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THE MAIN WELFARE ISSUES OF DAIRY COWS IN SMALL FARMS FROM ROMANIA

PRINCIPALELE PROBLEME DE BUNĂSTARE ALE VACILOR DE LAPTE ÎN FERME MICI DIN ROMÂNIA

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ABSTRACT | REZUMAT

This study aimed to highlight major welfare problems in dairy cows kept in small farms in Romania. One thousand eighty dairy cows housed in tie-stalls (10-25 cows/farm) were evaluated based on parameters regarding the cows' health (body condition score, fur condition, body cleanliness, skin lesions, and lameness), behaviour (flight distance), and management (watering). The parameter scores for each cow were summed and the animals were classified in categories according to their individual scores. The "acceptable" category had the highest number of cows, followed by the "proper" category, and the "unacceptable" and "excellent" categories had the fewest animals. Considering the welfare parameters assessed, more than half (693, 64.17%) of the cows were thin; 411 (38.06%) had dull hair on their back; 181 (16.76%) showed skin lesions in different body zones; 118 (10.93%) were moderately lame, 48 (4.44%) were lame, and 36 (3.33%) were severely lame; 707 (65.46%) were dirty and 198 (18.33%) showed fear at the observer's approach. The cows were watered either once *a day* (346 cows) or twice *daily* (734 cows). Thus, the major welfare problems in the assessed small dairy farms are inappropriate feeding and watering and also poor body hygiene of the cows. Due to the inappropriate fulfilment of the animals' basic needs, these problems can lead to a complex impairment of their overall health and welfare. The only way to avoid such an effect lies in the farmers' understanding and action to urgently and constantly address these problems.

Keywords: body cleanliness, lameness, skin lesions, watering

Scopul acestui studiu a fost să evidențieze problemele majore de bunăstare ale vacilor cu lapte din ferme mici din România. O mie optzeci de vaci întreținute în ferme mici (10-25 vaci/fermă),în stabulație legată, s-au evaluat pe baza parametrilor de sănătate (scorul condiției corporale, condiția robei, igiena corporală, leziunile pielii și șchiopătura), comportament (distanța de evitare) și management (adăpare). Scorurile parametrilor s-au însumat pentru fiecare vacă și animalele au fost clasificate în categorii pe baza acestor scoruri individuale. Cele mai multe vaci s-au încadrat în categoria "acceptabil", urmată de categoria "corespunzător", iar categoriile "neacceptabil" și "excelent" au avut cele mai puține animale. Conform parametrilor evaluați, peste jumătate (693, 64.17%) din vaci erau slabe; 411 (38.06 %) aveau părul mat pe spate; 181 (16.76%) au prezentat leziuni ale pielii în diferite regiuni corporale; 118 (10.93%) erau moderat șchioape, 48 (4.44%) erau șchioape și 36 (3.33%) șchiopătau sever; 707 (65.46 %) erau murdare și 198 (18.33%) manifestau frică la apropierea observatorului. Vacile erau adăpate fie o dată pe zi (346 vaci), fie de două ori pe zi (734 vaci). Rezultatele arată că problemele majore de bunăstare în fermele mici evaluate erau furajarea și adăparea necorespunzătoare precum și igiena corporală precară a vacilor. Din cauza neadresării nevoilor bazale ale animalelor, aceste probleme pot duce la deteriorarea complexă a sănătății și bunăstării lor. Singura modalitate de a evita astfel de efecte constă în înțelegerea acestor aspecte de către fermieri și implicarea lor activă în adresarea urgentă și continuă a acestor probleme.

Cuvinte cheie: igienă corporală, șchiopătură, leziunile pielii, adăpare

The welfare of dairy cattle represents an issue of increasing interest and concern for consumers worldwide. Although welfare is an attribute of the individual animal, whole groups of cattle kept on the same farm are impacted by the same conditions. To avoid and prevent welfare problems, the breeding systems have to be adequate to the health and behavioural needs of the animals, and each farm has to implement good animal keeping and rearing practices (17). More than just having an informative role in the problems faced by each animal, analysing the results of individual welfare assessments allows the identification of farm-level issues. Once such an issue is solved, the welfare degree of several (or even all) animals can be increased simultaneously.

Besides the large, industrial farms, Romania still uses extensive breeding systems, in small farms with tie stalls for dairy cows. It is assumed that the welfare

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of these animals is better than that of those kept in intensive breeding systems. The natural and species-specific conditions are essential for the welfare of the animals and these can be provided only in small-scale family farms (21). However, some of our research showed that the housing and management of the small farms have several deficiencies with negative effects on the dairy cows' health and welfare (8, 16). Larger herds are associated with increased stock per labour unit, increased stocking density, and less (if any) access to pasture (5). Thus, increases in farm size can have positive effects in the economic and labour management sense but they create welfare risks for the animals (18). Moreover, concerning farm capacity, herd size is considered by numerous studies as an influencing factor for animal welfare issues (2, 15). Contrary to these, in a recent study Lindena and Hess (2022) (11) prove that herd size has little, if any, effect on farm-specific animal welfare levels. Another study concludes that the characteristics of the housing conditions and the quality of the management practices seem to have a more significant effect on animal welfare than the number of dairy cows per farm (5).

In the attempt to gain a better insight into how the cattle perceive their living conditions and how they cope with the challenges posed by their immediate environment, several welfare assessment protocols were devised, using different welfare indicators (9, 16, 20, 28, 29). Some of these provide quantifiable scores, while others produce qualitative results. Because of the unequal practical value of the different welfare parameters (some welfare problems have a more negative impact on the animal than others), the calculation of individual welfare scores and inclusion of the animals in welfare categories is not usual. Yet, this type of data processing is important, to allow the comparison between animals, farms, or repeated assessments on the same farm. (to investigate the changes in dynamics). Thus, with the provision that the individual welfare scores show the general welfare degree for statistical processing, without indicating the necessary intervention domains, this type of classification is justified.

The aim of this study was to highlight the major welfare problems of dairy cows in small Romanian farms, both to have a general classification of the dairy cows' welfare degree and to identify the areas where welfare-improving interventions are needed.

MATERIALS AND METHODS

The study was conducted in 91 small farms (10-25 cows/farm) in Romania. The predominant breed was the Romanian Spotted Cattle (in 70 farms), followed by Holstein cattle (21 farms). The cows were housed in simple barns with tie-stalls, without endowments, mostly unsuitable from a hygienic point of view. Throughout the winter, the cows were kept inside the barns all the time, and in summer they were on the pasture during the day and housed for the night.

The cows' welfare was assessed based on several animal-related indicators: body condition score (BCS), body cleanliness, lameness, skin lesions, fur condition, avoidance distance, and by one management indicator, the frequency of their access to water. A total of 1080 dairy cows were assessed, before the beginning of the grazing season (April-October). Each cow was evaluated by two experimented researchers. For the assessment of some parameters (lameness, avoidance distance), the cows were untied and moved outside the barn. The body condition score (BCS) was assigned according to the method devised by Thomsen et al. (23). Thus, a fat cow is one with BCS≥ 4; a cow with normal body condition is one with 2.25≤ BCS≤3.75; a thin cow is one with 1.5≤BCS≤2, and an emaciated cow is one with BCS≤1.25.

The cows' body cleanliness was based on the observation of the corporal regions to record the presence (or absence) of manure soiling.

The skin lesions were assessed in all body areas (neck, shoulder and withers, knee, ribs, back/spine, tailbone, hipbone, point of hock, inside of the hock and stifle), using the method proposed by Leeb et al. (2004) (10). The presence and severity of lameness in the cows were evaluated based on the system elaborated by Sprecher et al. (1997) (22). To score the fur condition, the aspect of the hair on the dorsal region of the cow's back was verified according to the method proposed by Thomsen et al. (2007) (23): shiny hair or dull hair with little dust on the back or very dull hair with much dust on the back. The avoidance distance was recorded by measuring the distance (in meters) to how close the assessor can approach the cow before this withdraws, using the method described by Popescu et al. (2010) (16).

Table 1

The welfare parameters assessed and their assigned scores

Parameter	Score	Explanation
Body condition score	0	Fat or emaciated
	1	Thin
	2	Normal
Skin condition	0	Dull skin, dust on the back of the cow
	1	Shiny skin, little or no dust on the back
Body cleanliness	0	Dirty
	1	Clean
Skin lesions	0	Lesions
	1	No lesions
Lameness	0	Severely lame
	1	Lame
	2	Normal
Human-animal relationship	0	Fear of the assessor's approach
	1	No fear of the assessor's approach
Frequency of access to water	0	Once a day
	1	Twice a day
	2	Unlimited access

Some farm management data were provided by the farmer. Each welfare parameter assessed was scored, assigning the lowest score (0) to the worst result for the given parameter and the highest score for the best result (Table 1).

The results of the assessed welfare parameters were expressed as percentages. The parameter scores were summed to obtain an individual welfare score for each cow, which ranged from 0 to 10. According to their individual welfare scores, the cows were included in four welfare categories: unacceptable (scores from 0 to 2), acceptable (scores from 3 to 5), proper (scores from 6 to 8) and excellent (scores from 9 to 10).

RESULTS AND DISCUSSIONS

The results obtained for the animal-based parameters through the welfare assessment of the 1080 cows were recorded in tabular form (Table 2), and the prevalence of each parameter score was calculated.

Among the animal-based parameters, the most deviated from normality was the body condition score (BCS), with more than half of the assessed cows being evaluated as thin ($1.5 \leq BCS \leq 2$). The high percentage of thin animals may be due to insufficient feeding during the cold season (when the animals were not grazing) or to improper feed quality. The nutrition of dairy cows should provide energy for their milk production and, at the same time, support the maintenance of their weight. After periods of energy deficit, when the weight loss is more significant than the decrease in their production, feeding not only has to fuel maintenance but to ensure body weight gain, back to a normal BCS. Thus, the periodic evaluation of the BCS is a valuable tool in monitoring the dairy cows' nutritional management (19), enabling the farmer to make timely adjustments. Moreover, the BCS is an important part of most welfare assessment protocols as a valuable indicator of the dairy cows' welfare (28). The fat or thin state of cows can be a sign of nutritional and metabolic disorders, other health problems or it can indicate poor management at the farm level (29).

Another indicator showing changes was the skin condition. The assessment of this parameter was done to determine if the cow is able or not to keep her skin clean (23). The absence of self-grooming can indicate illness, poor general condition, or inability to perform certain movements. In this study, the result could be influenced by the length of the tethering chains, limiting the movement possibilities of the animals.

A high percentage of cows showed manure soiling of their bodies. Similar results were reported by other studies as well (8, 14). The cows' body can become dirty due to the decubital resting in the manure deposits in the stalls (4), in poorly maintained stalls having manure on the division elements, or by spillage from the dirty tails moved around the animals' hind quarters (30). Thus, the body hygiene assessment can provide more information about the cows' comfort and the care of the stockperson for the animal.

The skin lesions had a lower percentage than that reported by other researchers (23), but comparable to

Table 2

Results of the animal-based parameters assessed in 1080 dairy cows

Indicator	Number of cows	Percentage (%)
Body condition score (BCS)		
Fat: BCS ≥ 4.	9	0.83
Normal: 2.25≤BCS≤3.75	378	35.0
Thin: 1.5≤BCS≤2.	693	64.17
Emaciated: BCS≤1.25	0	0
Skin condition		
Shiny skin, little or no dust on the back	669	61.94
Dull skin, dust on the back of the cow	411	38.06
Body cleanliness		
Clean	373	34.54
Dirty	707	65.46
Skin lesions (only the most severe lesion found)		
No lesions	899	83.24
Lesions	181	16.76
Lameness		
Normal	878	81.30
Mildly lame	0	0
Moderately lame	118	10.93
Lame	48	4.44
Severely lame	36	3.33
Human-animal relationship		
Fear of the assessor's approach	198	18.33
No fear of the assessor's approach	882	81.67

the results of other studies conducted in Romania (14, 16). This finding is probably due to the characteristics of the housing and management. The skin lesions reflect the impact of the nearest environment on the animals' bodies (10). In the studied farms, the barns were simple, with only sparse equipment and divisions, lowering the risks of hitting. The straw bedding used offered padding while the cows were resting, lying down, and rising up. In small farms, the productive process is less expeditious, and the cows are less rushed than in a large farm. In several farms the milking was manual, in the stalls, thus the cows were not led to a milking parlour. Part of the skin lesions found (hair loss, small wounds), especially in the neck region, were possibly caused by the tethering chains. The skin lesions, generally, not only cause pain but can also indicate welfare and production problems. Therefore, the lesser their prevalence is, the better for the cows' welfare. Surprisingly, a small number of cows showed lameness of varying degrees of severity. We expected a higher prevalence of lameness because the cows' access to free movement was restricted during the whole cold season. Several authors showed that lack of exercise and pasturing lead to increasing foot problems (7, 17). Our finding is in line with those reported in other studies on small farms (8, 16). Lameness is one of the most severe and common problems encountered in commercial dairy farms around the world, with a strong negative impact on animal welfare and production (29). It lowers the milk production (29), progressively worsens the animals' body condition, and ultimately it shortens dramatically their productive lifespan (1).

In our study, a low percentage of cows showed fear at the approach of the assessor. This result expresses a good human-animal relationship in the majority of investigated farms. Similar findings were reported by Popescu et al. (2010) (16) in a previous study. The stockmanship is more friendly and considerate in extensive breeding systems for dairy cows than in intensive breeding. This is due to a smaller number of animals, which have regular contact (several times a day) with the workers. The human-cow interactions occur during feeding, watering, milking, cleaning the barn, and cleaning the animals, because all these works are performed manually. The animal's reaction to humans is assessed by a well-established method, the measurement of the flight distance (6, 25). The distance at which the animal withdraws shows the extent of that animal's "safety zone". The more comfortable and safer the animal feels around people, based on previous interaction experiences, the more indicative the test is for qualitatively and quantitatively proper animal handling by the workers. Yet, various elements can affect the outcomes of the assessment besides the experiences and disposition of the animal (lameness, the social environment of the testing, if the assessor is known or unknown for the animal, and even individual mental traits of the animal). If the assessor can approach within a close distance without the withdrawal of the cow, it means that the animal is not fearful. As a consequence, all daily procedures involving humans around will be less stressful for the animals. It has been established that the behaviour, milk production, and welfare of dairy cows are affected by the nature of the animal-stockman interactions (6, 25). Hemsworth et al. (2003) (25) demonstrate a correlation between workers' behaviour and a reduction in the animals' fear of humans and milk yield. The study suggests that the stockpersons' behaviour determines in the animal the level of fear of humans, which, in turn, can influence milk production. Most cows in the studied farms (67.96%, 734 cows) had twice-a-day access to water and a lower percentage (32.04%, 346 cows) only once a day (Fig. 1).

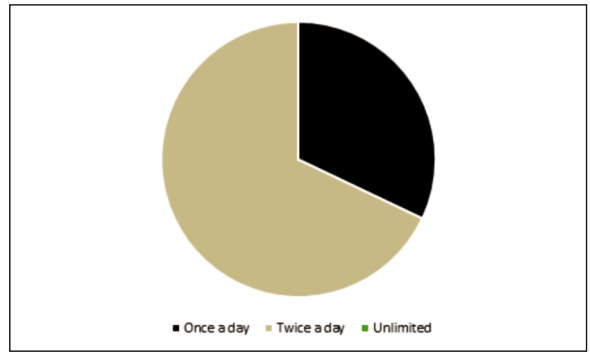

Fig. 1. The frequency of access to drinking water in the assessed cows

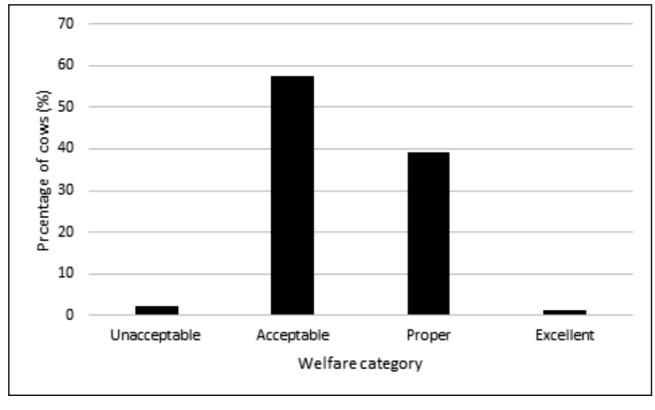

Fig. 2. The distribution of the assessed dairy cows in the four welfare categories according to their individual welfare scores

Restricting the water intake of cows by 50% results in 74% lower milk yield and more aggressive behaviour (12). Unlimited access of the cows to drinking water in adequate quantity is important for their health and production (12, 13, 28). Despite the attention paid to other nutrients, the quantity and quality of water are often overlooked by farmers. Prolonged thirst has a more severe impact on the animals' welfare than prolonged hunger, especially in milking cows, whose organism uses high quantities of water in order to produce milk. Cardot et al. (2008) (3) showed that lactating cows drink on average more than three times their mean daily milk yield, and other researc

chers reported that each kilogramme of milk produced requires from 1.24 to 1.30 litres of drinking water (13). Based on the individual welfare scores, the highest number of cows was in the "acceptable" welfare category, followed by the "proper" category. The "unacceptable" welfare category included only 2.22% of the cows, and even less (1.11%) were classified as being in the "excellent" category (Fig. 2).

This kind of classification is very useful to provide a quick view of the overall situation of a farm or of the whole or a segment of population assessed. The proportion of the cows in a given category is indicative for the improvement measures needed and for the degree of intervention urgency.

CONCLUSIONS

The major welfare problems of dairy cows in Romanian small farms are inappropriate feeding and watering and also the poor body hygiene of many cows. Taking into consideration that these problems impair the basic needs of the assessed animals, the situation can be considered severe and needs urgent improvement. The inappropriate fulfilment of vital necessities (such as eating and drinking according to their physiological and productive energetic demands) can cause overall health and welfare impairment through several interconnected mechanisms. Besides the direct negative impact, the more subtle but equally important body functions of the cows can suffer, such as their immune system. Thus, the understanding and active involvement of the farmers is paramount for these issues to be urgently and continuously addressed.

Despite the low percentage of cows displaying fear of humans, which was considered a positive effect of frequent handling of tethered cows, the tied housing system was concluded to harm the welfare of dairy cows. The improvement of this parameter (provision of regular access to free movement) could be more difficult to achieve due to lack of space and workforce in small farm settings, but it would bring considerable benefits for the health and welfare of dairy cattle.

However, the positive results of this study (such as the low prevalence of lameness in the assessed cows and the good human-animal relationship) are important, as these can build the basis of a positive dialogue with the farmers to increase their understanding and willingness to address the problems found for the better welfare of their animals.

