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Using blood's *Passer domesticus* as a possible bio-indicator of urban heavy metals pollution in Rabat-Salé (Morocco)

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Abstract

This study aims to evaluate the impact of heavy metals such as Zinc, Lead and Cadmium on the living organisms in industrial and urban areas, using for the first time in Morocco, the blood of *Passer domesticus* (House sparrow) as a bio-indicator of pollution by metals. For this purpose, a blood collection was performed 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: for Lead the highest levels were found in the town center > Kamra> algae >Agdal and the farm of Allal bureau (rural site for control). For Cadmium the most significant concentrations were detected in Oulja > Town center > Agdal > Kamra and A baharoui. As for the Zinc the highest rates were registered in the form of Sidi Allal baharoui.
These results suggest that we can have two possible sources of contamination by heavy metals: Road traffic, and Industrial activities.

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

1. Introduction

Heavy metals are a natural component of the Earth's crust, they are present in all compartments of the environment, but usually in small quantities.They are also included in the biogeochemical cycle of environment. Human activities, such as mining, industrial activities, have disturbed the exchanges in this natural cycle. 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 (smoke, sewers, cars) [1]. Some of the metals, at very specific doses, are essential for the life of organisms. There are called trace elements (which are necessary for biological processes such as iron, zinc, copper...), But any overdose has a toxic effect.While others are not essential, but extremely toxic to the living organisms as (Pb, Cd...ect) .

The metals are not biodegradable and are accumulating in the targeted organs of living organisms, it is the process of bioaccumulation, in particular, these metals can be amplified through the food chain from prey to predator, this is known as the phenomenon of bio-magnification [25].

In addition to the industrial activity , the road traffic is a chronic source of pollution of heavy metals in the city, in one way with the liquid material such as fossil fuels, Oils Lubricants, hydraulic fluids for brakes, battery acid and water of the radiator , and in the other one by the corrosion of solid material like tires, brake lining, paint, degradation of car bodies , and even the deterioration of infrastructures such as pavement (asphalt)

The table 1 below summarizes some earlier studies in the field which were used to estimate the rate of pollutants associated with these materials.

To evaluate the level of pollution by heavy metals and their impact on living organisms , and to prevent risks to public health in urban areas we must chose a biomonitoring and bioaccumulator species of toxic heavy metal [26,28], that respond to some specific characteristics [5]:

1-It must be (typical) of the ecosystem studied (e.g. Non migratory).
2-It must be ubiquitous and abundant.
3-Its size, biotype an behavior must be such as to make sampling easy.

4-It must bioconcentrate exobiotic substance to a level sufficient to perform a direct analysis without preconcentration .

5-It must be able to stand high concentrations of different toxic substances, so as to survive the pollutant studied.

Table 1: Principal Sources of pollution from road traffic.

Material	Trace Metals			Reference
	Zn	Pb	Cd	
Pneumatic µg/veh/km	10.3 - 615	0.05 - 105	0.01 - 85	[2]
Brake linings µg/veh/km	0.2 - 2	1.6 - 16	46 - 460	
Engine oil µg/g	1060	9		[3]
Lubricating grease µg/g	164	0		
Antifreeze µg/g	14	6		
Brake fluid µg/g	15	7		
Transmission fluid µg/g	244	8		
Gasoline mg/l	7.5	501		
Diesel mg/l	9.1	9.1		
Super leaded mg/l	0.11	140	0.0003	[4]
Unleaded mg/l	0.09	140	0.0001	
Diesel mg/l	0.19	0.9	0.0001	
Pavement µg/veh/km	0.1 - 4.3	0.01 -2.4	0.0004 - 0.02	[2]
Roadway coating (mg/kg)	150	100	1	[1]

Many previous studies have used pigeons as bioindicators of pollution in the world's biggest cities as London (Hutton, 1980, Amsterdam (Schilderman 1997), Seoul (Kim et al., 2001) and Rabat (El Abidi et al. 2010), but it was noted that the distribution of pigeons was not as wide as that of house sparrow (*Passer domesticus*) [28]. To complete the study initiated by Elabidi and al., 2010 and see the impact of pollution on other species like the house sparrow that adapts very well in Rabat city, we chose to use for the first time in Morocco these species as a biological matrix. They are androphile species that like to live in human modified environments. During their life most of the sparrows have only flown in a radius of 2 km from their place of birth [6].

