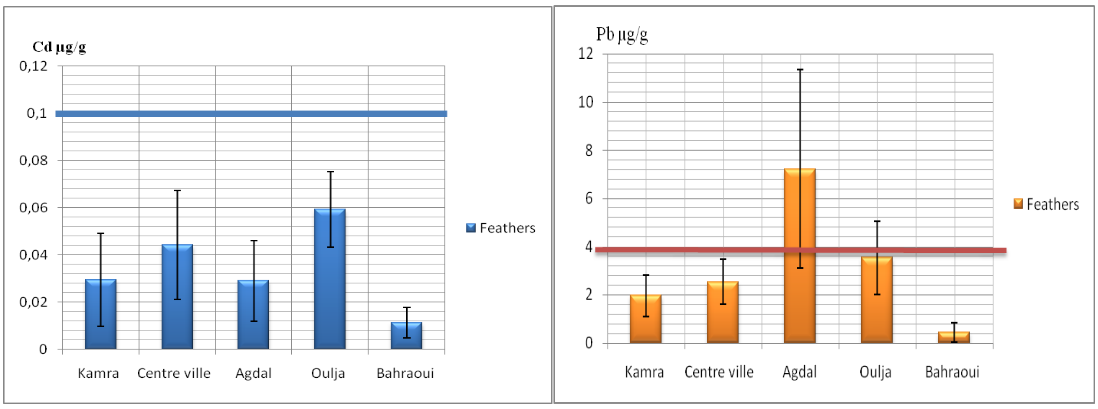


Fig 6: Diagram of the contents of (Pb and Cd) in the feathers

According to numerous studies [5, 8, 17] a concentration of about 4 ppm (or 4 µg / g dry weight) in feathers is associated with negative effects on behavior, parental and sibling recognition, thermoregulation, locomotion depth perception and lowered nestling survival. In this study, the results recorded at Agdal exceed greatly this threshold with an average of (7.22 ± 4.1 µg / g dry weight) (P = 0.0001) .The concentration found in the Oulja artisanal complex is close to this limit with an average of (3.54 ± 1.51), lastly Sidi Allal Bahraoui's farm which had a minimum score of

0.44 μg/g. The contamination in the sites of Agdal, Center City and Kamra could have as origin the road traffic, whereas in the industrial complex of Oulja could be due to the use of the galena in the potteries.

During their life most of the sparrows have only flown in a radius of 2 km from their place of birth [3], It's a sedentary species that helps us to map geographical distribution of metals and to prevent risk areas.

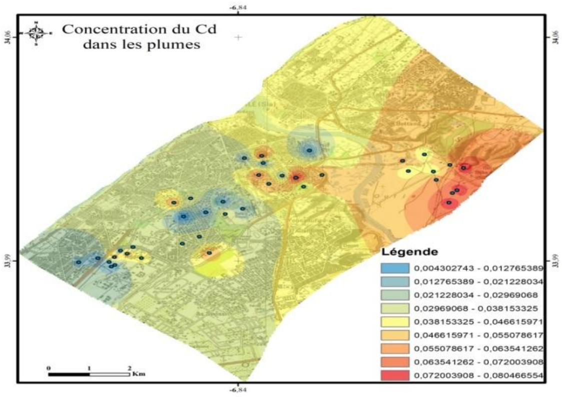


Fig 7: Map of the geographical distribution of Cd in the feathers.

Cadmium pollution is almost generalized for all study sites with a slight increase in the Oulja and the City-center.

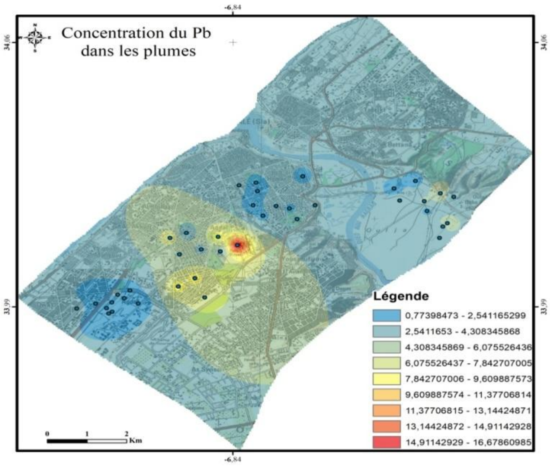


Fig 8:Map of the geographical distribution of Pb in the feathers.

According to the geographical distribution, it can be seen that the Agdal district and the Oulja artisanal complex have the highest Pb contamination in the feathers, exceeding the threshold of 4μg / g by weight. The birds contamination by metals is mainly done through respiratory and alimentary ways, also birds swallow a big quantity of gravels in their gizzards (66% of the gizzard's weight), these gravels help the birds during the digestion process, and they are contaminated by heavy metals [11], in a Norwegian study passerine are more influenced by diet consumption than by air pollution with Cd[13] , which draws our attention to the children who ingest the sand in the gardens, which is known as the pica disorder [14].

5. Conclusions

Feathers is the best indicator of heavy metal contamination for the whole body as a non-destructive method, because Birds sequester metals in their feathers on the one hand and on the other The proportion of body burden that is in feathers is relatively constant for each metal, and related to internal tissue levels [4,5].

However, the use of adult bird feathers to assess exposure to contaminants may be problematic as they are subject to atmospheric deposition for a long time and must be thoroughly cleaned before the analysis.

The road traffic is the major source of pollution by heavy metals in Rabat especially from older models of cars which do not respect the new norms adapted to the environment.

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