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WELFARE ASSESSMENT IN WORKING AND BREEDING HORSES USING BEHAVIOURAL INDICATORS

EVALUAREA BUNĂSTĂRII CAILOR DE MUNCĂ ȘI DE REPRODUCȚIE PE BAZA INDICATORILOR COMPORTAMENTALI

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ABSTRACT | REZUMAT

The aim of the study was to assess the behavioural response towards humans in different horse categories – breeding and working horses. The study was carried out over a period of 10 months on 90 horses (50 breeding horses and 40 working horses). Using specific methods, the general attitude of the horses was evaluated (apathetic or alert) and their reactions (aggressiveness, fear/avoidance, indifference, friendliness) to the assessors' approach and walking besides; the attempt of touching the animal. The data were analysed using the SPSS statistical software. The value of minimal significance was considered at $P < 0.05$. The proportion of apathetic horses was higher than of alert horses, with no statistically significant differences ($P > 0.05$) between the two categories included in the study. For each type of behavioural response (aggressiveness, fear, indifference, or friendly response), there were no statistically significant differences ($P > 0.05$) between the evaluated categories of horses. Among the behavioural indicators, a relatively high number of apathetic horses, especially in the reproduction category, indicates the need for a more detailed study of the causes.

Keywords: approach, human-horse relationship, response, behaviour

Scopul studiului a fost evaluarea răspunsului comportamental al cailor de diferite categorii - cai de reproducție și cai de muncă. Studiul s-a desfășurat pe parcursul a 10 luni, folosind o populație de 90 de cai (50 de cai de reproducție și 40 de cai de muncă). Cu ajutorul unor metode specifice, s-a evaluat atitudinea generală a cailor (apatic sau alert) și reacțiile lor (agresiv, fricos, indiferent, prietenos) la apropierea evaluatorului, la trecerea pe lângă animal, precum și la încercarea de a-l atinge. Datele au fost analizate cu ajutorul software-ului statistic SPSS, iar valoarea de semnificație minimă a fost stabilită la $P < 0,05$. Proporția cailor apatici a fost mai mare decât a celor alerți, fără diferențe semnificative statistic ($P > 0,05$) între cele două categorii incluse în studiu. Pentru fiecare tip de răspuns comportamental (agresivitate, frică, indiferență sau răspuns prietenos), nu au fost diferențe semnificative statistic ($P > 0,05$) între categoriile de cai evaluate. Dintre indicatorii comportamentali, un număr relativ ridicat de cai apatici, în special din categoria de reproducție, indică necesitatea unui studiu mai detaliat al cauzelor.

Cuvinte cheie: apropiere, relație om-animal, răspuns, comportament

Horses exhibit a consistent set of behavioural parameters or indicators when they encounter situations that involve pain, fear, or stress (24). These behavioural parameters can manifest in explicit and potentially hazardous ways for humans (6), often resulting in corrective measures by the handler and contributing to the horse's distress (29). However, they can also be observed at a much subtler level. The incorporation of behavioural indicators in conjunction with physiological markers is acknowledged as a critical element in identifying pain (11).

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A variety of analogous behaviours have been linked to fear and stress reactions, including actions like eye wrinkling, twitching, and blinking (17), wide or triangulated eyes, ear position, muscular tension, defecation, avoidance, and tail swishing (16, 13). Numerous obstacles exist when it comes to learning how to identify and understand the nuanced body language involved. These behaviours can be individualised, as some horses exhibit more overt signals than others (19, 28). Distinguishing "relaxation" from the behaviour of a horse that is "shut down," depressed, and/or moving towards a state of learned helplessness can be challenging (1, 5, 3, 4).

The manner in which a horse is managed can elicit various types of responses. Various forms of restraint, such as the twitch, hobbles, chutes, and bits with

chains, are frequently employed to prevent flight or aggression. However, the use of a twitch, for instance, has been identified as a significant cause of accidents for equine veterinarians (18). Moreover, early forced contact can lead to foals showing aversion to human interaction at later stages (10).

It is evident that further research is required to investigate how to approach horses in a better and safer manner. This includes studying factors such as body position, posture, gaze, and the timing of approaches. Research should also explore the types of approaches and their timing that can contribute to developing a positive bond with horses. Additionally, it is crucial to examine how human management and care practices affect the human-horse relationship and identify ways to adapt these practices to positively influence the relationship (8).

Nonetheless, how horses react to interactions with humans primarily emerges from the dynamic interplay between their individual temperament, the temperament and capabilities of the human handler, and the cumulative experiences they've had with humans (8). The development of methods for assessing how horses respond to humans is a relatively recent endeavour, with various approaches emerging, particularly through behavioural testing. Various tests have been developed to assess how horses respond to human interactions. These include tests where a human remains stationary and waits for the horse to approach (31, 27, 14, 9), tests where the human moves around (12, 23), tests where the human approaches the horse suddenly (7) or slowly (15), and tests where the human attempts to touch the horse (27, 9). The aim of the study was to assess the behavioural response towards humans in different horse categories – breeding and working horses.

MATERIALS AND METHODS

This study evaluated 90 horses from various categories and husbandry systems. The research was conducted over a period of 10 months. The two main cate-

Table 1

The assessed behavioural indicators

Indicator	Assessment method	Score
Alert/ Apathetic	The animal is observed from a distance of two to three metres for 60 seconds without drawing its attention.	0 = Apathetic (inactive, half-closed eyes, head down, lack of attention to the environment); 1 = Alert (attentive to the stimuli of the environment).
Response to the human approach	The assessor approaches the horse at an angle of approximately 20° to the sagittal plane of the animal, gently addresses it with a few calm words, and stops at a distance of about 30 cm from the horse's shoulder. The evaluator remains in a relaxed but still-body position for 30-60 seconds. The horse's behavioural response is then recorded.	0 = Aggressiveness (ears flat, any attempt to kick or bite); 1 = Fear or avoidance (taking a step in the opposite direction or turning the head away from the human / tense muscles, immobile body position, ears pointing towards the assessor); 2 = Indifference (present in the environment but does not pay attention to the observer); 3 = Friendliness (movement of the head towards the human, relaxed facial expression, eyes normally open, ears turned forward, no wrinkling around the mouth or nostrils).
Response to human walking beside	The assessor walks alongside the horse towards its rear and back again, maintainning a distance of approximately 30 cm from its body, recording any sign of the animal's attention.	The scoring is similar to a human's approach test: 0 = Aggressiveness; 1 = Fear/avoidance; 2 = Indifference; 3 = Friendliness.
Response to a human attempt to touch	At shoulder level, approximately 30 cm from the animal, the evaluator extends his hand towards the chin region, with the hand pointing upwards, as if offering food. He stays like this for about 30 seconds, then, if the animal does not initiate physical contact, he raises his hand towards its chin, trying to touch it. The behavioural response is recorded.	The scoring is similar to a human's approach test: 0 = Aggressiveness; 1 = Fear/avoidance; 2 = Indifference; 3 = Friendliness.

gories of animals investigated included breeding horses (n= 50) from state stud farms and working horses (n=40) from the private sector. The horses kept on the stud farm were Lipizzaner and Romanian Draft horse breeds. The private working horses were of varying age categories and genders.

The general attitude of the horses (apathetic or alert) was assessed, as were their reactions (aggressiveness, fear/avoidance, indifference, and friendliness) in three specific situations: (i) to the assessors' approach; (ii) walking beside; and (iii) the attempt to touch the animal using the methods outlined by Popescu & Diugan, 2013 (20; Table 1).

Each horse was assessed by two experimented assessors trained together in a preliminary study until they achieved a minimum of 80% reliability both within and between them (20). The obtained data were analysed with the statistical programme SPSS (version 17).Comparisons between breeding and working horses were made with the Kruskal-Wallis test, as the data did not follow a normal distribution. Differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 2 contains the results obtained after evaluating the behavioural indicators described before.

The behavioural tests were conducted to investigate the relationship between horses and humans.

The sources of fear and stress can undoubtedly be numerous, depending on the individual experiences of each horse in relation to their temperament and reactivity. However, given the conditions of care and use, humans can represent a constant source of these negative states, depending on how horses are handled. On the other hand, for the safety of the caretaker personnel, it is essential that the horse's perception of humans is not negative. The proportion of apathetic horses was higher than that reported in other studies (2, 20-23), with no statistically significant differences ($P > 0.05$) between the two categories included in the study. General apathetic behaviour, the lack of response to environmental stimuli, occurs in sick horses but also in cases where they are exhausted from work (30). Analysing the results, it can be observed that for each type of behavioural response (aggressiveness, fear, indifference, or friendly response), there were no statistically significant differences ($P > 0.05$) between the evaluated categories of horses.

The frequency of aggressive responses in the breeding horses increased from the second to the third test, and in the working horses it increased from the first to the second test, after which it decreased, probably due to more frequent human contact. Correlations have been reported between aggression and the comfort of animals in the shelter (25).

The number of horses that showed fear increased from the first test to the second and then decreased in

Table 2

Prevalence of evaluated indicators in breeding and working horses

Indicators	% (No. of animals)		P
	Breeding horses	Working horses	
The general attitude			
Apathetic	32 (16)	25 (10)	0.469
Alert	68 (32)	75 (30)	
Response to the human approach			
Aggressiveness	0 (0)	2,5 (1)	0.079
Fear/avoidance	22 (11)	27.5 (11)	
Indifference	42 (21)	52.5 (21)	
Friendliness	36 (18)	17.5 (7)	
Response to human walking beside			
Aggressiveness	0 (0)	5 (2)	0.141
Fear/avoidance	36 (18)	30 (12)	
Indifference	12 (6)	37.5 (15)	
Friendliness	52 (26)	27.5 (11)	
Response to a human attempt to touch			
Aggressiveness	2 (1)	0 (0)	0.395
Fear/avoidance	20 (10)	12.5 (5)	
Indifference	12 (6)	15 (6)	
Friendliness	66 (33)	72.5 (29)	

$P < 0.05$ - the difference is statistically significant

both categories of horses. The fear response in animals towards humans is likely a result of inappropriate attitudes from individuals with whom the animal has had unpleasant experiences before. If the interaction between horse and human has negative connotations for the animal (stressful, fearful, painful interactions), then the horse will avoid similar interactions in the future and will show a more pronounced fear behaviour (8). In working horses, correlations have been reported between the lack of response to humans and certain health and management issues (2, 21). These findings suggest that the observed indifference in working horses is a negative state. The friendly response of horses to human presence progressively increased from one test to another. From a general welfare perspective, a horse's friendly behaviour towards humans is the most desirable behavioural response. Sankey et al., (2010) observed a direct correlation between repeated positive interactions between humans and horses and the positive reactions and attitudes of horses towards people (26). Furthermore, horses have the ability to generalise positive experiences with an individual by developing positive mental associations and extending these to display positive attitudes even towards unknown individuals and people in general, even in different situations than those in which the mental associations were formed. These generalisations can persist even after a relatively long period of time has passed.

CONCLUSIONS

Among the behavioural indicators, a relatively high number of apathetic horses, especially in the reproduction category, indicates the need for a more detailed study of the causes. Regarding behavioural responses to humans, no statistically significant differences were demonstrated between the categories of studied horses. Unfortunately, issues related to the relationship between horses and humans are the most challenging to remedy, as they require a change in the mindset of those caring for the horses.

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animals

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Article

Changes in Management, Welfare, Emotional State, and Human-Related Docility in Stallions

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Simple Summary: Regardless of age, breed, physiological condition, or gender, all horses are social animals, requiring the company of their own kind. Due to their natural instincts to fight each other, adult stallions are often kept in isolation. Several studies have shown the possibility of free group housing and recommended methods to reduce the detrimental effects of limited social contact on their physical and/or mental wellbeing. Nevertheless, the beneficial effect of transitioning from tie-stalls to free housing on adult stallions' overall welfare has not been researched before. According to our results, in only two weeks after the management change, the studied stallions had significantly better welfare, improving further over time. Additionally, their human-related responses improved, and their docility did not decrease, despite minimal human–animal contact during the study. Although positive emotional states were identified within the study, these did not correlate consistently with the other parameters assessed. Given the findings in this paper and accounting for all precautions required while making such a management change, we conclude that adult stallions can and should be kept in free group housing to provide them with the best possible conditions to support their optimal welfare.

Abstract: Despite an increase in awareness of their essential needs, many stallions continue to be kept in conditions limiting their social interactions and movement. To supplement the studies which highlight the effects of these practices on selected aspects of equine mental and physical wellbeing, we aimed to monitor a group of 32 adult intact stallions during their transition from tethered housing with limited outdoor access to free group housing through the lens of their overall welfare, perceived emotional status, and docility toward humans. Over three visits (before the management change, two weeks, and three months after, respectively), their welfare, qualitative behavior, and docility were assessed. Analysis of the data collected showed an improvement in the stallions' overall welfare and no decrease in their docility after their group-release, with a constant correlation between these two aspects. The evaluation of their emotional states was less relevant, lacking consistency between the assessments for most of the descriptors used, warranting further research in similar conditions. Although our study covered a relatively short period of time, our results provide encouraging support for stallion owners in deciding on a similar management change for the welfare of their animals.

Keywords: stallions; group housing; horse welfare; human-related docility; qualitative behavioral assessment

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1. Introduction

A widely known definition [1] states that animal welfare is the state of an individual regarding its attempts to cope with its environment. The degree of coping, or adaptation, concerns simultaneously three aspects of welfare: the state of the animal's body, mind,

and the extent to which its nature is satisfied [2]. For domestic animals, including horses, their housing conditions define their close environment, and the implementation of specific management decisions represents a major factor in shaping environmental challenges for the animal.

The traditional management of adult stallions relies mostly on stabling, with more or less outside access, but in isolation or with limited interaction with other equines [3]. Although this is an efficient way to avoid fighting (and, thus, the occurrence of injuries), unwanted mating, and the spread of diseases, the natural behaviors and social interaction of these animals are sacrificed [4]. Many authors agree that solitary confinement still practiced in adult stallions can lead to stress [5] because it disregards their need to display natural behaviors [6], negatively impacts their mental and physical health, and, consequently, their welfare [7]. The use of tie-stalls that are no longer accepted in many countries [8] is associated with an increased incidence of health problems in stallions, probably due to lack of exercise and prolonged standing on wet and soiled bedding [9]. Although more appropriate compared with the tied system, individual box-housing also has deficiencies, such as social isolation, a significant limitation of normal grazing behavior, and severe lack of exercise, which can trigger abnormal behaviors and health problems in stallions [10]. The majority of adult stallions in Romania, both in state-owned and private facilities, are kept tethered or in individual boxes, with or without paddock access.

Some authors observed fewer behavioral disorders in horses living in paddocks or on pasture [4,11–14], although in stallions, this housing system may favor fights which can cause severe lesions and even the death of the animals in some cases [3,4]. More recently, Gehlen et al. [5] concluded, based on an extended literature review, that group-housing of stallions represents the most adequate system if a few characteristics regarding the exercise area (size and design), the composition of the group, and the horses are considered. Several benefits of this management system have been studied in the reviewed papers on different aspects of equine health or behavior, but not on their overall welfare status considered holistically.

The aim of the present study was to monitor a group of adult intact stallions during their transition from tethered housing with limited outdoor access in individual paddocks to free group housing through the lens of their overall welfare, perceived emotional status, and docility toward humans. Additionally, several correlations between the studied parameters were investigated.

2. Materials and Methods

Disclaimer: This paper presents an observational study of a stallion group in their transition from tethered to free group-housing conditions. All management decisions were planned and implemented solely by the owner of the animals. Although the paper fully describes the sample characteristics and the context, in order to ensure research repeatability, the authors do not encourage or suggest exact replication of the presented conditions to any lay person, especially where thorough supervision of the animals is not possible.

2.1. The Stallions and Their Housing Conditions

The study was performed in the spring of 2022 (April–July) in a privately owned group of 32 stallions (aged between 5 and 18 years). Concerning breeds, they were Hutzul horses (n = 7), Pure Bred Arabians (n = 3), Romanian Draft horses (n = 11), Ardennes (n = 7), and local horses with unregistered genealogy (n = 4). Purchased gradually over the previous five years, the stallions had been housed in a progressively extended wooden barn, chain-tethered on two sides, with periodic access to free movement in the six paddocks next to the barn. These square-shaped outside exercise areas fenced with wooden rails were on flat, rocky terrain, and each had a surface of 100 m^2. The 2 m space between adjacent paddocks did not allow physical contact between the stallions.

The stallions' daily management was performed manually by the owner and two employees. Cleaning the barn, partially replacing the wood shavings and sawdust used as bedding on the compacted earth flooring, and checking the cleanliness of the water buckets mounted on the sides of the feeder boxes were carried out once per day. The stallions were fed four times per day (three meals of hay and one of a mixture of equal amounts of corn, oat, and barley). At these times, the water in the buckets was also replenished as needed. The stallions were hand-led to the paddocks and back to the barn. Most of them were haltered and fitted with a bit, except for two of them, considered the most docile by the owner, which were led only using a rope attached loosely around the base of their necks. Except for five stallions used for work almost every day (forestry, wood hauling), each stallion had access to free exercise for an average of three hours every three days.

This type of management allowed limited social contact between the animals: a certain degree of visual, olfactive, and auditive contact was always possible, especially for stallions tethered next to each other or let out in neighboring paddocks at the same time, but, generally, tactile interaction (physical touch) was not possible. The five stallions used for forestry work were the only exception. Although never unsupervised by humans, during their work (sometimes in pairs) or while resting at the workplace, these animals used to be left close enough so that they could touch each other. For their whole stabled history, the stallions were kept in the same places in the barn and let out in the paddocks in the same order. All the purebred stallions purchased from national stud farms were bought either directly from the breeding category or had been previously in that category. Although the owner used four of them for breeding his own mares, the main reason to keep the stallions intact was the traditional belief that castration causes loss of physical strength and workforce in the animal.

The large outside paddock (approximately rectangular shaped) where the stallions were released for their management change had an approximate area of 15 ha. Its terrain varied (approximately 70% flat, 15% slopes with less than 5° inclination, and 15% slopes with more than 5° inclination), and was mostly covered by grass (approximately 80% of the land had a natural mountain-grass coverage), with the bare ground (covered by fallen leaves) only under the mixed species trees (conifers and broadleaf trees) which had grown in patches (on a total surface area of around 20% of the land). The paddock was protected by double electric fencing (3 m distance between the two fences), with two wire-rows on wooden posts, and a third three-row fence (1 m distance from the outer electric fence-line) made of only bright-white rope between wooden posts. The water source was a shallow streamlet (50 cm at its deepest and between 1 and 1.5 m wide) crossing the terrain lengthwise in a rock bed, with two purposefully constructed watering places where the stream course had been widened to approximately 10 m^2 water-eyes (with a depth less than 50 cm, with additional rock consolidation on the margins around). In addition to these drinking places, the whole length of the streamlet was accessible for the stallions to drink from.