2-Materials and Methods

After receiving a license for capture, a specific materiel was used for the hunting sparrows in urban areas like the mist nets (Japanese net), traps with springs, folding nets, trap cages. A total of 50 house sparrows (*Passer domesticus*) males and females were captured between 2010 and 2011, in five different sites from the region of Rabat- Salé (ten specimens by site) according in the density of traffic and industrial activities (figure 1):**1** kamra
2 town center (Bab chellah), **3**Agdal ,**4** Complex Oulja (pottery and craft industry), and **5**th is a rural site for control (Sidi Allal Bahraoui) .

Blood samples were taken from the jugular vein using an insulin syringe , we must wash the birds'neck carefully using alcohol and distilled water to prevent any external contamination ,then they will be put in EDTA tubes to avoid coagulation , this blood collection must be kept cold until the analysis in the laboratory .

After the homogenization of blood samples by the vortex, 1 ml of blood was taken from each sample, and digested by 3 ml of nitric acid 65% (Merck) in a Teflon vessel which was very well closed and put in the sand bath at 120° for 4 hours,until the solution became clear and no fumes were observed,each sample was then diluted to 25 ml with doubly distilled water [7].

The dosage of Pb and Cd in blood samples was accomplished by (VARIAN GTA 120 AA 240 Z) atomic absorption spectroscopy with graphite furnace, the background correction was made by a Zeeman effect, while the dosage of Zn was performed by atomic absorption spectroscopy (VARIAN AA40 FS) with flame, in the accredited laboratory of toxicology in the National Health Institute (Rabat , Morocco).

To reduce chemical interferences and volatility of Cd and Pb in a furnace, a matrix modifier was used (mixture of PdCl2 and MgNO3) .

The calibration curve was made by the "MSA (Method of Standard Addition)", the validity of this method was verified by internal control using blood spiking samples,afterwards, the curve linearity was checked by knowing concentrations in these spiking samples, the precision was verified by three successive readings for each sample, the average of these measures would be taken into consideration if the RSD (relative standard deviation) was under 10% .