The management change was implemented over a few hours in a single day. Three men with whom the stallions were familiar (the owner and two employees) haltered and led by hand three stallions at the same time, taking them in the order they had been tethered inside the barn (one side of the barn, then the other). This way, each stallion was released at the same time with at least one of his previous neighbors. The animals were led from the barn to the paddock at a distance of about 7–8 m from each other. After entering the paddock, the stallions were led to approximately 45–50 m from each other and then released by removing the halters from their heads. Four additional persons were present, equipped with long whips and ropes, ready to intervene in case of any fighting between the stallions. At the request of the owner, none of the researchers were present at the release of the horses. The description of the procedure is based fully and exclusively on the owner's declaration.

Before the management change, all the stallions had been unshod and had their hooves trimmed. During the study, hoof trimming was repeated in some of them, as decided by the

owner. To avoid fighting as much as possible, the stallions received no additional feeding to supplement grazing.

All the procedures required by the study were performed with the consent and in the presence of the owner. The welfare assessment protocol's application was completely non-invasive, and no animal was stressed in order to be studied.

2.2. Animal Assessments

The farm was visited three times during the stallions' transition from tethered housing to free group keeping. The first assessment was performed a couple of days before the management change, the second two weeks after the stallions were released on pasture in a single group, and the third evaluation three months after the second visit.

For the welfare assessment of the stallions, five freedoms were explored [9] through 25 mostly animal-based parameters (Table 1), following mainly the protocol described by Popescu et al. [9]. Differences from the protocol included the omission of some parameters, the inclusion of the water cleanliness assessment, and the fact that for the freedom from fear and distress evaluation, the interaction between the horses and their owner was observed without the involvement of an unfamiliar assessor.

Table 1. Parameters and scoring in the welfare assessment of the 32 studied stallions.

No	Parameter	Assessment Description
Freedom from hunger and thirst		
1	Body Condition Score (BCS)	Visual and palpatory, on the 5-point scale [15] and scored as 0—improper BCS (emaciated, thin, fat, and obese conditions); 1—good body condition
2	Water cleanliness	Visual, a filled 2 L transparent glass bottle assessed for any change in color and/or turbidity compared with drinking water (0—dirty; 1—clean)
Freedom from discomfort		
3	Body soiling	Visual, assessing the haircoat for any foreign material sticking, which covers an area bigger than the palm of a hand (0—present; 1—absent)
4	Hip point lesions	Visual, assessed on both sides (0—skin lesion; 1—scar, thickened skin with alopecia; 2—absent)
Freedom from pain, injury, or disease		
5	Hair coat condition	Visual, assessed to identify dull, matted hair with or without skin debris and/or alopecia (0—abnormal on extended areas, more than 20 cm in diameter, the presence of several small alopecic spots included; 1—abnormal on limited areas, less than 20 cm in diameter; 2—normal)
6	Hair quality in the mane/tail	Visual, assessed to identify missing and/or broken hairs, skin debris, and dull hair (0—abnormal; 1—normal)
7	Body lesions	Visual and palpatory, assessed to identify interruptions in skin integrity, except for the feet (0—deep lesions interrupting at least the whole thickness of the skin; 1—superficial lesions, without complete penetration of the skin; 2—the absence of lesions)
8	Feet lesions	Visual and palpatory, assessed below the knees and hocks (0—deep lesions interrupting at least the whole thickness of the skin; 1—superficial lesions, without complete penetration of the skin; 2—the absence of lesions)
9	Lip corner lesions	Visual and palpatory, assessed for any visible lesion (0—at least one lesion; 1—the absence of lesions)

Table 1. *Cont.*

No	Parameter	Assessment Description
10	Lesions at harness contact points	Visual and palpatory, assessed in the body areas where the specific harness would be in contact with the body (0—at least one disruption of the skin integrity; 1—missing hair at the harness contact points with no skin interruption; 2—the absence of lesions)
11	Swollen tendons/joints	Visual, assessing the legs and feet (0—both tendons and joints swollen in at least one area; 1—at least a tendon or joint swollen; 2—the absence of swellings)
12	Hoof horn quality	Visual, looking from above, without uplifting the feet, previously washed as needed (0—abnormal, with interruptions, rough surface, lacking periople; 1—normal hoof horn)
13	Hoof shape	Visual, as above (0—abnormal including all possible deviations of shape; 1—normal)
14	Gait	Visual, assessed in the horse walked for at least 10 m on even terrain and turned in both directions (0—lame; 1—abnormal gait; 2—sound)
15	Dyspnea	Visual, assessing the nostrils (straight from the front) and lateral body areas (at an angle of 45° from behind) (0—present; 1—absent)
16	Cough	Auditory, assessed to record any cough over the whole assessment (0—present; 1—absent)
17	Nasal discharge	Visually assessed during the dyspnea assessment (0—present; 1—absent)
18	Ocular discharge	Visual (0—presence of mucopurulent or purulent ocular discharge;1—presence of serous ocular discharge; 2—absence of ocular discharge)
19	Diarrhea	Visual, assessed on the medial and/or caudal aspect of the hindlegs for any fecal soiling (0—present; 1—absent)
Freedom to express normal behavior		
20	Company of other horses	Visual (0—none; 1—limited; 2—unrestricted)
21	Access to free exercise	Visual and owner declaration, assessing the possibility of free exercise in a space that allows minimum 5 steps at gallop in minimum 2 directions for a minimum of 1 h per day (0—none; 1—limited; 2—unrestricted)
Freedom from fear and distress		
22	General alertness	Visual, assessed by observing the horse's body position and response to environmental stimuli (0—apathetic/depressed; 1—alert)
23	Response to the familiar person approaching	Visual, assessed by observing the horse's body language (0—aggressiveness; 1—fear/avoidance; 2—indifference; 3—friendliness)
24	Response to the familiar person walking beside	Visual, assessed by observing the horse's body language (0—aggressiveness; 1—fear/avoidance; 2—indifference; 3—friendliness)
25	Response to the familiar person touching	Visual, assessed by observing the horse's body language (0—aggressiveness; 1—fear/avoidance; 2—indifference; 3—friendliness)

All assessors had attended a previous training exercise in which at least 80% inter- and intra-assessor repeatability was achieved for each parameter of the welfare assessment protocol. The assessors worked in pairs (one assessing, the other writing the results) by rotation.

At the end of this assessment, the qualitative behavioral assessment (QBA) of the stallions was performed, as described and recommended by the AWIN welfare assessment protocol for horses [16]. This QBA relies on the ability of humans to integrate perceived

details of behavior, posture, and context into descriptions of an animal's body language using 13 descriptors with expressive, emotional connotations (aggressive, alarmed, annoyed, apathetic, at ease, curious, friendly, fearful, happy, look for contact, relaxed, pushy, and uneasy—in this order) to provide directly relevant information to animal welfare as a useful addition to information obtained from quantitative indicators. To apply this tool, the horses were observed from 5 to ten10 meters, without disturbing them, for approximately 30 s to one minute. Then, the observer departed to a quiet spot and scored the list of the descriptors using visual analogue scales (VAS), one for each term. Each VAS is a horizontal line between a left "minimum" and right "maximum" point, with the expressive quality indicated by the term entirely absent in the whole observation period at the minimum and dominant during the whole observation period at the maximum. The intermediate scores ticked with a mark on the line depending on the intensity and duration of a behavior. The measure for each term is the distance in millimeters from the minimum point to the point where the VAS is ticked.

In order to score the two parameters which evaluated their human-related docility (Table 2), the stallions were observed by an assessor while being handled by their owner.

Table 2. Parameters and scoring in the docility assessment of the 32 studied stallions.

	Docility	
1	Response to the familiar person putting a rope around the base of the horse's neck	Visual, assessed by observing the horse's body language (0—aggressiveness; 1—fear/avoidance; 2—indifference; 3—friendliness)
2	Response to the familiar person attaching a halter on the horse's head	Visual, assessed by observing the horse's body language (0—aggressiveness; 1—fear/avoidance; 2—indifference; 3—friendliness)

2.3. Data Processing and Statistical Analysis

As in the work described by Popescu et al. [9], an individual welfare quality score (IWQS) was calculated for each stallion in each of the three evaluations performed by adding up the scores of the welfare assessment parameters. The range of this score was from a theoretical zero to a maximum of 41, showing better welfare quality with the increase in the score.

To provide qualitative significance to the numerical results of the welfare assessment, each stallion was included in one of the four qualitative welfare classes according to their IWQS: not classified (scores from 0 to 15), acceptable (between 16 and 25), enhanced (from 26 to 35), and excellent (ranging from 36 to 41). The number of stallions in each qualitative welfare class was calculated as a percentage in each of the three assessments.

The calculation of the docility score (DS) was performed by adding up the scores recorded for the two parameters of the human-related docility test by each stallion in each of the assessments. The higher the DS was, the more docile the animal was considered.

The results were analyzed using SPSS (SPSS version 17, SPSS Inc., Chicago, IL, USA) software. The mean, standard error of the mean, median, minimum, and maximum were calculated as descriptive statistical parameters. The normality distribution of the data was tested by the Kolmogorov–Smirnov test, and Friedman and subsequent Wilcoxon tests were used to evaluate the parameter changes in relation to time. A principal component analysis (PCA), using a correlation matrix and applying no rotation, was conducted on the QBA descriptors. Because the data were not normally distributed, the Spearman rank correlations were used to study the relationships between the assessed parameters. Differences and correlations were considered statistically significant at a p-value < 0.05.

3. Results

3.1. Welfare Parameters of the Studied Stallions

The results of the welfare assessments were compared between the three evaluations (Table 3), before (assessment 1—A1), and after the stallions' management change (assessment 2—A2, assessment 3—A3). For the majority of the evaluated parameters, there were significant differences, except for those which investigated the freedom from fear and distress.

Table 3. Results for the welfare parameters (improper) of the stallions in each of the three assessments and the significance of differences between them.

Parameter	Percentage of Stallions (Number of Stallions)			*p* Value (Friedman Test)
	A1	A2	A3	
Freedom from hunger and thirst				
BCS (improper)	21.88 (7)	25 (8)	12.5 (4)	0.197
Water cleanliness (dirty)	21.88 (7)	0	0	0.001
Freedom from discomfort				
Body soiling (present)	31.25 (10)	12.5 (4)	18.75 (6)	0.135
Hip point lesions (present)	34.38 (11)	25 (8)	3.13 (1)	<0.001
Freedom from pain, injury, and disease				
Hair coat condition (abnormal)	25 (8)	15.63 (5)	3.13 (1)	0.008
Hair quality in the mane/tail (abnormal)	3.13 (1)	9.38 (3)	9.38 (3)	0.368
Body lesions (present)				0.003
- deep	6.25 (2)	15.63 (5)	0	0.05
- superficial	6.25 (2)	34.38 (11)	31.25 (10)	0.016
Feet lesions (present)				0.001
- deep	9.38 (3)	3.13 (1)	0	0.164
- superficial	18.75 (6)	15.63 (5)	0	0.043
Lip corner lesions (present)	15.63 (5)	3.13 (1)	0	0.015
Lesions at harness contact points (present)	28.13 (9)	25 (8)	6.25 (2)	0.001
Swollen tendons/joints (present)	34.38 (11)	15.63 (5)	6.25 (2)	<0.001
Hoof horn quality (abnormal)	9.38 (3)	6.25 (2)	3.13 (1)	0.135
Hoof shape (abnormal)	12.5 (4)	12.5 (4)	9.38 (3)	0.368
Gait				0.045
- lame	12.5 (4)	9.38 (3)	0	0.05
- abnormal	18.75 (6)	25 (8)	21.88 (7)	0.834
Dyspnea (present)	12.5 (4)	6.25 (2)	0	0.05
Cough (present)	9.38 (3)	6.25 (2)	0	0.174
Nasal discharge (present)	15.63 (5)	6.25 (2)	0	0.004
Ocular discharge (present)	12.5 (4)	15.63 (5)	3.13 (1)	0.074
Diarrhea (present)	0	15.63 (5)	0	0.007
Freedom to express normal behavior				
Company of other horses				<0.001
- none	0	0	0	<0001
- limited	100 (32)	0	0	<0.001
Access to free exercise				<0.001
- none	71.88 (23)	0	0	<0.001
- limited	28.13 (9)	0	0	<0.001

Table 3. *Cont.*

Parameter	Percentage of Stallions (Number of Stallions)			*p* Value (Friedman Test)
	A1	A2	A3	
Freedom from fear and distress				
General alertness (apathetic/depressed)	6.25 (2)	0	0	0.368
Response to the familiar person approaching				0.368
- aggressiveness	0	0	0	-
- fear/avoidance	3.13 (1)	0	3.13 (1)	0.603
- indifference	31.25 (10)	43.75 (14)	28.13 (9)	0.383
- friendliness	65.63 (21)	56.25 (18)	68.75 (22)	0.561
Response to the familiar person walking beside				0.819
- aggressiveness	0	0	0	-
- fear/avoidance	6.25 (2)	6.25 (2)	3.13 (1)	0.812
- indifference	43.75 (14)	40.63 (13)	37.5 (12)	0.802
- friendliness	50.00 (16)	53.13 (17)	59.38 (19)	0.748
Response to the familiar person touching				0.595
- aggressiveness	0	0	0	-
- fear/avoidance	6.25 (2)	6.25 (2)	9.38 (3)	0.859
- indifference	62.5 (20)	34.38 (11)	37.5 (12)	0.068
- friendliness	31.25 (10)	59.38 (19)	53.13 (17)	0.063

A1—first assessment; A2—second assessment; A3—third assessment. If the *p*-value is less than 0.05, the difference between assessments is significant.

3.2. Dynamics of Individual Welfare Quality and Docility Scores of the Stallions during Their Management Transition

The IWQS median increased significantly from the first to the second and third evaluations, while the DS had similar values in the three assessments (Table 4).

Table 4. Descriptive statistics of the individual welfare quality and docility scores in the assessed stallions in each of the three assessments performed.

Parameter	Individual Welfare Quality Scores (IWQS)			Individual Docility Scores (IDS)		
	A1	A2	A3	A1	A2	A3
Mean	32.28	36.03	38.41	4.71	4.50	4.47
Standard Error of the mean	0.72	0.54	0.36	0.20	0.21	0.21
Median	33.5 [a]	36.00 [b]	38.5 [c]	5.00	4.50	5.00
Minimum	24.00	31.00	33.00	2.00	2.00	2.00
Maximum	37.00	41.00	41.00	6.00	6.00	6.00

[a,b,c] Values in a row with no common superscript are significantly different ($p < 0.05$).

Without exception, the individual welfare quality scores for the third assessment were higher than those recorded in the first assessment, even where the second assessment score was lower (stallion no. 4) or equal to the first one (stallions no. 13 and 32), or when the second assessment score exceeded (stallions no. 5, 17, 18, 25, and 28) or equaled (stallions no. 7, 10, and 11) the IWQS recorded in the third assessment (Figure 1).

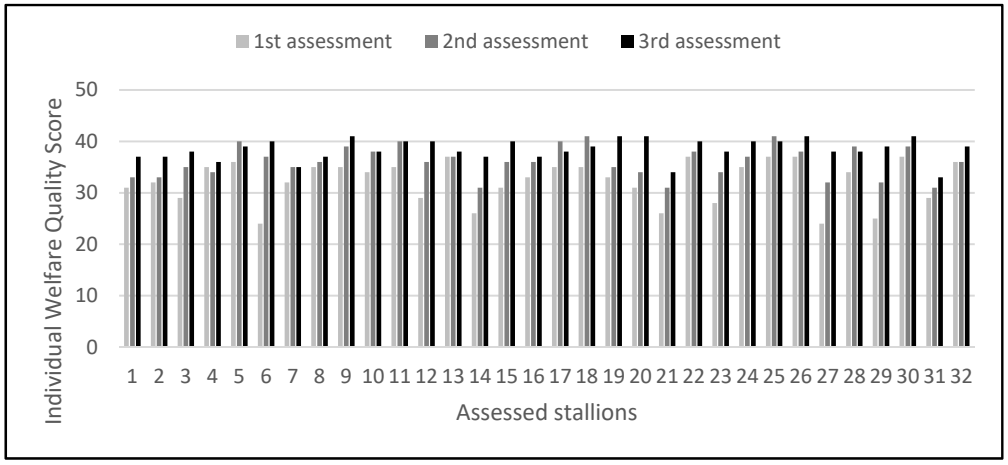

Figure 1. Evolution of the IQWS in the 32 stallions within the three welfare assessments performed.

There were no stallions in the 'not classified' qualitative welfare class (according to their IWQS) in any of the assessments. The percentage of stallions included in the other three classes and their variation from one assessment to the other are shown in Figure 2.

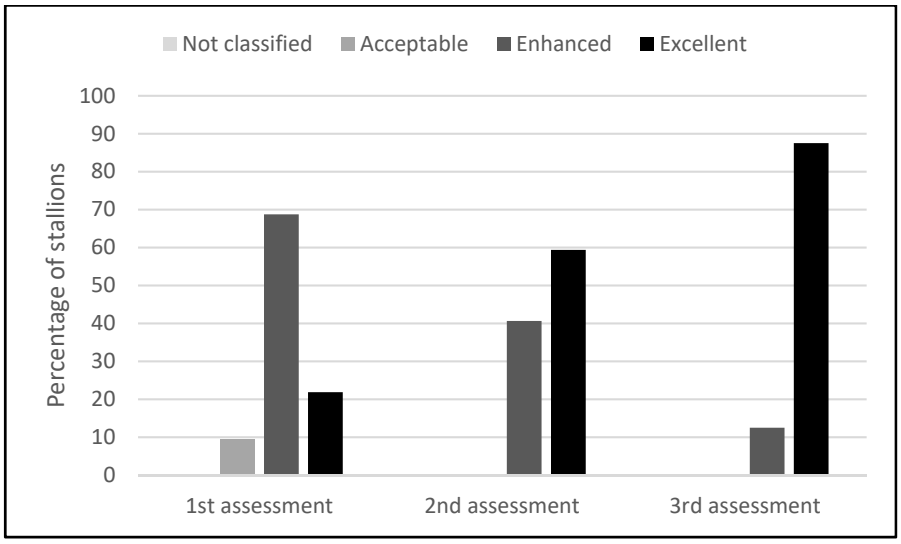

Figure 2. Percentages of stallions in the established qualitative welfare classes (acceptable, enhanced, and excellent) in the three welfare assessments performed.

Three stallions were included in the 'Acceptable' class in the first assessment but none in the second and third assessments. The rest of the stallions were included in the two superior classes (enhanced and excellent), and their percentage in the 'Excellent' class increased from the first to the third assessment (Figure 2).

For the 'Acceptable' class, a statistically significant difference was found between the first and third assessments ($p = 0.047$). The number of stallions in the 'Enhanced' class differed significantly between the first and second ($p = 0.025$), between the first and third ($p < 0.001$), and between the second and third assessments ($p = 0.025$). The differences in numbers within the 'Excellent' class were significant between the first and third ($p < 0.001$), first and second ($p = 0.002$), and second and third assessments ($p = 0.011$).

3.3. Principal Component Analysis of the Qualitative Behavioral Assessment Descriptors

The PCA was applied to the first and third assessments performed in the stallions before the management change and three and a half months after their release in a single free-housed group. Three principal components have been identified, as shown in Table 5.

Table 5. Loadings for the QBA descriptors on the first three principal components.

Descriptor	A1			A2			A3		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Aggressive	−0.183	0.190	-	-	0.721 *	-	-	0.694 *	0.130
Alarmed	−0.779 *	-	−0.149	−0.781 *	0.168	−0.307	−0.862 *	-	−0.245
Annoyed	−0.526 *	0.646 *	0.104	−0.471 *	0.605 *	0.132	−0.607 *	0.608 *	-
Apathetic	−0.170	−0.325	**0**.861 *	−0.273	−0.379	−0.833 *	−0.134	−0.455 *	−0.815 *
At ease	0.593 *	−0.380 *	0.433 *	0.559 *	−0.394	0.321	**0**.737 *	−0.354	0.129
Curious	**0**.664 *	0.389	-	0.717 *	0.462 *	-	0.815 *	0.447 *	-
Friendly	**0**.842 *	0.283	-	**0**.886 *	0.259	0.129	**0**.879 *	0.269	-
Fearful	−0.896 *	-	−0.250	−0.824 *	-	−0.357	−0.910 *	-	−0.276
Happy	−0.627 *	-	−0.589 *	0.637 *	0.135	0.628 *	0.445 *	0.277	**0**.731 *
Look for contact	**0**.595 *	0.213	-	0.731 *	0.495 *	-	0.724 *	0.487 *	-
Relaxed	**0**.791 *	−0.272	−0.252	0.720 *	−0.413 *	−0.210	**0**.757 *	−0.326	−0.210
Pushy	0.311	**0**.659 *	0.344	0.402	**0**.627 *	0.354	0.318	**0**.736 *	0.348
Uneasy	−0.534 *	0.641 *	-	−0.546 *	0.630 *	0.257	−0.651 *	0.602 *	0.189
Eigen values	5.005	2.914	1.575	5.117	2.722	1.695	5.761	2.784	1.585
Variance explained (%)	38.501	22.418	12.112	39.364	20.938	13.040	44.313	21.417	12.193
Cumulative variance explained	38.501	60.919	73.030	39.364	60.302	73.342	44.313	65.730	77.923

The numbers with asterisks (*) indicate higher (positive or negative) loadings in the three assessments within each PC.

In the first assessment, the QBA descriptors on PC1 ranged from negative emotional states, such as fearful and alarmed, to positive, such as friendly and relaxed; on PC2, from pushy to apathetic; on PC3, from apathetic to happy. In the second assessment, the PC1 arranged the QBA descriptors from fearful/alarmed to friendly/relaxed/curious, the PC2 from aggressive to uneasy/pushy, and the PC3 from apathetic to happy. In the third evaluation, the QBA descriptors on PC1 ranged from fearful/alarmed to friendly/curious/relaxed/look for contact, on PC2 from aggressive to pushy, and on PC3 from apathetic to happy.

Table 6 shows the first three principal components and their coefficients. Generally, on all components, the correlation coefficients have been low (less than 0.2). The strongest correlations were found for the apathy and happiness descriptors (Table 6).

Table 6. Component score coefficient matrix for three principal components in the three qualitative behavior assessments of the stallions.

Descriptor	A1			A2			A3		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Aggressive	−0.03	0.17	−0.01	−0.01	0.26 *	0.01	−0.01	0.25 *	0.08
Alarmed	−0.15	0.02	−0.09	−0.15	0.06	−0.18	−0.15	0.02	−0.15
Annoyed	−0.10	0.22 *	0.07	−0.09	0.22 *	0.08	−0.10	0.22 *	0.05
Apathetic	−0.03	−0.11	0.55 *	−0.05	−0.14	0.49 *	−0.02	−0.16	0.51 *
At ease	0.12	−0.13	0.27 *	0.10	−0.15	0.19	0.13	−0.13	0.08
Curious	0.13	0.20 *	0.06	0.14	0.17	0.05	0.14	0.16	0.02
Friendly	0.17	0.10	0.04	0.17	0.09	0.08	0.15	0.11	0.02
Fearful	−0.18	−0.02	−0.16	−0.16	0.03	−0.21 *	−0.16	0.01	−0.17
Happy	0.13	0.03	−0.37 *	0.12	0.05	−0.37 *	0.08	0.10	0.46 *
Look for contact	0.12	0.18	0.04	0.14	0.18	−0.04	0.13	0.18	0.05
Relaxed	0.16	−0.09	−0.16	0.14	−0.15	−0.12	0.13	−0.12	−0.13
Pushy	0.06	0.23 *	0.22 *	0.08	0.23 *	0.21 *	0.05	0.26 *	0.22 *
Uneasy	−0.11	0.22 *	0.06	−0.11	0.23 *	0.15	−0.11	0.22 *	0.12

Extraction method: Principal component analysis; * coefficients beyond 0.2 reflect a considerable positive/negative relationship between the principal component and the original variable [17].

3.4. Correlations between the Assessed Parameters

To explore the relationship between the stallions' welfare degree, docility, and their emotional states, correlations have been calculated, as shown in Table 7.

Table 7. Correlations between the first three principal components (PCs) and the IWQS and DS in the studied stallions.

Assessment	A1		A2		A3	
Principal Component	IWQS	DS	IWQS	DS	IWQS	DS
PC1	0.35 *	0.32	0.33	0.19	0.36 *	0.32
PC2	0.05	0.18	0.11	0.11	0.14	−0.09
PC3	0.48 *	0.38 *	−0.25	0.43 *	0.00	0.24

* Correlation is significant at the 0.05 level (2-tailed).

The PC1 correlated significantly with the IWQS in the first (A1) and third (A3) assessments. The PC3 correlated with the IWQS and the DS in the first assessment (A1) and in the second assessment (A2) only with the DS.

Constantly significant correlations have been found in all three assessments between the stallions' IWQS and DS (A1, $r_s = 0.386$, $p = 0.01$; A2, $r_s = 0.357$, $p = 0.05$; A3, $r_s = 0.521$, $p = 0.01$).

4. Discussion

4.1. Dynamics of the Welfare Parameters during the Stallions' Management Change

According to the results of a recent literature review performed by Gehlen et al. [5], the group husbandry of stallions is not only possible but also a desirable response of horse breeders to the increasing social awareness of animal welfare. There is a considerable body of research relating to the provision of husbandry in such a way as to allow species-specific and natural conditions for all horses, including adult stallions. Several housing systems and transition methods have been tested around the world, more or less traditionally [18], to overcome the popular belief that isolation is an acceptable method for keeping adult stallions safe.

The transition from tie-stalls to free group housing has generally had a positive influence on the welfare parameters assessed in our study, measurable in as little as two weeks from the implementation of the management change (Table 3).

4.1.1. Freedom from Hunger and Thirst

One of the important indicators relevant to freedom from hunger and thirst is the body condition score (BCS). Similar to the results reported by Yngvesson et al. [19], no statistically significant differences were found in the BCS of the stallions between the assessment before the management change and the two measurements after it. Nevertheless, in our study, the prevalence of stallions with improper BCS decreased in three months (at the third assessment—A3) of free group housing outdoors. There are many different factors and possible ailments contributing to improper body weight in horses, whether the BCS is too low (thin or emaciated animals) or too high (fat or obese animals). However, in the studied healthy stallions kept in relative inactivity, the access to a more natural lifestyle of outside exercise and grazing seemed to normalize their body weight, promoting fitness in both the formerly overweight and underweight individuals. In a comparative study on breeding horses kept in tie-stalls (stallions) and loose group housing (mares), Sanmartín Sánchez et al. [20] found a higher risk for low BCS in the free-housed mares, despite them having free access to a greater variety of forage than the stallions, in conditions allowing more natural equine behavior. Possible factors of influence on the free-range horses' BCS highlighted by the mentioned study [20] are lower pasture quality, competition for feeding, and possibly better teeth condition in the stabled stallions. In our study, these factors

were not present, and most probably, the fat stallions lost weight because of more physical activity in freedom, and the thin ones ate more, having continuous free access to good quality pasture.

In our study, the husbandry system change also had a positive impact on water cleanliness due to the water source the stallions had access to while free-housed. Water is an essential nutrient for all mammals, especially horses, who need considerable amounts of water for their hindgut microbiota for proper fermentation [21]. Thus, fresh and clean drinking water should be available for horses at all times, not only because of its nutritional importance but also for its role in preventing digestive problems with potentially life-threatening severity.

4.1.2. Freedom from Discomfort

The hip point assessment is used to reveal possible lesions, present wounds, or healed scars indicative of housing discomfort. Especially in sick horses which spend prolonged periods lying down, the pressure of a bone on a reduced skin surface causes tissular ischemia and subsequent necrosis [20,22]. The hip point (external angle of the ilium) is an anatomical area predisposed to such types of wounds. Similarly, in healthy horses, hip point lesions occur while lying on hard, abrasive surfaces without bedding or when the bedding is insufficient, dirty, or wet [23]. The pre-existent individual conditions that precipitate the occurrence of decubital ulcers include the loss of subcutaneous fat padding in thin equines [22], but also inappropriate hygiene of the skin [24]. It seems that the main factor triggering these lesions is improper resting surfaces because these injuries were identified even in obese horses, which had no other body lesions [23]. The results of the present study seem to confirm this assumption, as the prevalence of hip point lesions decreased significantly after the stallions had access to softer and less abrasive resting surfaces of their choice outside the barn (Table 3).

4.1.3. Freedom from Pain, Injury, and Disease

Most health problems were more common in the tie-stalled stallions, and many of these improved significantly in time after their transition to free group housing (Table 3). For example, the abnormal hair coat condition noticed in eight stallions in the first assessment persisted in only one of them until the final (third) evaluation, a significant ($p < 0.05$) improvement (Table 3). Body condition and coat quality have been widely used indicators in welfare assessment protocols (e.g., the Working Equine Welfare Assessment protocol or Standardized Equine Based Welfare Assessment tool—SEWBAT [25] and Animal Welfare Indicators—AWIN [16] protocols), both in working and recreational horses [26]. As for body lesions, their prevalence increased significantly after the stallions' transition to free group housing (from the first to the second assessment—A1 to A2), and then it decreased from the second to the third assessment. As opposed to several studies reviewed by Gehlen et al. [5] on adult stallions' transition from isolated to free group housing, minimal previous acclimatization was provided to the animals in the present study. As stated above, we do not encourage the described conditions to be reproduced, especially without continuous and close monitoring of the stallions. The owner's choices and decisions nevertheless allowed us to observe the consequences of this type of transition. The large open space enabled escape possibilities for the animals from each other, a very important positive aspect, but, on the negative side, the lack of more diverse previous social contact triggered aggressive encounters, especially in the first few days after their release. Group dynamics can change rapidly in this type of setting. As Briefer Freymond et al. [27] describe, ritual and affiliative interactions do not involve physical aggression, but agonistic interactions have the potential to do so, and all three occur within a group of free stallions. In only three to four days, the frequency of agonistic and ritual behaviors decreases in the group, according to Briefer Freymond et al. [27], and certain previous experiences in group housing may minimize aggression between free-housed stallions (when their management is periodically switched between individual and group housing). In our opinion, the visual,

olfactory, and auditive contacts possible between the studied stallions led to a certain degree of acclimatization, even if each animal could best experience these with only two neighbors (both while tethered inside the barn and outside in the paddocks), due to the farm's daily routines. These practices were meant to minimize aggression in the given setting but rotating the stallions without being allowed tactile interaction could have been beneficial at the moment of their release (a higher number of horses acclimatized with each other previously). Furthermore, closer social contact, with the possibility of tactile interaction, such as the use of "social boxes" [5], could have lowered aggression during the stallions' group integration. However, after the initial increase in the frequency of body lesions (recorded two weeks after the housing system change), the number of deep wounds decreased significantly ($p = 0.001$) to none in three months, even if the frequency of superficial body lesions remained five times higher than before the management system change. According to Briefer Freymond et al. [27], a stable hierarchy is established and measurable in two to three months after the stallions are released as a group. This is consistent with our results regarding the decreasing frequency of more serious (deep) wounds, mainly produced by aggressive encounters between the stallions on the first days of free group housing. The high prevalence of superficial body lesions recorded at three months from the management change included several wounds which have been found to be deep at the previous welfare assessment but healed meanwhile.

Some of the health parameters of freedom from pain, injury, and disease improved as a consequence of the management change but not of the group housing in itself. The significantly lower prevalence ($p < 0.05$, Table 3) of lip corners, harness contact points, and feet lesions occurred through the healing of previous wounds and non-exposure to new ones, as the stallions have not been used for work at all during the study. The improvement of their hoof horn quality may have been related to nutrition with higher biotin and vitamin quantities (grass vs. hay), although for a pertinent conclusion, a longer study period would be needed. As Josseck et al. [28] describe, biotin supplementation does not accelerate hoof growth (7 mm/28 days, complete hoof renewal in 11 months), but it leads to significant hoof condition improvement in nine months; only a third of that period was covered by the present study, and our differences were not statistically significant ($p > 0.05$, Table 3).

The condition of more than half of the horses with swollen tendons/joints improved in only two weeks, and 82% of those with leg swellings at the first assessment were free of this problem in three months (Table 3); the difference between successive assessments being highly significant ($p < 0.001$). Although no diagnostic was established for the volume and shape modifications of the legs, based on their quick and spontaneous remedial (without any treatment or movement restrictions), the cause might have been mainly circulatory (peripheral edemas) due to insufficient exercise for some of the stabled stallions. However, volume changes in horse limbs were shown to occur quite quickly in relation to exercise. Using an optometric device, Siewert et al. [29] measured a significant decrease in limb volume immediately after exercise (compared to the inactive value measured after a 12 h standing period) but also an increase after one hour of rest following the exercise, with significantly bigger changes in male horses compared to females. Thus, in our study, the edematous limb swellings could have also been caused by excessive effort (in the stallions used for wood-hauling). For possibly similar reasons, the lameness prevalence decreased between successive assessments, but the number of stallions with abnormal gait did not.

Respiratory problems (dyspnea, cough, nasal discharge) had a higher frequency in our first assessment while the stallions were tie-stall housed later (Table 3) while kept outside. It was previously found that poor indoor air quality can negatively impact the respiratory health of both horses and humans [30]. The tight link between equine respiratory health and air quality was recently reinforced once again through an interdisciplinary effort over 19 years (between 2000 and 2019) to study equine airway inflammation, to clarify the phenotype and terminology involved, and to finally introduce the term 'equine asthma' for horses with chronic respiratory signs, previously referred to as 'inflammatory airway disease' and 'recurrent airway obstruction'. The 2019 report [31] on this topic states that

the role of exposure to environmental dust in the pathophysiology of both mild and severe equine asthma is supported by strong evidence. Thus, the conclusion that "the pasture is the best housing" drawn more than 20 years ago by McGorum et al. [32] still holds ground.

4.1.4. Freedom to Express Normal Behavior

In addition to better air quality, group housing on a pasture has tremendous benefits regarding the freedom to express normal behavior. As expected, compared to the previous (tie-stall) management system in the present study, both parameters (the company of other horses and access to free exercise, Table 3) showed statistically significant differences. Daily free-running is a behavioral and physiological requirement for all horses [33] as neither work nor the use of training devices can fulfill their need for free exercise [34]. In addition to the general health-preserving benefits of free exercise on the respiratory tract, locomotor apparatus, and immune system of horses, and against the traditional misbelief that performing horses need rest rather than free movement [35], the positive consequences of active recovery have been shown not only in terms of recovery time and performance but also of general welfare improvements, even in horses on intensive physical effort programs [33].

In respect of the importance of company of the same species Dierendonck [36] concludes in the light of the large amount of available literature that social positive physical interactions (allogrooming, play) represent an ethological need for horses, a highly motivated behavior, so important for the animal that husbandry systems that do not provide access to it cause chronic stress. The author states that all horses need physical and social contact [36]. Although with a special focus on horse–human interactions, Rørvang et al. [37] describe the importance of each sensory ability in equine communication with individuals of the same or different species, highlighting the possible reinforcing value of tactile stimulation during affiliative interactions (mutual grooming, swishing flies for one another, and standing in close proximity while grazing or resting). These findings indicate that free interactions between horses are much better than olfactory, visual, and auditive interactions in stabled or individually exercised animals. Of course, limited interaction with their own kind was proved to still be better than the lack of it (complete isolation), as Houpt and Waran [38] admit for mares chronically confined in tie-stalls with severe movement restrictions (in the pregnant mare urine production industry) developing fewer stereotypies compared to box-stalled Thoroughbreds.

4.1.5. Freedom from Fear and Distress

This section of the welfare assessment explored the dynamics of the horse–human relationship during the management transition. Our results did not reveal any significant impact of the pasture release on the stallions' responsivity toward humans (the owner or caregivers). However, a reduction of the indifference to humans can be observed (Table 3) when the results of the first assessment were compared to those of the third (alongside the increasing welfare degree of the stallions). Some authors [39,40] found the strongest correlations between indifference and the absence of free exercise, barn dirtiness, poor body condition, and health problems in working equines.