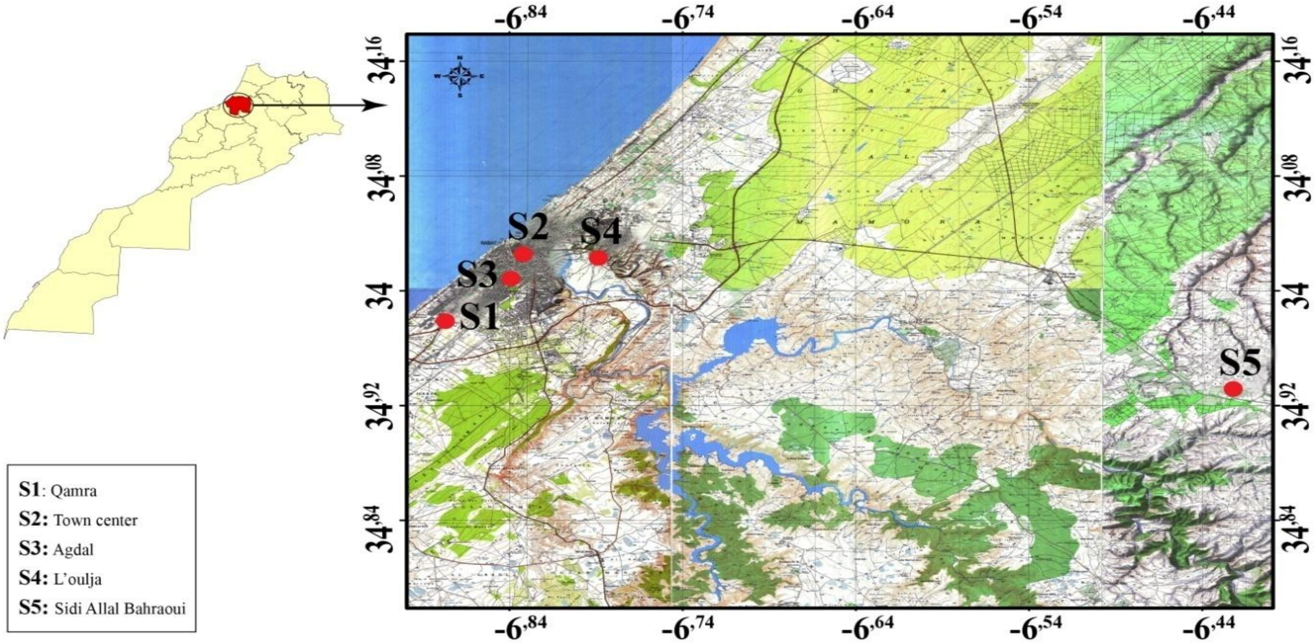

Figure 1:Map of sampling sites

As for external control, the precision and the validity of the obtained results were verified using reference material provided by various international agencies such as : 11PLO4 from the **AFSSAPS :***Agence Française de securité sanitaire des produits de santé* (for blood lead levels) , then DORM-2 Dogfish Muscle and Liver Certified Reference Materials for Trace Metals was given by **CNRC**: *Conseil National de Recherches Canada*, and SRM 2976 (Standard Refence material as a marine bivalve mollusk tissue) that was made by Marine Environment Laboratory of **IAEA:***International Atomic Energy Agency, Monaco*. The obtained results are summarized in Table 2.

Table 2: comparison between Certified values 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	% Recovery
11PLO4	Pb	166,3±38,20	173,28±12	104,2
S R M 2976	Pb	2,2±0,07	1,9±0,18	86,36
	Cd	0,89±0,04	0,82±0,16	92,13
	Zn	148±10	137±13	92,56
DORM-2	Zn	25,6±2,3	22,3±3,5	87,1
	Pb	0,065±0,007	0,060±0,007	92,3

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, but this relative loss of analyte could be caused by manipulating mistakes during the stages preparation of the samples, or by the effect of physical and chemical interferences which are very well known in this field of study and must be continuously correcting.

The most known physical interference are:

1-Viscosity and surface tension inside the graphite tube

2-Attenuation of the incident hollow cathode radiation due to light scattering or to molecular absorption in the graphite tube can cause a false analytical signal unless it is corrected. The non atomic absorption is typically called background and The Zeeman background correction technique is designed to correct these errors

The most chemical interferences encountered is :The volatile compound formation, where the analyte element is lost at a relatively low temperature, perhaps during a dry or ashing stage, without undergoing atomization in this case the matrix modification can be used successfully in changing the appearance temperature and improving the sensitivity of the analyte [29].

3- Statistical analysis

Statistical analysis of data was performed using xlstat 2010 for Windows software, In addition to descriptive statistics the test of ANOVA (one way) was used and the normality of samples was tested by shapiro- wilk, and Jarque-Bera tests with a P-P plots.To make a correlation between the locations and trace metals studied we used the Principal Component Analysis with Pearson's correlation.