The ability of horses to recognize familiar humans (based on a global, integrated, multisensorial representation of the person) and to adapt their behavior to expectancies (based on previous experiences) in a familiar situation with that person has been proven by several studies [41,42]. Another important scientific finding along the same lines was the first-time discovery that horses are capable of spontaneous cross-modal recognition of individuals from a morphologically very different (and phylogenetically very distant) species, matching visual and auditory information from familiar humans [43]. More recent research revealed their ability to differentiate between familiar and unfamiliar humans from photographs of faces [44] and the fact that they employ a holistic mental approach rather than processing the images as simple abstract shapes [40], clarifying even more that horses recognize and remember their caregivers, even when they have no interaction with

them for longer periods of time [45]. Our results regarding the response of the stallions at the approach, walking beside, and touch of the familiar person (Table 3) are explained by this equine ability to recognize and remember a human, and also to adapt their behavioral reactions according to their previous experiences—neutral or positive in the present study. As Boissy et al. [46] proved, positive interactions and experiences with humans have beneficial effects on improving the animals' welfare degree, and, we could add, the effects persist even through management changes which provide more freedom (and avoidance possibility) to the stallions in our case.

4.2. Effects of the Management Change on the Stallions' Overall Welfare and Docility

Changing the housing and management system from tie-stalls with limited outside access and social contact to free group housing had a positive influence on the studied stallions' overall welfare, their individual welfare quality scores (IWQS) being significantly higher in the second and third assessments than in the first one (Table 4, Figure 1). The overall improvement in the stallions' welfare was also visible when they were assigned to welfare categories based on their IWQS in each of the three assessments (Figure 2). This finding is in accordance with other studies which show that living in a paddock or on a pasture is more appropriate than living in a box for the welfare of stallions [4,11,13]. Providing horses with a living environment more similar to their natural conditions is part of welfare improvement [13], especially when the simultaneous contribution to each of the five freedoms are considered. However, certain elements of the environment and management system influence several aspects of the overall welfare, being framed in more than one freedom. The free group housing on a pasture compared to the tie-stall management improved at the same time the physical, mental, and natural components of the stallions' welfare, bringing measurable benefits to each freedom. Under natural conditions, horses spend between 75% and 90% of their time grazing [10,47], usually walking continuously at a slow pace [48]. According to McGreevy [49], this way, they can travel as much as 65–80 km per day, having all the benefits of the social interactions in the group. After the management change, the studied stallions had constant access to pasture and a water source, a clean and comfortable surface to rest on—the soft, warm, and dry earth of the pasture under the protection of dense tree canopy—access to the company of the same species, being allowed natural behaviors such as grazing, running, and rolling, and not being frightened and stressed by human interventions. These conditions fulfilled four out of the five freedoms of animal welfare. The only less addressed freedom was the one from pain, injury, and disease, although frequent monitoring and surveillance enabled timely intervention and treatment when required, even prevention at all times. In the present study, an improvement in the stallions' welfare quality was observed in as little as two weeks from their transition to free group housing. This proves a positive influence of the free system on the overall welfare of the horses, an aspect that has been previously suggested but not proved by other studies which focused only on selected elements (health, behavior, human–horse relationship, and so on).

One of the widespread concerns about horses kept with less frequent human interactions is whether they maintain (and for how long) their willingness to remain 'submissive' to humans or if their docility decreases when handled less, and there is not much systematical research available to answer these questions. The timing of the decrease in the frequency of human manipulation, how well the previous training has been conducted, and for how long no element of it is recalled are all relevant factors. However, with regard to human safety around horses, Rivera et al. [12] noted that horses housed in paddocks are less aggressive toward humans than those kept in boxes. The importance of docility has been recognized in horses since the early times of domestication and selective breeding and later on, during the development of different classical horse breeds [50]. Still highly desirable in the entire horse industry, docility is continuously studied today. As recent genetic sequencing research [51] shows, through the discovery of a genomic region that may have influenced it, docility and a strong back were even highly valued traits in the Bronze Age.

Other studies, using the term trainability for docility, attempted to determine a possible connection between this trait and hormones such as oxytocin and serotonin [52,53]. In our study, the housing system's change did not influence the docility score of the stallions. Given the numerous studies proving that horses are able to recognize humans [41–45] and remember familiar persons for longer periods of time even without seeing them [45], the plausible explanation of this finding was the fact that the people interacting with the stallions were the same with whom they had been habituated previously.

4.3. Effects of the Management Change on the Qualitative Behavioral Assessment (QBA) Descriptors of the Studied Stallions

Although no significant differences were found regarding the qualitative behavioral assessment (QBA) of the stallions in the successive evaluations (transition from one management system to another, time passed since the management change), some aspects were still notable. As Tables 6 and 7 show, considering only the variables for which the correlation coefficients between the original variables and PCs were above 0.2 (as recommended by Bassler et al. [17]), the stallions were more annoyed, apathetic, uneasy, pushy, and less at ease and happy, than later. In the second and third evaluations, the animals seemed happier and less apathetic than during the first determination. As their welfare improved, the stallions seemed to be happier. These findings support the statement of Boissy et al. [46] that if an animal is in a happy emotional state, then its needs are being met, and its welfare is good.

The QBA was developed and promoted to determine and include indicators of the animals' positive state in welfare assessments. An intrinsically holistic and dynamic tool, QBA addresses the whole animal in terms of its behavior [54], allowing the assessor to integrate these behavioral expressions using descriptors that reflect the animal's putative emotional experiences [55]. Applied in an impressive number of studies on many domestic animal species, QBA was even proposed as a potential 'first pass' screening method to decide if a further in-depth assessment may be warranted on a specific farm [56]. Far from being generally accepted as an "ideal method", QBA was also firmly disputed in the scientific literature for its limitations [57,58].

Specifically in horses, QBA was found to be useful in identifying more positive affective states [54]. Indeed, irrespective of the assessment in our study (before the management change, two weeks later, or three months later), the tendencies of almost all principal components (PCs) showed a direction from negative to positive emotional states, except for the first assessment, where on PC2 the tendency was from pushy to apathetic. As the overall welfare of the stallions improved, the tendency on PC1 became richer (Table 5), and the stallions added progressively to the friendly and relaxed attitudes (first assessment), curiosity (in the second assessment), and then a keenness for contact (look for contact, in the third assessment). Less relevant, on PC2 in the second and third assessments, the tendency gravitated around aggressiveness and a pushy attitude (defined as assertive or forceful, not leaving space, head butting, exhibiting dominant behavior, possibly mouthy or nippy [AWIN, 2015]), with a rather negative connotation. On PC3, the tendency was constant, from apathetic to happy, irrespective of the housing system and conditions the stallions were in. Analyzing these results, we note that the QBA identified positive states in each assessment of the studied stallions, but their management transitioning and change in their overall welfare were not closely reflected by the dynamics of the emotional states identified.

Moreover, when looking at the relationship between the PCs and the original variables (Table 6), a constant considerable relationship (with a correlation coefficient above 0.2 as recommended by Bassler et al. [17]) was found only for apathy and happiness on PC3, and for pushy attitude on PC2 and PC3, with the strongest correlations for the first two (apathy and happiness), lessening the importance of the other 10 descriptors (Table 6) for our study. As Hausberger et al. [59] note in a comprehensive review of equine welfare assessment methods, the validity of QBA remains in question. The same review shows that this method's validation has not been tested for adult horses. The same authors [59] warn

that human representations of behaviors' significance may be influenced by a variety of factors (culture, access to a reference population, personal experience), which can introduce a bias-induced error at the very early stages of data collection.

4.4. Interrelations between the Studied Parameters during the Stallions' Management Change

To explore the significance of the QBA descriptors in the specific setting in our study further, the PCs were studied in relation to the IWQS and DS of the stallions (Table 7). When it comes to the statistically significant correlations between QBA scores and physiological or other quantitative measures relevant to welfare proved by many authors (reviewed by Fleming et al. [56]), not all research performed is congruent. Andreasen et al. [60] show weak correlations of QBA scores to Welfare Quality® measures (and no meaningful pattern of relationship between these measures) in dairy cows. Similarly, Hausberger et al. [59] note that the relationship between welfare indicators and QBA results is not straightforward for donkeys. In our study, the correlations were found to be inconsistent: the PC1 correlated significantly with the IWQS in the first (A1) and third (A3) assessments; the PC3 correlated with the IWQS and DS in the first assessment (A1), and in the second assessment (A2) only with the DS (Table 7).

Although QBA is a promising tool to complement horse welfare assessments in situations of multiple emotional dimensions of both positive and negative valences [55], and it is sensitive to the quality of the horse–human relationship [54], in light of our results of weak correlations and low relevance for the assessment for the stallions' management transition, we consider that most QBA descriptors did not fit the type of study described in this paper. More specific research in similar conditions is required.

Regarding the relationship between the IWQS of the stallions and their DS, interestingly, these showed constantly significant positive correlations in all three assessments, enforcing the hypothesis that the docility of the stallions did not decrease as their welfare improved, even if they had been handled by humans less frequently during the period of our study.

5. Conclusions

Given the above results, we conclude that the free group housing of adult stallions improved their overall welfare and did not reduce their docility toward familiar humans compared to their previous management system (tie-stall housing). These are two important aspects that have not been studied in this manner before. The successive assessments showed the improvement of the stallions' IWQS in only two weeks from the change in the management system, with further improvements in time once the group's hierarchy stabilized. The constant positive correlation between the DS and IWQS of the stallions in the successive assessments (despite less frequent human–animal contact after the management change) is a promising result, further providing incentives to stallion owners who consider implementing similar management changes for their animals. Many of the assessed qualitative behavioral descriptors showed no relevance for our study and followed neither the welfare degree nor the docility level of the stallions closely. Thus, we consider that most QBA descriptors require more specific studies in similar conditions to those described.

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RESEARCH ON THE QUALITY OF THE LIVING ENVIRONMENT AND ITS INFLUENCE ON THE HEALTH OF STURGEONS IN A FISH FARM IN EAST ROMANIA

CERCETĂRI PRIVIND CALITATEA MEDIULUI DE VIAȚĂ ȘI INFLUENȚA ACESTUIA ASUPRA SĂNĂTĂȚII STURIONILOR ÎNTR-O FERMĂ PISCICOLĂ DIN ESTUL ȚĂRII

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ABSTRACT | REZUMAT

The quality of the sturgeon's living environment, the equipment used in the farm, the microclimate factors (noise, lighting), the biosecurity of the area where the fish farm is located, and the quality of the staff working in these units influence the health of the fish. During the study, water and blood samples were taken from sturgeons. The physicochemical parameters (temperature, turbidity, pH, dissolved oxygen, nitrogenous compounds, organic substance - KMnO4, total hardness, residual chlorine, iron, copper, phosphorus, sulphates, phenols, and detergents) were determined from the water samples with the help of the photocolorimeter "Nova 60". The results were compared with the norms in force regarding the quality of surface waters (Order 146/2006) and the reference values for sturgeons. From the blood samples, haematological parameters (haematocrit, haemoglobin, number of erythrocytes, leukocytes, and platelets) and biochemical parameters (urea - BUN, creatinine - CRE, phosphorus - PHOS, total proteins - TP, albumin - ALB, globulin - GLOB) were determined, alanine aminotransferase - ALT/GPT, aspartate transferase - AST/COT, glutamyl transpeptidase GGT, glucose - GLU, lactate dehydrogenase – LDH, and triglycerides - TRIG). The haematological parameters were determined by classical methods, and the biochemical ones with the Vettest 8008 device (dry biochemistry). The obtained results were compared with reference values for sturgeons. As a result of the research, the water quality for most of the determined parameters is adequate, except for the degree of oxygen saturation. The haematological parameters are within the reference values, and among the biochemical parameters, slightly increased values were determined for GPT, GLU, PHOS, LDH, and TRIG as a result of handling stress. Correlating the results obtained for the three categories of parameters, the final conclusion is that the sturgeons benefit from appropriate welfare reflected in a good health status of the evaluated fish.

Keywords: water, biochemical parameters, haematological parameters, sturgeon, fish farm

Calitatea mediului de viață al sturionilor, utilajele folosite in fermă, factorii de microclimat (zgomotele, iluminatul), biosecuritatea zonei unde este amplasată ferma piscicolă și calitatea personalului care lucrează în aceste unități influențează sănătatea peștilor. În cadrul studiului efectuat au fost prelevate probe de apă și sânge de la sturioni. Din probele de apă au fost determinați parametrii fizico-chimici (temperatura, turbiditatea, pH-ul, oxigenul dizolvat, compușii azotați, substanța organică - KMnO4, duritatea totală, clorul rezidual, fierul, cuprul fosforul, sulfații, fenolii și detergenții) cu ajutorul fotocolorimetrului „Nova 60”. Rezultatele au fost comparate cu normele in vigoare privind calitatea apelor de suprafață (Ordinul 146/2006) și valorile de referință pentru sturioni. Din probele de sânge au fost determinați parametrii hematologici (hematocritul, hemoglobina, numărul de eritrocite, leucocite și trombocite) și parametri biochimici (uree - BUN, creatinină - CRE, fosfor - PHOS, proteine totale - TP, albumine - ALB, globuline - GLOB, alantaminotransferaza - ALT/GPT, aspartaminatransferaza AST/COT, glutamiltranspeptidaza GGT, glucoza - GLU, lactatdehidrogenaza - LDH și trigliceridele - TRIG). Parametrii hematologici au fost determinați prin metode clasice, iar cei biochimici cu aparatul Vettest 8008 (biochimie uscată), rezultatele obținute au fost comparate cu valori de referință pentru sturioni. În urma cercetărilor calitatea apei pentru majoritatea parametrilor determinați este corespunzătoare cu excepția gradului de saturație în oxigen. Parametrii hematologici sunt în valorile de referință, iar dintre parametrii biochimici s-au determinat valori ușor crescute la GPT, GLU, PHOS, LDH și TRIG, ca urmare a stresului de manipulare. Corelând rezultatele obținute la cele trei categorii de parametrii , concluzia finală este aceea că sturionii beneficiază de bunăstare corespunzătoare reflectată într-o stare de sănătate bună a peștilor evaluați.

Cuvinte cheie: apa, parametri biochimici, parametri hematologici, sturioni, piscicultura

As a result of the ever-increasing demand for caviar, the profitability of sturgeon growth is affected primarily by the quality of their living environment. Fish are in permanent intimate contact with the environment through the large surface of the gills, so water quality is the most critical aspect of their living environment (7, 9). Sturgeons are species of chondrostean fish taxonomically classified in the Family *Acipenseridae*, Order *Acipenseriformes*, which includes

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fish species originating in the Triassic (8). Sturgeons are long-lived fish; over time they have undergone few morphological changes, tolerate different temperatures, have few predators, The recent large losses among sturgeons were due to environmental pollution, overexploitation of natural resources, and changes in hydromorphological parameters (2, 3). The breeding of sturgeons in fish farms must take into account certain requirements, such as avoiding the noises produced by different machines in the farm, adequate lighting, building the breeding tanks so that the faeces of the fish are easily removed, ensuring the optimal concentration of oxygen in the water, biosecurity of the area, protection against predators, disease prevention, etc. (4, 8). The quality of the personnel working in these fishing units is very important to be able to identify in time the deterioration of the water quality due to inadequate fish components and the deterioration of the functionality of the technical systems (3, 5).

MATERIALS AND METHODS

In a fish farm in the east of the country, water and blood samples were taken from sturgeons raised on the farm. The following physic-chemical parameters were determined from the water samples taken: temperature, dissolved oxygen, pH, nitrogen compounds (N-NO2-; N-NO3-; N-NH4+); organic matter (Kmn O4), total hardness, iron, copper, phosphorus, sulphates, phenols, detergents, and residual chlorine. The water temperature was determined with the help of the aquatic thermometer with a float, and the other water quality parameters were determined with the help of the Nova 60 photocolorimeter, which has as its working principle the establishment of the optical density at various wavelengths depending on the monitored parameter. The results were compared with the norms in force, Ord. 161/2006 - regarding the quality of surface waters and the limits provided for the growth of sturgeons. The evaluation of the health of the fish was done after taking blood samples by puncturing the caudal vein with a ventral point of choice. The following haematological parameters were determined from the blood samples taken: haematocrit (Hct), haemoglobin (Hb), number of erythrocytes, number of leukocytes, number of platelets. The determinations were made by classical methods. Biochemical parameters were also determined: (urea-BUN, creatinine - CRE, phosphorus - PHOS, total proteins - TP, albumin - ALB, globulin-GLOB, Alanine Aminotransferase-ALT/GPT, aspartate transferase AST/COT, glutamyl transpeptidase GGT, glucose - GLU, lactate dehydrogenase-LDH and triglycerides -TRIG). The biochemical analyses were performed using the Vettest 8008 device (dry biochemistry), and the obtained results were compared with the reference values (parameters) provided for sturgeons.

RESULTS AND DISCUSSIONS

The average results of the physic-chemical parameters of the water in the sturgeon breeding ponds are shown in Table 1.

Analysing the obtained results, a series of statistically significant elements were found. The favourable water pH for sturgeons is between 7 - 8.5 pH units, and the average value determined was 8.57. pH values between 6 - 7 and 8.5 - 9 can influence the toxicity of nitrates in the water. Extreme values of the pH of the water can irritate the brachial tissue of the sturgeons, and in order to protect themselves from the harmful effect of the pH, they produce a greater secretion of mucus that covers the body of the fish, making it difficult for them to breathe. Also, the acidic pH of the water negatively affects the feeding processes of the fish, which leads to the insufficient use of nutrients and the slowing down of their development. In the acid environment, a reduction in the mobility of the fish with surface swimming and air "piping" is observed.

Regarding the organic substance, the results ob-

Table 1

The average values of the physic-chemical water quality parameters for the sturgeon breeding ponds

The determined parameter	Values obtained	Reference values
Water pH	8.57 µg	6.5 - 9 µg
The organic substance ($KMnO_4$)	30.57 mg/l	5 - 60 mg/l
Total Water Hardness	17.38 °D	8 - 20 °D
Nitrites (NO_2)	0.036 mg/l	0 – 0.2 mg/l
Nitrates (NO_3)	3.06 mg/l	1 – 5 mg/l
NH_3	0 mg/l	0 – 0.2 mg/l
Residual chlorine	0.003 mg/l	0 – 0.005 mg/l
Dissolved oxygen	7.64 mg/l	4 – 14 mg/l
Oxygen saturation	78.27%	80 – 95 %
Temperature	20.11 °C	19 – 24 °C
Fe (iron)	0.2 mg/l	1 mg/l
Cu (copper)	0.1 mg/l	< 0.3mg/l
phosphorus	absence	2 mg/l
Sulphates	absence	2 mg/l
Phenol	0.05 mg/l	0.2 mg/l
Detergents	0.01 mg/l	< 0.1 mg/l

Table 2

Average values of haematological parameters

The determined parameter	Values obtained	Reference values
Haematocrit (Hct)	35.4 %	26 – 46 %
Haemoglobin (Hb)	6.8 g/dl	5.7 – 8.7 g/dl
No. of erythrocytes	0.98 * 10^6 µl	0.65 – 1.09 * 10^6 µl
No. of leukocytes	42.16 µl	28.37 – 90.78 µl
No. of platelets	60.15/ µl	32.10 – 122.1/ µl

tained are the reference values. Amounts of organic substances below 5 or above 60 mg KMnO4/l water can directly lead to the appearance of sturgeon respiratory problems, the development of bacteria as a subsequent source of infections, asphyxiation of eggs in incubators, and as indirect effects the reduction of light, the decrease in the amount of oxygen from water, and the decrease in productions.

The average value of total water hardness (17.38 ºD) falls within the reference limits (8 - 20 ºD). Total hardness values below 8.0 ºD and above 20 ºD of the water can lead to whitening of the eggs and deformations of the protein sac and can indicate the presence of some toxic metals in the water. The average value of nitrites in water was 0.036 mg/L, a value that falls within the reference limits (0 - 0.2 mg/L). The higher values of nitrites in the water slow down the growth rate of sturgeons. Nitrates determined from the water samples recorded an average value of 3.06 mg/L, a value that falls within the reference limits of 1-5 mg/L, and ammonia was absent in all water samples.

Analysing the values obtained, the content of free chlorine in the water is found to be within the reference limits allowed for sturgeons. A long-term exposure of sturgeons to concentrations higher than 0.04-0.2 mg/L of active chlorine can cause agitation, spasmodic movements of the fins, suffocation, congestion, and haemorrhages in the gills. The average value of dissolved oxygen in the water was 7.64 mg/l, a value that falls within the reference limits for sturgeons (4-14 mg dissolved oxygen/L of water), and the degree of oxygen saturation was 78.27% close to the minimum limit of 80% of the reference values. The lack of oxygen in the water represents an important stress factor for sturgeons, which leads to a decrease in the performance of the fish, and the degree of saturation in oxygen below 80% can lead to changes in the colour of the gills and their "fringing". The water temperature for growing sturgeons must have values between 19 - 24ºC. The average value of the water temperature, according to the data in table no. 1, was 20.11 ºC, which shows us that it was within the reference limits allowed for sturgeons. The water temperature above 24 ºC influences the increase in the metabolic rate and the fertility of the fish. Sudden temperature fluctuations of the water in the sturgeon breeding pools influence their ability to adapt to quite serious changes in their health status (5, 11).

Table 2 shows the mean values of the haematological parameters. The average values of iron, copper, phosphorus, sulphates, phenols (polyphenols), and detergents are within the limits provided for the growth of sturgeons. The determined values fall within the optimal parameters and thanks to the existing filtration systems at the level of the growth basins.

Analysing the values obtained, it is found that all the indicators are within the reference limits for sturgeons. The average results of blood biochemical parameters (urea - BUN, creatinine - CRE, phosphorus - PHOS, total proteins - TP, albumins - ALB, globulins - GLOB, ala-

Table 3

Average values of blood biochemical parameters

#	The determined parameter	Um	Average value determined	Reference values
1	BUN	mg/dl	3.15	3.740
2	CRE	mg/dl	-	0.342
3	PHOS	mg/dl	11.83 ↑	10.69
4	ALB	g/dl	1.21	1.26
5	TP	g/dl	4.83	5.00
6	GLOB	g/dl	3.62	4.07
7	GPT	U/L	106.63 ↑	100.65
8	GOT	U/L	243.33	265.16
9	GGT	U/L	-	0.02
10	GLU	mg/dl	132.00 ↑	91.08
11	LDH	U/L	2124.66 ↑	2007.15
12	TRIG	mg/dl	726.00 ↑	6,99 – 9,66

nine aminotransferase - GPT, gamma-glutamyl transferase - GOT, lactate dehydrogenase - LDH, glucose - GLU, triglycerides - TRIG, are shown in Table no. 3.

Analysing the obtained results, it is found that for most of the determined parameters, the values are within the reference limits for sturgeons, with the exception of: PHOS, GPT, GLU and TRIG. Thus, urea (BUN) is included in the reference values for *Acipenserides*. Changes in this parameter appear in renal or brachial disorders of fish. The increase in blood urea reflects an increased concentration of carbon dioxide and ammonia in the water. Creatinine did not register values, being below the sensitivity limit of the kits. Phosphorus (PHOS) is an important mineral element that recorded average values above the reference value by 1.11 times. Changes in this parameter occur in sturgeon kidney disorders. Total proteins (TP) recorded values that fall within the reference values. Total proteins were determined to investigate the degree of hydration and hepato-renal function of fish. The role of these proteins is to intervene in maintaining the osmotic pressure of the blood and in the transport of vitamins, hormones, and minerals. Plasma proteins decrease in correlation with changes in water pH, intensification of catabolism, and periods of fish starvation, as a result of haemorrhages or the presence of infectious agents (5). Albumin (ALB) represents the largest part of total serum proteins and is synthesised by the liver. The average values determined are normal according to the reference values. Changes in this parameter appear in anaemias as well as in liver or kidney diseases.

Serum globulins (GLOB) recorded average values of 3.62 g/dl, which are within the reference limits. A decrease in this parameter shows a deficient immune system of sturgeons as a result of chronic infections (3, 4, 11). GPT (alanine aminotransferase) recorded slightly increased values compared to the reference values as a result of the intense muscular effort of the sturgeons at the time of their capture, but also due to the increase in ammonia concentration, the alkaline pH, or the decrease in the oxygen concentration in the water (1, 3). Blood glucose (GLU) exceeded the reference value by approximately 1.5 times, this increase may be due to the stress of sturgeon handling and as a result of the demands of the fish's living environment (12). Lactate dehydrogenase (LDH) as an average determined value exceeded the reference value by 1.06 times. The increase in LDH reflects possible liver, heart, or muscle damage. Triglycerides (TRIG), according to the values in Table 3, exceeded the reference limits by 1.06 times, exceedances that can be due as in the case of the other parameters of handling stress.

CONCLUSIONS

The average water pH value of 8.57 pH units ensures an alkaline living environment for sturgeons. The physicochemical parameters of the water (nitrites, nitrates, total hardness, ammonia, free chlorine, temperature, dissolved oxygen, and organic substances) are included in the reference values for the sturgeon. The degree of oxygen saturation of the water had an average value of 78.6%, a value located below the lower limit allowed. The average values of the haematological parameters (haematocrit, haemoglobin, no. of erythrocytes, no. of platelets, and no. of leukocytes) were included in the reference values. Most of the biochemical parameters (urea, phosphorus, total proteins, albumins, globulins, and GOT) are within the reference values for sturgeons: slightly increased values were recorded for GPT, blood glucose, PHOS, LDH, and TRIG as a result of handling stress. Correlating the results regarding the water quality with the haematological and biochemical parameters of the evaluated sturgeons, an average well-being of the fish can be found in the fish farm studied.

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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Efficiency of four currently used decontamination conditionings in Romania against *Aspergillus* and *Candida* strains

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KEYWORDS
Aspergillus;
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Biocides;
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SR EN1657:
2006 adapted

Summary
Introduction. — Efficacy of four commercial biocidal products (noted A to D), using manufacturers' recommendations, and a contact time of 30 minutes, were evaluated in the purpose of standard SR EN1657: 2006 adapted.
Methods. — Were used four strains, two as reference: *Aspergillus brasiliensis* (*niger*) (ATCC 16404) and *Candida albicans* (ATCC 10231), and two isolates: *Aspergillus flavus* and respectively *Aspergillus fumigatus*. The inoculum plates containing Malt Extract Agar (MEA) were incubated 48 h for *C. albicans* and 72 hours for *Aspergillus* spp. The standard SR EN1657: 2006 adapted was conducted in two phases: the test cultures preparation and the method validation. Method validation included: the control of experimental conditions and of neutralizant solution, and the method verification.

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Results. — Results revealed that three from the four tested products (A, B and D) had exerted biocidal effect on the studied strains at the recommended concentrations, the registered CFU values being reduced by more than 4 $\log_{10}$, conversely in the case of the product (C), applied against *A. fumigatus* at the recommended concentration of 2%, the biocidal effect was not detected, fact confirmed also by the CFU's value (3.59 $\log_{10}$). The biocide retested at a greater concentration (of 5%), showed a biocidal effect against *A. fumigatus* after 30 minutes, the CFU value being reduced, by more than 5.29 $\log_{10}$, evidencing the resistance emergence of *A. fumigatus* under the repeated pressure of biocides.
Conclusion. — It is re-confirming that merely the ''chemical'' defense measures to defuse the fungi's strategies become unproductive.

Introduction

While many studies have been conducted on fungi or the accompanying contaminants charge, only a few reported comparatively the biocides efficacy [1,2]. In order to survive to the biocides' deleterious activity, fungi can act via activation of resistance mechanisms, in order to reduce the concentration of the chemical combinations [3]. In parallel, an alarming escalade of the resistance to one, or more than one, pharmaco-chemical structures: antibiotics/antifungals, biocides/decontaminating products has been observed, all followed by negative repercussions in the control of fungal infections, both in humans and animals. Furthermore, of an important interest, is the persistent presence of the biofilm-related infections, which are more and more frequently signaled, but difficult to be eradicated [4,5].

In the last decade, especially the infections presence with *Candida albicans*, regularly combined with the biofilm formation became a major threat, intensified by resistance tendency of these fungi [6,7]. Studies of the biocides activity on fungi populations suggested that, unlike antibiotics, which are acting selectively on cellular targets, these structures exploit one or more than one location as: the cell wall, plasma membrane, thiol groups of the proteins and enzymes, the ribosomes and the DNA [8,9]. All these were followed by significant steps, several techniques: from non-standardized to the standardized ones were being suggested in the last decade, and so the testing of anti-fungal susceptibility (AST) became a well-recognized method for the human and veterinary medicine. In general, all methodologies are built on the principles used in the antibiograms and antifungigrames, which now can be accomplished in compliance with the international standards in the aim to choose the most appropriate product to use. [10—13].

The present study propose an efficacy assay of four commercial biocides (the most commonly used in the Romanian decontamination programs), accomplished on reference fungi and strains sampled from representative pig and poultry units and tested after the standard SR EN1657: 2006 adapted.

Materials and methods

From our previous study carried out in eight Romanian great poultry and swine farms, we have identified a total of 544 strains, from 9 genera of filamentous fungi, respectively: *Aspergillus*, *Penicillium*, *Mucor*, *Lichtheimia* (*Absidia*), *Rhyzopus*, *Alternaria*, *Ulocladium*, *Cladosporium*, *Fusarium* and two yeast genera, namely *Rhodotorulla* and *Candida*. From the total strains isolated, *Aspergillus* and *Candida* have had a meaningful share of 42.4%, respectively: 29% *Aspergillus* and 13.4% *Candida* [14]. Analyzing the incidence of the *Aspergillus* and *Candida* strains in the indoor environment of the farms, we have ascertained that, the bedding and surfaces that came in contact with animals, respectively the watering and feeding systems were the most important infection sources, confirming the studies stating that, these are decisive in generating and maintaining the contamination, in the poultry and swine units [14—16].

Biocides

For the experiment, four commercial biocide products (the most used in the Romanian decontamination programs), noted with A, B, C and D, have been applied after the recommended concentrations by manufacturers, the efficacy being measured after 30 minutes contact, time specified in the used standard (Table 1).

Fungi strains

Four strains were used: *Aspergillus brasiliensis* (niger) (ATCC 16404) and *C. albicans* (ATCC 10231) as reference, and respectively two isolates identified from the visited units: *Aspergillus flavus* and *A. fumigatus*.

Media cultures, reagents and apparatus

Were those usual for the lab of mycology: dried Malt Extract Agar (MEA) (Oxoid), tryptone sodium chloride solution (HiMedia Laboratories); the neutralizant was a mixture of: polysorbate 80 (30 g/L), saponin (30 g/L), L-histidine (1 g/L), lecithin (3 g/L) and sodium thiosulphate (5 g/L) added in the diluent.

The interfering substance (Merck) was prepared from a bovine fraction V albumin, 3 g/L, sterilized through membrane filtration for a low level of dirt.

As apparatus: pipettors and different sizes graduated pipettes, 90 mm Petri dishes, incubator (Thermoscientific), autoclave (Multilab), water bath (Multilab), stirrer vortex (Multilab), pH meter (Multilab), Millipore filters (Merck) (0.11—0.45 μm), and a spectrophotometer (LabSytems) have been used.

Table 1 The composition of tested commercial biocides.

Crt. No.	Decontaminant product tested at recommended concentration (%)	Composition (active substances)	Contact time (minutes)
1	A-1	Glutaraldehyde Alkyl dimethyl benzyl ammonium chloride Dodecyl dimethyl ammonium chloride Etilic alcohol	30
2	B-2.5	Alkyl dimethyl benzyl ammonium chloride Dodecyl dimethyl ammonium chloride	30
3	C-2 (and 5)	Chlorhexidine digluconate	30
4	D-5	Formaldehyde Alkyl dimethyl benzyl ammonium chloride	30

Methodology

The standard method EN1657: 2006 [17] adapted was accomplished in two phases.

Phase A

The test cultures preparation and validation. For *Candida*, in a test tube were pipetted 10 mL of sodium chloride-tryptone and *C. albicans* culture. Content was vortexed for 3 minutes, after which cell number from the suspension it was estimated using diluent at a value between 1.5×10^7—5.5×10^7 CFU/mL. Knowing that the corresponding values of the Optic Density (OD) are comprised between: 0.200 and 0.350, in order to adjust the number of the fungi cells, a spectrophotometer set to the wavelength of 620 nm it was used.

For *Aspergillus* spp., the spores were gathered and suspended in 10 mL sterile solution of polysorbate 80 (0.05% w/v in freshly distilled sterile water) and mixed for 1 minute. In order to estimate the spores number in the suspension between 1.5×10^7—5.0×10^7 CFU/mL, the resulting suspension was filtered to separate the mycelium fragments by the individualized spores.

The validation of the experimental conditions it consisted in verifying: of the absence of any lethal effect on the fungal cultures (noted ''A''), the toxicity absence of the neutralizant on fungal cultures (noted ''B'') and the validation for the dilution-neutralization method (noted ''C'').

Phase B

The verification of fungicide effect, (noted as ''Na''), consisted in the test conditions compliance, in terms of temperature; the contact time; presence of interfering substances, (bovine albumin). Method's validation included: the control of experimental conditions; the control of the neutralizing process, and respectively the method verification (Fig. 1).

From stock culture, strains distributed in MEA subcultures were incubated for 48 h for *C. albicans* and respectively 72 hours for *Aspergillus* spp., and at the incubation time expiration, only the well individualized fungi colonies were counted, the confluent ones being removed. Also, as control, to each efficiency test, blank fungal cultures were incubated.

To interpret the results accurately, for the filamentous fungi, only the culture plates on which the colonies increased between 14 and 165, and respectively between 14 and 330 for yeasts, were considered. The sum of the colonies it was expressed as Vc value for each plate. If the number of the colonies per plate was greater than 165 (in the case of filamentous fungi), or of 330 (for the case of yeasts), the sum was noted as '' $>$ 165'' and respectively '' $>$ 330'', and if Vc values were smaller than 14, it was registered as ''$<$ 14''. Data obtained from all colonies grown on MEA plates were used for the efficacy calculations. For each of the four strains used, values of CFU/mL in test suspensions N; Na; N_0, and respectively R, were calculated. For each experimental situation, the reduction as decimal logarithm ($\log_{10}$) it was calculated and recorded. The abbreviations used for the calculation are presented in the legend below:

- N — CFU/mL of the tested fungi suspension
- Nv — CFU/mL of the tested fungi suspension for validation
- N_0 — CFU/mL of the test mixture at the contact time beginning (time ''zero'' = 0)
- Nv_0 — CFU/mL of the validation test mixture at the contact time beginning (time ''zero'' = 0)
- Na — CFU/mL per 1 mL of the test mixture, after the contact time expiry and before neutralisation
- Vc — CFU/mL
- A — CFU/mL at controlling the experimental conditions, after the 30 minutes contact time passing
- B — CFU/mL to the Neutralizant's control, after the 5 minutes contact time passing
- C — CFU/mL to the method's validation, after the 30 minutes contact time passing
- R — Reducing of the CFU degree of decrease after the disinfectant action

Indicators estimation was established using the following formula:

$$N = \frac{C}{(n_1 + 0.1n_2)10^{-5}}$$

Where:

C = sum of the Vc values taken into consideration

n_1 = number of Vc values taken into consideration for the lowest dilution, i.e. 10^{-5}

n_2 = number of Vc values taken into consideration for the highest dilution, i.e. 10^{-6}

10^{-5} = dilution factor, corresponding to the lowest dilution.

To validate the method, the N value must to be comprised between: 1.5×10^7 and 5.0×10^7 ($7.17 \leq \log N \leq 7.70$), the

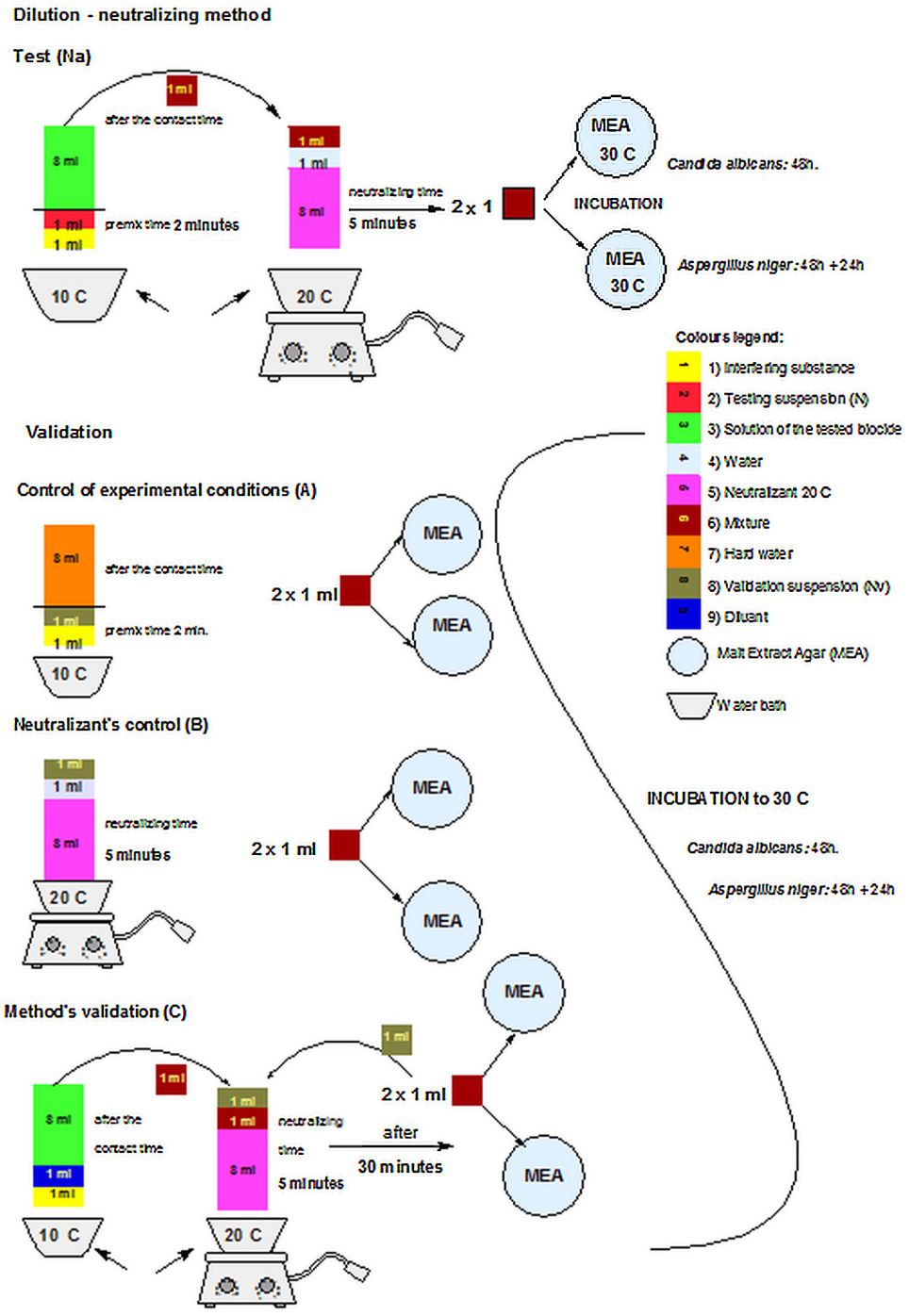

Figure 1 The adapted procedure for determination of biocides' concentration and the method's validation scheme.