4- Results and discussion

Table 3: Descriptive statistics of metal concentrations in blood of House Sparrows (levels represent means of 10 samples ±standard deviation) range of maximum and minimum values are shown in parentheses

Region	Sites	Number of specimens	cd µg/l	Pb µg/l	Zn µg/l
High road way	Kamra S1	10	3,783± 1,208 (6,33 - 2,35)	35,418±13,09 (42 - 1,201)	3310,70± 589,62 (4354,35-2100,52)
Urban	Town center S2	10	5,63± 2,94 (10,19 - 1,44)	40,12±9,5 (55,65 - 23,5)	3261,17± 915,06 (5555 - 2200)
Medium road way	Agdal S3	10	3,88± 3,32 (10,07 -0,5153)	32,03±17,29 (64,7-17,65)	2127,4±1109,7 (4204,2- 1200)
Industrial	Oulja S4	10	8,34± 0,532 (9,20 - 7,8)	33,72±0,945 (35,10 -32,5)	2845,59± 482,72 (3406- 2250,56)
Rural	A, Bahraoui S5	10	3,74± 2,82 (9 - 1,2)	2,70±1,169 (4,2 - 1,201)	4037,04± 1043,17 (5400- 2455)

4-1. Results of normality tests

Table 4: Shapiro- Wilk, and Jarque-Bera tests

(Zn µg/l) [A]		(Pb µg/l) [A]		(cd µg/l)[B]	
P-value	0,374	P-value	0,143	P-value	0,068
Alpha	0,05	Alpha	0,05	Alpha	0,01

A: Shapiro- Wilk B: Jarque-Bera

A) In the case of Zn and Pb, at the level of significance α=0, 05 the decision is not to reject the null hypothesis according to which the sample follows a normal distribution, In other words, the non normality is not significant, because the p-values (One-tailed p-value) are widely superior at α=0,050
B) Because the cadmium had low concentrations we take α=0,01 , so the distribution of samples follow subnormal law , when the p value was superior at α.

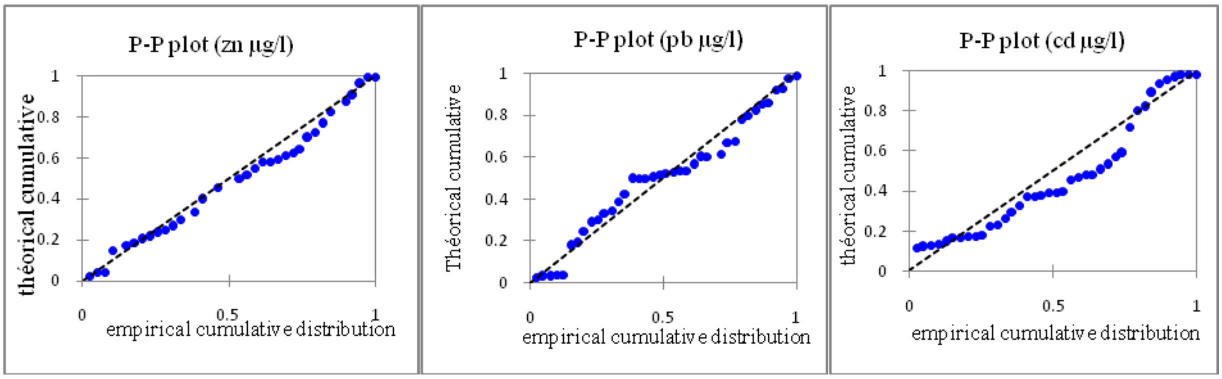

Figure 2: Probability–probability plots

The observed probability distribution was sub aligned to the theoretical model of probability distribution for three metals (suggesting that the observed distribution of the samples follows a normal distribution law).

4-2. Results of ANOVA test

Table 5 : ANOVA test

ANOVA Single Factor at α = 0,05		
Cd	Pb	Zn
P value= 0,002063	P value = 0,00001371	P value= 0,02490

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. The P -value (One-tailed p-value) is widely smaller than α = 0.05, thus we reject the null hypothesis and conclude that there is a significant difference for each metal in the five study sites with a significance level (risk of error) 5%

4-3. Results of Principal Component Analysis

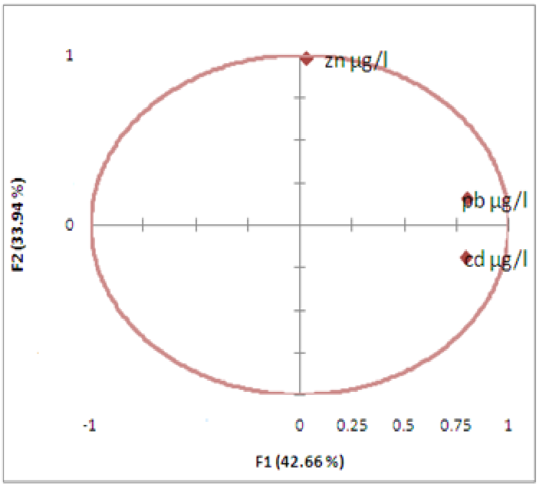

Fig 3 : Correlation's circle

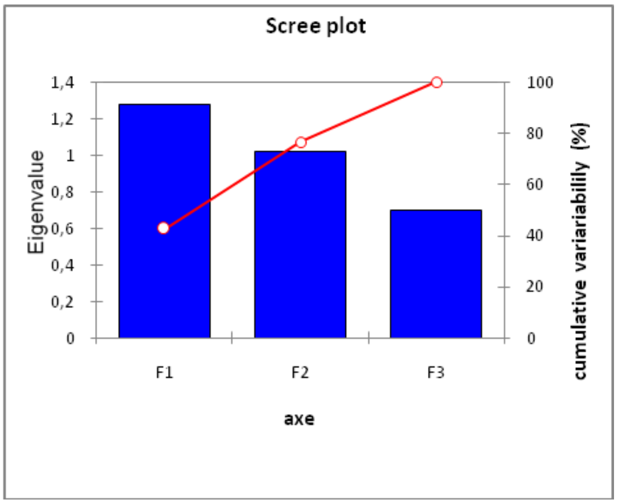

Fig 4 : Eigenvalue and cumulative variability

Table 6 : Matrix of Pearson's correlation.

Variables	cd µg/l	pb µg/l	zn µg/l
cd µg/l	1	0.280	-0.047
pb µg/l	0.280	1	0.058
zn µg/l	-0.047	0.058	1

The projection of the sites on axes F1 and F2 shows that the contamination by Pb and the Cd mainly affects the town center and kamra, this a sign of the pollution by road traffic,concerning Oulja, we can distinguish two zones, the first one is on the positive side of axis F2,which means that, this zone is contaminated by Pb and Cd.While the second zone is less affected by pollution, since it approaches the negative part of the same axis ,this is probably due to the craft industry, the less contaminated samples were captured in July,a month of the holiday season, marked by a decrease of industrial activities,whilst the most contaminated ones were caught in October, the month with the highest rate of industrial production. Agdal is the least polluted site, it's a residential area , where the industrial activities are almost absent , and the road traffic is relatively lower.

In all the analyzed blood samples (Table 3), the concentrations of zinc were higher than Pb and Cd. This result is totally normal, since zinc is an essential trace element that has a biological function (enzymatic processes, for example) but, in case of overdoses it becomes toxic.While lead and cadmium have no biological role and are toxic to the living organisms, the higher rates of Zinc's concentrations found on the farm of Sidi Allal Bureau can be explained by the use of phosphate fertilizer [8].