Nv_0 value must to be comprised between: 3×10^2 and 1.6×10^3; the A, B, C $\geq 0.5 \times Nv_0$; and logR = $logN_0 - logNa$.

It was considered that a disinfectant has an efficient fungicidal activity if a reduction of at least 4 log_{10} will occur in 30 minutes or less.

Results

Our findings about the antifungal efficiency of the four commercial products using the adapted SR EN1657: 2006 standard are shown in Supplementary Table S1 and Fig. 2.

The obtained data presented that the studied commercial products generated a cidal effect on strains of *A. brasiliensis* (*niger*), *C. albicans* and *A. flavus*. At the concentrations specified by manufacturer and after 30 minutes contact, the ascertained CFU values were reduced by more than 4 log_{10}. In consequence, the test was validated as they have fulfilled all criteria for classification within the reference range provided in the testing standard for Nv, Nv_0, N_0, respectively N values and also A, B, C $\geq 0.5 \times Nv_0$.

In the case of *A. fumigatus*, it was observed that, after 30 minutes contact time, in three from the four studied

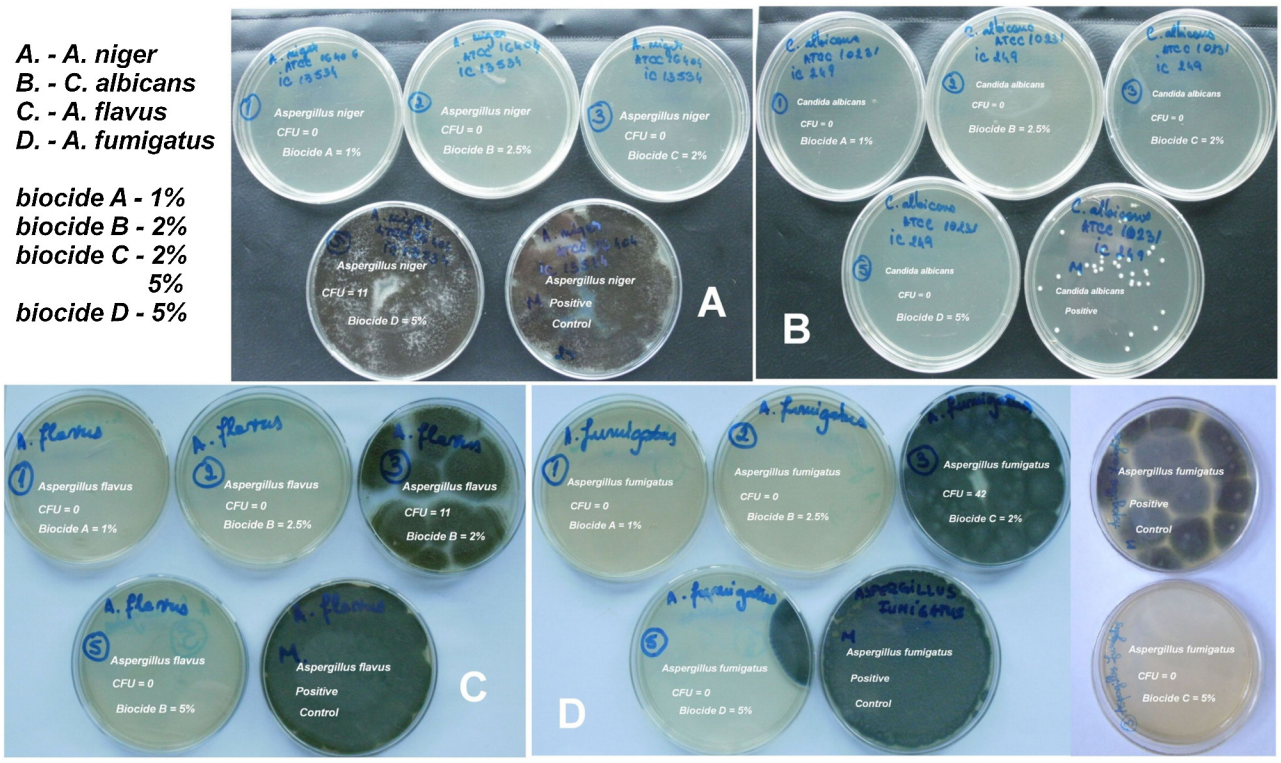

Figure 2 Biocidal activity of products A—D tested. A. *Aspergillus brasiliensis* (*niger*): 1 = biocide A, (0 colonies); 2 = biocide B, (0 colonies); 3 = biocide C, (0 colonies); 4 = biocide D, (14 colonies); control plate C = positive (*A. niger* colonies). B. *Candida albicans*: 1 = biocide A, (0 colonies); 2 = biocide B, (0 colonies); 3 = biocide C, (0 colonies); 4 = biocide D, (0 colonies); plate C = positive (*C. albicans* presence). C. *Aspergillus flavus*: 1 = biocide A, (0 colonies); 2 = biocide B, (0 colonies); 3 = biocide C, (11 colonies); 4 = biocide D, (0 colonies); plate C = positive (*A. flavus* colonies). D. *Aspergillus fumigatus*: 1 = biocide A, (0 colonies); 2 = biocide B, (0 colonies); 3 = biocide C (2%), (42 colonies); 4 = biocide D, (0 colonies; colony increased on the edge of plate is considered contamination); plate C = positive control (*A. fumigatus* colonies); 5 = biocide C (5%), (0 colonies); plate C = positive (*A. fumigatus* colonies).

biocides (A 1%; B 5% and respectively D 2.5%), the CFU value was reduced more than 4 $\log_{10}$ (4.18 $\log_{10}$).

In the case of product C, at the indicated concentration of 2% and after a 30 minutes contact period against *A. fumigatus*, was not ascertained any biocidal effect, the CFU value being of 3.59 $\log_{10}$, so in this case, because criteria classification within the reference range provided by the standard ($C \geq 0.5 \times Nv_0$), were not fulfilled, the testing it was not validated. The product C retested at a superior concentration, in this case 5%, was exerted a biocidal effect against *A. fumigatus* after a 30 minutes contact period, the CFU value being reduced to 5.29 $\log_{10}$, proving the efficiency of this concentration rise.

Discussion

Majority of the researchers who had studied this theme have observed that the fungi's remarkable success is due to their structural polyvalence; to the prompt dimorphism and to miscellaneous associations or to their specific features in the cellular wall biosynthesis [18,19], and from the ability to generate biofilms, or to adapt metabolically to the pharmacons presented abundantly on the market [20,21].

Théraud et al. (2004) proved that the global efficacy of antiseptic and disinfectant solutions against the fungi isolates was unlike, when the cells were grown under planktonic conditions or in biofilms. Authors had observed that eight out of nine disinfectants investigated were ineffective against *Candida* growing in biofilms, situation that could be easily overlapped in the human, but especially in the veterinary field [22].

In our case, the product C has confirmed these studies; the concentration rise, from 2 to 5%, had reinstated the product efficiency and has validated that the resistance phenomenon is a palpable and dynamic one. We consider, that a ''re-evaluation'' of these tenacious microorganisms, considering: the individual structure, the pathological associations and a fine-tuning investigation of the lifecycle, will be the most appropriate key to diminish their detrimental activity.

In this respect, there are several approaches thought to assure an efficient disinfection and an antifungic protection like: the phyto-therapeutic approaches [23], biotechnological [24,25], proteomics-based [26], to physico-chemicals [27], or even to develop durable antimicrobial surfaces [28].

All practical aspects linked to the fungi isolation and identification, respectively to reveal the incidence per each fungi gender/species and livestock category, can be considered as helpful to understand and conceive the most appropriate and coherent steps in future antifungal strategies, including the biocides' periodical efficiency assays. In this

aim, our study presented how a modified standard methodology can be useful in the efficacy assays of the commonly used biocidal structures, underlying the necessity of seasonal assays in different field conditions, in order to augment the active substance concentration in the biocidal product, or, in numerous cases, to abandon its use where this phenomenon is frequently quantifiable.

Concluding, our study has suggested the presence of the evolutionary resistance amongst the *A. fumigatus* isolated strains, sampled from representative swine and poultry farms, against a currently used conditioning in the Romanian decontamination programs. This proves that only the concentration change will become shortly fruitless, other added means being required to reinstall the biocides efficiency.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mycmed.2017.04.013.

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Researches Regarding the Presence of some Mycotoxins in Different Vegetal Products Used as Food stuffs or Fodders

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Abstract. The mycotoxin contamination of some products which constitutes vegetal substrates in different foodstuff or fodder was often ignored, despite the fact that the consumption of such products may seriously jeopardize the health. Different food stuffs and fodders samples were collected from a county in the South of Romania and it was studied the concentration of ochratoxin A, zearalenone, fumonisin, deoxynivalenol (DON) and total aflatoxin. Mycotoxins identification was made by using ELISA method and the results interpretation was done according to the provisions of Commission Regulation (EC) no. 1881/2006. Following the investigations there were recorded values of 1,28-5,40 times higher than the admitted limits for DON concentration in 7 out of 194 analyzed samples and of 1,40-5,00 times higher for ochratoxin A in 2 out of 93 analyzed samples. For zearalenone, total aflatoxin and fumonisin, the concentrations ranged within the admitted limits in all samples. During this study, mycotoxin contamination was noticed for a large number of samples. Even if the levels of contamination recorded values lower than the admitted limit, they provide a clear picture regarding the quality of raw materials used for various food and fodder and their processing, transport, storage and marketing conditions.

Keywords: vegetal products, mycotoxins, admitted limits

INTRODUCTION

Fungal infestation and mycotoxin contamination are quite frecuent lately, so that almost all vegetal substrates which get in animals' or man's diets carry such undesirable elements (Coman and Popescu, 1985; Coman et al., 2007).

Mycotoxicology researches try to provide data on vegetal substrates quality, on their nutritional valence and, finally, to establish the extension and intensity of both foodstuff and fodder contamination with mycotoxins, in order to ensure high quality nutritional substrates which wouldn't affect consumers' heath.

The risk associated with consumption of mycotoxins that can occur naturally or not in people and animals food is often ignored although it can lead to serious epidemiological aspects (Fink-Gremmels, 2005; Fung and Clark, 2004; Lund and Frisvad, 2003; Pestka, 2007).

MATERIALS AND METHODS

From the territory of a county in the South of Romania there were collected samples of: bread, maize products, pasta, flour, breakfast cereals, oil, tea, wine, spices, dried fruits, rice and

cereals used in some mixed fodder obtaining. From the collected samples, there was made the mycotoxic examination examination for establishing the levels of ochratoxin A, zearalenone, fumonisin, deoxynivalenol and total aflatoxin. The method used for mycotoxins' identification was ELISA test. The results interpretation was made according to EC Regulation no. 1881/2006, which provides the maximum admitted levels for certain contaminants in foodstuffs (***, CE 1881/2006).

RESULTS AND DISCUSSION

The results of the analysis are presented below in 5 tabs. In Tab. 1 are given the values of deoxynivalenol in maize products, bread, pastries, pasta, flour, cereals (wheat) and breakfast cereals. 8 samples were collected from maize out of which none exceeded the maximum value of 750µg/kg, as provided by EC Regulation 1881/2006.

Tab. 1

Deoxynivalenol values in different foodstuff

Sample type	Total no. of samples	No. of samples with undetectab values	No. of samples with values below the admitted limit/values	No. of samples with values above the limit/values	Maximum admitted limit CE 1881/2006 Reg.
Maize products	**8**	**6**	**2/µg/kg: 58.8; 140**	**0**	**750 µg/kg**
Bread	**29**	**13**	**13/µg/kg: 418.8; 19.2; 64; 23; 102; 97; 99; 69.5; 405; 190.6; 20.5; 328; 38.2**	**3/ 1140 µg/kg; 650 µg/kg; 847 µg/kg**	**500 µg/kg**
Pastries	**22**	**7**	**13/µg/kg: 331; 43; 251; 69.6; 258; 315; 48; 23; 251; 306.8; 74.4; 30.8; 298**	**2 (pretzels)/ 1330 µg/kg; 815 µg/kg**	**500 µg/kg**
Pasta	**28**	**8**	**20/µg/kg: 28; 20.5; 20.5; 321; 21; 126; 77.8; 45; 75; 34.6; 38; 35; 24; 95.5; 26; 27; 27.4; 83.5; 53; 147**	**0**	**750 µg/kg**
Flour	**22**	**13**	**7/µg/kg: 494; 25; 604; 35.3; 41.5; 23.5; 241**	**2/ 1215 µg/kg 964 µg/kg**	**750 µg/kg**
Cereals	**74**	**46**	**27/µg/kg: 467; 30; 440; 20.4; 710; 48.6; 586; 55.2; 35; 23.7; 290; 29.7; 140.4; 101.3; 51.32; 27; 70; 22; 82.6; 46.5; 35; 82.6; 46.5; 35; 82; 45; 337; 220.5; 73.5; 80.3; 41; 56**	**1/ 6750 µg/kg (wheat)**	**1250 µg/kg**
Breakfast cereals	**11**	**6**	**5/µg/kg: 35.4; 124; 32.6; 20.5; 385.4**	**0**	**500 µg/kg**

Of a total of 29 samples of bread out of which deoxynivalenol was measured, 3 samples had values above the maximum admitted limit of 500µg/kg, exceeding being by 2.28 times, 1.3 times and 1.7 times. From the 22 samples of pastries, two samples of pretzels exceeded the limit of 500 µg/kg by 2.66 and 1.63 times respectively. In none of the 28 samples of pasta and 11 of breakfast cereals was not exceeded the admitted limit for deoxynivalenol.

Of the 22 samples of flour were recorded overvalues in two of them by 1.62 and 1.28 times. From the 74 cereals samples, in 46 was not detected deoxynivalenol. A sample of wheat exceeded the maximum admitted limit of 1250µg/kg by 5.4 times and in 27 samples the values recorded were below the limit. The amount of zearalenone in samples of cereals, flour, pastries, bread, maize products, breakfast cereals and oil are presented in Tab. 2.

Tab. 2

Zearalenone values in different foodstuff

Sample type	Total no. of samples	No. of samples with undetectab values	No. of samples with values below the admitted limit/values	No. of samples with values above the limit/values	Maximum admitted limit CE 1881/2006 Reg.
Maize products	**38**	**31**	**7/ µg/kg: 14; 5; 3.14; 4.36; 22.8; 8; 65**	**0**	**75 µg/kg**
Cereals (maize)	**69**	**58**	**11/ µg/kg: 17; 2.83; 79.6; 27.3; 250; 1.9; 19.28; 3.75; 2.3; 146; 5.35**	**0**	**350 µg/kg**
Flour	**25**	**19**	**6/ µg/kg: 28; 17.4; 2.97; 10.6; 6.6; 2.76**	**0**	**75 µg/kg**
Pastries	**47**	**31**	**16/ µg/kg: 4.35; 2.16; 7.85; 5.44; 2.16; 1.91; 9.98; 2.93; 7.95; 3.04; 11.66; 2.66; 2.9; 2.53**	**0**	**50 µg/kg**
Bread	**29**	**25**	**4/ µg/kg: 4.09; 4.33; 4.43; 11.3**	**0**	**50 µg/kg**
Breakfast cereals	**28**	**24**	**4/ µg/kg: 6.7; 6.14; 25.5; 63**	**0**	**50 µg/kg**
Oil	**9**	**0**	**9/ µg/kg: 59.23; 136; 135; 112; 8.12; 17.5; 15.2; 51.9; 149.4**	**0**	**400 µg/kg**

Analyzing the results in the tab it is noticed that from the total of collected samples, regardless of product, there was none with values above the admitted limit as provided in EC

Regulation 1881/2006. Thus, out of 69 cereals samples, in 58 zearalenone was not detected as for the other 11 samples the values were much below the admitted limit.

From the 38 maize samples, 31 were negative and 7 below the limit.

Regarding the other samples, it was noticed that: from the 25 flour samples, 19 were negative and 6 below the admitted limit; from the 47 pastries samples, 31 were negative and 16 below the limit (50 µg/kg); from the 29 bread samples, 25 were negative and 4 below the limit; from the 28 breakfast cereals samples, 24 were negative and 4 below the limit and in all 9 oil samples there was detected zearalenone below the 400 µg/kg admitted limit.

In Tab. 3 are shown the values of fumonisin out of 15 maize and 72 maize products samples.