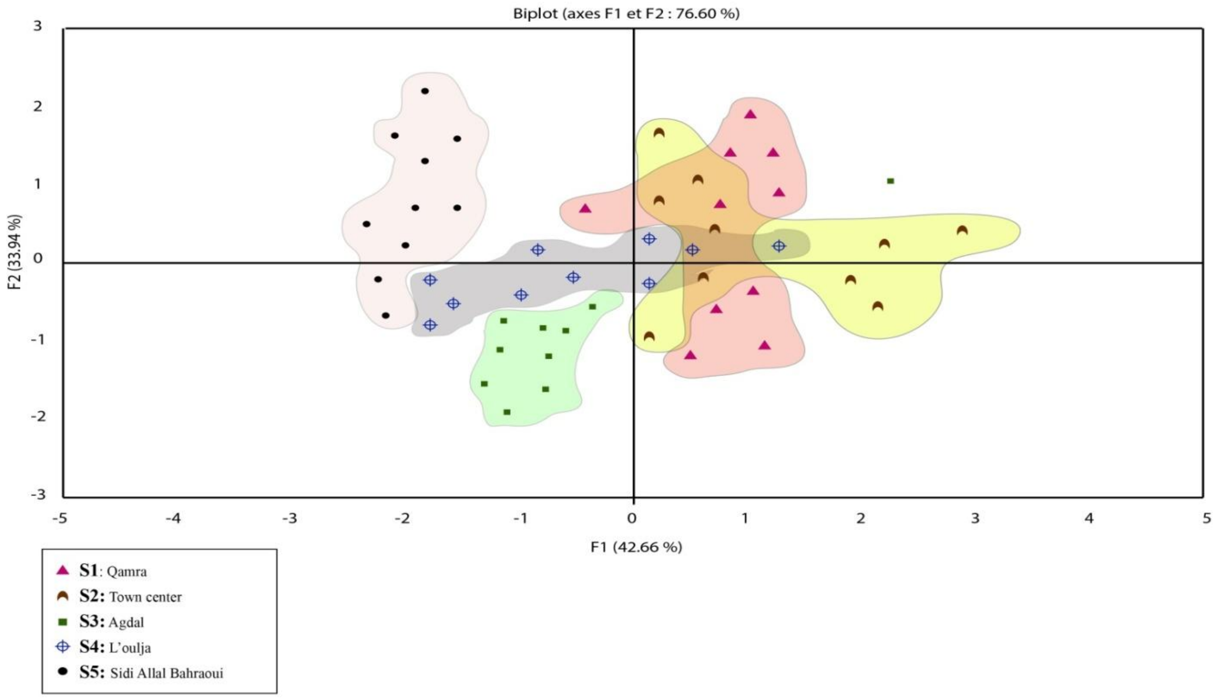

Fig 5: Distribution of sites on the F1xF2 plane

After a time of suspension in air, the particles of zinc, lead and cadmium in dust fall down to the ground and they are essentially concentrated on the superficial layers (0-10 cm) , where they are absorbed in clays and organic materials [9,4] .The birds contamination by metals is mainly done via respiratory and alimentary ways, studies carried out on rats showed that intestinal absorption of Pb was increased by vitamin D and sunshine , and even with a diet that is rich with fats , it should be noted that the small sized particles suspended in air and inhaled by birds can easily transfer from lungs to the blood.

Birds have a very high rate of breathing and metabolism compared to humans which increases the bioaccumulation of heavy metals [24].

The house sparrow makes a large stock of energy in the form of fat, this bird swallows in its gizzard a big quantity of gravels (66% of the gizzard's weight), which helps it during the digestion processes, these gravels are contaminated by heavy metals [11].

The sparrow has an omnivorous diet and it could accumulate more fat, which explains the relatively high levels of lead in its body compared to the pigeon's that has a granivorous diet in the study of Abidi and Al

For humans, the legal limit of lead or the maximum tolerated concentration is 100 µg/l, while it was 400 µg/l in 1976 [12].

According to many bibliographic references the avian blood lead exceeded largely human's blood lead levels we can say that birds are more tolerant to lead than humans, and those previous studies have focused on polluted areas to test the impact of lead's pollution , using birds as a bio-indicators. The study of bird lead's poisoning is very difficult because avian blood lead is not well known and differs from one species to another [27] , besides, the half-life of lead in the blood is relatively short (35 days for humans) [13] once absorbed almost all of Pb is attached to hemoglobin in red blood cells, where it replaces the atom of iron , so the oxygen transport is disturbed, then lead well be accumulated in others target organs such as the bones, liver, kidney…ect.

A few studies of avian poisoning were made in shooting areas, these lands were contaminated by lead shot which, afterwards, was ingested by birds.

According to Scheuhammer's studies 1989, the concentration of lead in the blood is an indication of a strong recent exposure. Lead levels in the blood of (48 ± 33 µg /l) are considered normal , while a level of 150 µg / L indicates the absence of abnormal lead exposure , a level of about 200 µg / l is a sign of a slightly elevated lead level in the blood. [14].

Table 7: comparison of concentration's metals with other studies done on different species of birds

Cd µg/l	Pb µg/l	Zn µg/l	Bird species	Region	Reference
8.34± 0.532 3.88± 3.32 3.74± 2.82	33.72±0.945 32.03±17.29 2.70±1.169	2845.59± 482.72 2127.4±1109.7 4037.04± 1043.17	***Passer domesticus***	Rabat (industrial zone) High road traffic urban Rural	Present study
1.13±0.3 1.58±0.35 0.34±0.04	31.33±2.85 16.4±1.6 11.17± 2.14	6998 ± 396 3463±256 5608±224	***Columbia livia***	Rabat (industrial zone) High traffic road urban rural	[21]
4.8 1.5	208 71	3300 1900	***Anas playrhynchos*** ***Ciconia ciconia***	Doñana Park Spain	[22]
6.24±2.4 6.2±2.96	263± 102 73.3±10.6	1600±100 2212± 200	***Columbia livia***	Amsterdam high traffic Medium traffic	[23]
	1011±132 162±55		***Columbia Livia***	Chelsea Heathrow	[24]

(Note that all values in other studies were converted to µg/l)

Other authors consider a concentration higher than 200 µg /l as an indicator of sub lethal exposure [15]. In this study, blood lead levels in sparrows have not reached critical or alarming values in any site which mean that the prohibition of leaded gasoline was effective. And the rates of lead ,considered as disturbing for humans , come from industrial sources like the pottery Oulja where the galena PbS has been used for paint utensils tools (recently this was banned), and also the ceramic production .As for urban areas , road traffic remains the main source of pollution by heavy metals.

The Zn is mainly generated by the engine oil, liquid Cooling system, brake and tire, but the rate detected in this study does not interfere with biological processes, on the contrary it seems to play an antagonist role like the intoxication by Pb and Cd .

The zinc concentrations in human whole blood vary between 4760±520 µg/l [8] and 5300 µg/l [16] which seems to be in the normal rates .As for Cd , the normal rate in human blood is of about 10µg/l ,70% of which is in the erythrocytes binding to hemoglobin [17]. Cadmium in human blood normal rate is of about 10 µg/l , in other references it is lower than 0.5 µg/l and can reach 5µg / l among smokers [18] .It is in the industrial complex Oulja that we found the highest average 8.34 ± 0.532 µg / l this may be related to the crafts industry of ceramics and earthenware , we do not know much about poisoning by Cd in the wild .However some other studies were carried out in the Japanese quail: Richardson et al. (1974) [19, 20]. Fed diets containing 75 µg/g of Cd to Japanese quail from hatching to 4 or 6 weeks of age and observed significant toxic effects, on a variety of organ systems. Cd-induced anemia, bone-marrow hyperplasia, and cardiac hypertrophy were similar to the effects used by Fe deficiency.

Conclusion

The house sparrow contamination levels didn't reach the critical values like the ones found at the time when the leaded gasoline was still used . Toxic metals do not remain for a long period in the blood because they have a short half-time , after that , metals would be accumulated for a long time in other target organs such as liver , kidneys, bones ...Etc. So it's very difficult to detect lead level's blood in avian wildlife. Hence, it is important to make a dissection and a dosage of heavy metals in these organs in order to detect cases of chronic contaminations.

The concentrations of toxic heavy metals accumulated in the organs can be multiplied by hundred through the food chain from prey to predator. This is the phenomenon of bio-magnification.

The house sparrow is a very good bio-indicator of pollution in urban areas. The use of any form of Lead in gasoline, was definitely banned by the Minister of Energy and Mines:The order (No. 1546-07 of 3 August 2007) also galena (PbS) was replaced by other coatings in craft industry that has greatly reduced the emission of Pb in the atmosphere .

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

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