Tab. 3

Fumonisin values in different foodstuff

Sample type	Total no. of samples	No. of samples with undetectab values	No. of samples with values below the admitted limit/values	No. of samples with values above the limit/values	Maximum admitted limit CE 1881/2006 Reg.
Maize	**15**	**10**	**5/µg/kg: 70; 201; 500; 1830; 68.4**	**0**	**4000 µg/kg**
Maize products	**72**	**59**	**13/µg/kg: 236; 95; 233; 821; 103; 67; 841.5; 1572; 823; 324; 106; 191**	**0**	**4000 µg/kg**

Analyzing the results, it can be noticed that in 10 of the 15 maize samples fumonisin could not be detected and in the other 5 the concentration was below the admitted limit of 4000 µg/kg. In 59 of the 72 maize products samples, fumonisin was not detected unlike the other 13 where the values were below the limit. Ochratoxin A was measured in 93 samples: 59 of cereals, 5 of tea, 26 of wine and 3 of spices, respectively.

The results of the measurement in the above samples are shown in Tab. 4.

Tab. 4

Ochratoxin A values in different foodstuff

Sample type	Total no. of samples	No. of samples with undetectab values	No. of samples with values below the admitted limit/values	No. of samples with values above the limit/values	Maximum admitted limit CE 1881/2006 Reg.
Cereals	**59**	**55**	**2/ µg/kg: 1.129; 2.04**	**2 (wheat)/ µg/kg: 7; 25**	**5 µg/kg**
Tea	**5**	**2**	**3/ µg/kg: 1.14; 2.74; 4.26**	**0**	**20 µg/kg**
Wine	**26**	**26**	**0**	**0**	**2 µg/kg**

Spices	**3**	**2**	**1/ µg/kg: 1.73**	**0**	**15 µg/kg**

Analyzing the results in the tab, it can be seen that in 55 of the cereals samples there was not detected ochratoxin A, in 2 of the samples the values ranged below the admitted limits of 5 µg/kg and in 2 samples of wheat are above the admitted limit by 1.4 and 5 times respectively.

From the tea samples, 2 did not contain the mycotoxin and 3 recorded values below the admitted limit of 20 µg/kg. In any of wine samples there was not detected ochratoxin A.

Concerning the spices samples, only in one the concentration was detected and below the admitted limit of 15 µg/kg. In Tab. 5 are presented the results of total aflatoxin measured in different vegetal foodstuff samples. There were collected 139 samples: 18 of dried fruits, 36 of spices, 2 of flour, 1 of instant rice, 61 of cereals and 21 of oil seeds and oleaginous fruits.

Tab. 5

Total aflatoxin values in different foodstuff

Sample type	Total no. of samples	No. of samples with undetectab values	No. of samples with values below the admitted limit/values	No. of samples with values above the limit/values	Maximum admitted limit CE 1881/2006 Reg.
Dried fruits	**18**	**14**	**4/µg/kg: 0.47; 0.28; 0.28; 0.58**	**0**	**4 µg/kg**
Spices	**36**	**12**	**24/µg/kg: 0.52; 1.19; 1.1; 0.83; 0.57; 1.05; 1.12; 0.9; 0.88; 0.46; 0.54; 0.45; 0.37; 0.34; 0.27; 0.4; 0.48; 2.84; 0.38; 0.95; 0.4; 0.43; 0.616; 3.55**	**0**	**10 µg/kg**
Flour	**2**	**1**	**1/µg/kg: 1.76**	**0**	**4 µg/kg**
Instant rice	**1**	**0**	**1/µg/kg: 0.86**	**0**	**4 µg/kg**
Cereals	**61**	**33**	**28/µg/kg: 2.75; 1.36; 2.38; 1.86; 1.93; 2.135; 1.9; 2.1; 2; 1.7; 1.9; 2.7; 2.94; 2.65; 2.64; 2.63; 2.53; 2; 2.48; 2.52; 2.55; 2.18; 2.04; 2.93; 2.1; 2.8; 2.9; 2.7**	**0**	**4 µg/kg**
Oil seeds and oleaginous fruits	**21**	**15**	**6/µg/kg: 1.6; 0.31; 0.27; 0.34; 0.35; 0.33**	**0**	**4 µg/kg**

Analyzing the obtained results, it can be concluded that in any of the samples the concentrations were not above the admitted limits although in 64 samples total aflatoxin could be detected as follows: 4 of dried fruits, 24 of spices, 1 of flour, 1 of instant rice, 28 of cereals and 6 of oil seeds and oleaginous fruits. The present study highlighted a serious issue concerning the mycotoxin contamination given the large number of samples affected. The concentrations below the admitted limits could still be dangerous due to the epidemiologic potential which can jeopardize consumer health, mostly the people with immunologically vulnerable background (children, older people and suffering from chronic diseases).

It also should be taken into account mycotoxins accumulation in liver because enzymatic detoxification of aflatoxin is a complex process with multiple stages in which a wide spectrum of enzymatic compounds participate successively or simultaneously. Thus, hepatic detoxification is not carried out immediately and the repeated exposure of the organism to such toxic compounds even in low dosage could lead to chronic mycotoxicoses. Mycotoxins' synthesis processes and especially the extent of their evolution depend on the substrate of fungi development and multiplication, on environmental factors mainly the temperature and humidity and on technological conditions of collection, storage and processing. Aflatoxin-contaminated foodstuff (including animal origin ones) and fodders may cause serious conditions in humans and animals, even lethal, such as hepatocellular carcinoma, more and more frequently.

CONCLUSIONS

From the 194 foodstuff samples, DON exceeded the admitted limit provided by EC Regulation 1881/2006 in 3 bread samples, 2 pretzels samples, 2 flour samples and 1 wheat sample by 1.28 to 5.40 times.

Zearalenone did not exceed the limit in any samples of the 245 analyzed ones.

Fumonisin was not detected in 69 samples and overvalues were not recorded in any of the 87 samples.

Ochratoxin A, measured in 93 different vegetal foodstuff, was detected in 8 of them, out of which in 6 the values ranged below the admitted limit and in 2 wheat samples exceeded it by 1.4 to 5 times. Total aflatoxin did not record overvalues in any of the 139 samples regardless their nature.

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Water Quality and Blood Biochemical Parameters as Indicators for Welfare Assessment in a Sturgeon Private Farm

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Summary: Lately, *Acipenseriformes* are considered to be in danger due to over fishing, dams building and to life environment degradation, which is why the focus is on the development of sturgeon farms by accessing European funds.

This study aims to assess the sturgeons' welfare in such a farm from Calarasi County.

In order to assess the water quality there were taken samples from several checkpoints in farm and there were determined by using Nova 60 photocolorimeter the following physical and chemical parameters: temperature, pH, turbidity, dissolved oxygen, chlorine, nitrates, nitrites, phosphorus, iron, copper, ammonia, sulfates, phenols and detergents.

There were also determined the water microbiological parameters, respectively total coliforms and fecal ones.

For biochemical examination there were taken blood samples from several sturgeon specimens and were analyzed by using dry biochemistry technique (Vettest 8008 analyzer) the following: blood urea nitrogen, creatinine, uric acid, calcium, total proteins, albumin, globulins, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, triglycerides, carbohydrates, lactate dehydrogenase, phosphorus, magnesium, alkaline phosphatase, total bilirubin, cholesterol, ammonia, amylase, lipase and creatine kinase. The results interpretation was made according to the reference values for sturgeon.

Following the researches, it was found that most physical and chemical parameters of water were within normal limits accepted for sturgeon, except nitrates and sulphates. Water microbiological parameters exceeded the limits in all samples.

Regarding blood serum biochemical parameters, the obtained values were normal, except alanine aminotransferase, aspartate aminotransferase, triglycerides and alkaline phosphatase.

Linking the water quality parameters values with those for blood biochemical parameters, the welfare of sturgeons in the farm may be rated as average.

Introduction

Sturgeons are valuable fish, known for their nutritional qualities and taste of both meat and roe, as well as for their size compared to other species, and for being among the most ancient fish on earth. In our country, there is evidence that on the tables of the Geto-Dacian state founders could be found sturgeon steaks and caviar.

Sturgeon meat is easily digestible, with great nutritional value, considering the high content of amino acids, vitamins A, B and D, phosphorus, nonessential fatty acids.

Now, due to over fishing, water pollution and dams building, *Acipenseriformes* are in danger, which is why the focus is on the development of sturgeon farms by accessing European funds in order to breeding, raising and restocking natural basins in our country.

Researches in the present paper were conducted in such a fish farm from Calarasi County.

Material and methods

Sturgeon welfare assessment from Tamadau farm was made considering two indicators, namely: the quality of fish living environment and blood biochemical parameters.

In order to assess water quality, samples were collected of which have been determined physical-chemical parameters (temperature, turbidity, pH, dissolved oxygen, nitrates, nitrites, phosphorus, residual chlorine, iron, copper, ammonia, sulfates, phenols, detergents), and microbiological parameters (total coliforms and fecal coliforms).

Blood samples were taken from sturgeons by caudal vein puncture with ventral election point and there have been determined 22 serum biochemical parameters: blood urea nitrogen (BUN), creatinine, uric acid, calcium, total proteins, albumin, globulins, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), triglycerides, carbohydrates, lactate dehydrogenase (LDH), phosphorus, magnesium, alkaline phosphatase (ALP), total bilirubin, cholesterol, ammonia (NH_3), amylase, lipase and creatine kinase (CK).

Water quality parameters were determined by using NOVA 60 photocolorimeter and blood biochemical

parameters by Vettest 8008 dry biochemistry analyzer.

Results interpretation was made according to the reference values provided for sturgeon [3,4].

Results and discussion

Average values of physical and chemical parameters of water are presented in Table 1.

Table 1 Average values of water physical and chemical parameters

Determined parameters	Sampling points					Admitted limits[3,4,6]
	Water source	Stilling basin	Water inlet	Pond center	Water outlet	
Dissolved oxygen (mg/l)	7.0	7.0	8.0	7.5	7.2	>6.0
Residual chlorine (mg/l)	0.14	0.14	0.14	0.09	0.12	<0.3
pH	7.7	7.7	7.7	7.6	7.4	7 – 8
Nitrates (mg/l)	8.0	8.0	8.0	12.0	13.0	10.0
Nitrites (mg/l)	0.063	0.063	0.063	0.078	0.093	<0.1
Phosphorus (mg/l)	0.8	0.8	0.8	1.4	0.9	2.0
Iron (mg/l)	0.07	0.07	0.07	0.07	0.07	1
Copper (mg/l)	0.09	0.09	0.06	0.05	0.05	<0.3
Ammonia (mg/l)	not detected	not detected	not detected	not detected	not detected	<0.05
Sulphates (mg/l)	20.0	20.0	20.0	25.0	20.0	2 – 7
Phenols (mg/l)	0.10	0.10	0.10	0.20	0.19	1 – 2
Detergents (mg/l)	0.05	0.05	0.05	0.05	0.04	<0.1

Analyzing data from the table, it is found that dissolved oxygen ranges both within the limits stipulated by Order 161/2006 [6] and into the reference values for sturgeon [3, 4].

Dissolved oxygen content in the fish living environment is very important for sturgeon life because only in clean water it tends toward the saturation value depending on temperature and atmospheric pressure. Excess fish food may decrease dissolved oxygen content due to biological and biochemical processes in the water.

Residual chlorine and water pH are within the reference values for sturgeon.

Water reaction is neutral. Any change in water pH may adversely affect the health of fish: water acidic reaction can lead to respiratory changes, lack of interest in feed, reduced mobility, gasping at the surface, slowing growth and, ultimately, sturgeon's death [2].

Nitrites, phosphorus, iron, copper, ammonia, phenols and detergents recorded values falling within the reference standards for sturgeon.

Deviations from the reference values were recorded for nitrates, the results exceed the limit approximately 2 times, which is not a threat to fish health.

For sulfates, there was recorded significant exceeding, causing nervous disorders and skin lesions in fish [1].

In Table 2 are shown water microbiological parameters values, namely total and fecal coliforms in water samples taken from three points.

Table 2 Average values of water microbiological parameters

Sampling points	Determined parameters	
	Total coliforms (No./ml water)	Fecal coliforms (No./ml water)
Water inlet	620	428
Pond center	905	920
Water outlet	800	700
Admitted limits [5]	500	100

Analyzing the results, it is found that the values obtained are above the admitted limits in all sampling points. This is explained by the high microbiological load of the water source that supplies the sturgeon farm.

Blood biochemical parameters values are shown in Table 3.

Analyzing the results, it is found that for the most of the determined biochemical parameters (BUN, uric acid, calcium, total protein, LDH, phosphorus, total bilirubin, cholesterol, ammonia, creatine kinase, amylase, lipase, GGT) values obtained are within the standard limits for *Acipenserids*.

The changes of the determined values are represented by significant decrease of triglycerides and slight decrease of magnesium and globulins, related to the retention stress during blood sampling, and by increased alkaline phosphatase generated by adaptation stress, given that fish had been recently transferred from one pond to another and had had feeding problems [7].

There were also noticed a slight decrease of ALT and an increase of AST activity, most likely related with fish muscular injuries caused by blood sampling.

Table 3 Values of blood serum biochemical parameters

Determined parameters	Obtained values		Reference values Female/male
	Female	Male	
BUN (mg/dl)	4.0	3.0	3.69 ± 0.64/3.78 ± 0.84
Creatinine (mg/dl)	0.0	0.0	0.344 ± 0.048/0.34 ± 0.06
Uric acid (mg/dl)	< 0.1	< 0.1	0.02 ± 0.003/0.03 ± 0.005
Calcium (mg/dl)	5.8	6.9	8.52 ± 2.76
Total proteins (g/dl)	3.5	4.2	4.51 ± 1.0/5.5 ± 0.94
Albumin (g/dl)	1.0	1.0	1.26 ± 0.29
Globulins (g/dl)	2.8	2.9	3.63 ± 0.84/4.5 ± 0.69
ALT (U/l)	100.0	92.0	100.65 ± 1.18
AST (U/l)	220.0	459.0	265.6 ± 56.55
GGT (U/l)	0.0	0.0	0.02 ± 0.0035
Triglycerides (mg/dl)	250.0	270.0	699.6 ± 22.94
Carbohydrates (mg/dl)	62.0	52.0	61.62 ± 15.13/120.54 ± 26.74
LDH (U/l)	2300	2800.0	2007.15 ± 521.97
Phosphorus (mg/dl)	8.0	11.4	12.39 ± 0.267/9.009 ± 2.07
Magnesium (mg/dl)	1.81	2.27	2.79 ± 0.63/3.67 ± 0.85
ALP (U/l)	59.6	360	69.05 ± 13.04
Total bilirubin (mg/dl)	0.5	< 0.1	0.616 ± 0.0234
Cholesterol (mg/dl)	70.0	91.0	90 ± 40
NH_3 (mmol/l)	449	457	300 ± 170
Amylase (U/l)	0.0	0.0	0.001 ± 0.0003
Lipase (U/l)	0.0	0.0	0.004 ± 0.001
CK (U/l)	411	> 2000	2700 ± 1150

Conclusions

1. Physical and chemical parameters of water quality in the studied sturgeon farm have ranged, in most cases, within the reference values, except nitrates and sulfates.

2. Microbiological parameters of water recorded overvalues due to pollution of the farm water supply source.

3. Most of the blood serum biochemical parameters determined recorded appropriate values for sturgeon, except for the high alkaline phosphatase and the decrease of triglycerides. The increase of AST activity is most likely related with fish muscular injuries during blood sampling.

4. Correlating the results for the physical chemical parameters and microbiological parameters with the ones for the blood biochemical parameters, it results that the welfare of the sturgeons in this farm is rated as average.

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RESEARCHES ON LEAD POLLUTION AND ITS INFLUENCE UPON THE ANIMALS IN THE EASTERN AREA OF BUCHAREST

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SUMMARY

Lead is one of the pollutant elements emitted in the eastern part of Bucharest. It has a high binding capacity of various proteins and minerals from organs, its toxicity being obvious at vascular, neurological and kidney levels.

In the area of influence of "Acumulatorul" industrial unit lead was measured in air, water samples, forages, soil and organs (liver and kidney). Determination of lead was achieved by atomic absorption spectrometry, and the interpretation was based on the regulations in force. For animals, analysis methods consisted of clinical and histopathologic examinations.

Lead concentrations exceeded maximum admitted limits in "Acumulatorul" area both in air samples and in the soil, water, forage and organs ones, the values decreasing with the distance from the pollution source. High doses of lead established from samples collected were correlated with symptoms of neural and gastrointestinal disorders and lesions located in the filtering organs (liver and kidney).

Correlation of lead concentrations in air, soil, water, plants and organs with clinical signs as well as the anatomopathological changes confirms the status of chronic lead toxicosis in the eastern part of Bucharest.

INTRODUCTION

In the areas nearby the polluting units, neglecting the environmental protection measures can often cause air, water and soil degradation with severe consequences on plants and animals [8]. Among other elements produced by "Acumulatorul" plant (the main polluting agent in the Eastern area of Bucharest), lead is also present.

Lead has no proved physiological role in organism and it is considered having a toxic activity at vascular, neurological and renal levels due to its capacity of binding different proteins and minerals [2, 6].

The present study is meant to be an alert signal due to the fact that pollution has reached such a level that could affect humans, animals and their environment [5].

MATERIAL AND METHODS

In the Eastern area of Bucharest, near "Acumulatorul" plant there were collected samples of water, soil, forage and organs (liver and kidneys) both from dead and slaughtered animals in case of necessity.

From each sample there was established lead concentration. There was also measured lead in air samples, considering that this heavy metal has a harmful effect by massive inhibition of the Delta-aminolevulinic acid dehydratase.

The analyze method consisted in atomic absorption spectrophomometry. Results interpretation was made according to the provisions in force: Regulation 1881/2006, Government Resolution (G.R.) 128/2002, Law 311/2004, Order 161/2006 of Environment and Water Management Ministry.

Soil samples were collected from different distances from the main pollution source: 200, 500, 1000, 2000 meters and different depths (between 0 and 100 cm).

For animals, there were run clinical examinations and anatomopathological and histopathological exams for the organs.

RESULTS

The average lead concentration levels in air are shown in table no. 1 for three directions from the pollution source: East, South and West, at three distances: 50 m, 500 m, respectively 1500 m. Analyzing the data in the table, it can be noticed that the highest overvalues in relation with the maximum limit have been recorded for the samples collected from the East of the polluting source, at 50 m distance. Lead exceeding in the air samples, collected at 50 m distance of "Acumulatorul" plant was: East – by 16 times, South – by 2.5 times and West – by 9.3 times; while for the samples collected at 500 m distance, it was: East – by 5.56 times, South – by 2.58 times and West by 1.8 times.

Table 1: Average lead values in air samples – Acumulatorul Area

Sampling point	The distance from the pollution source		
	50 m	500 m	1500 m
East	7,96 mg/m^3	2,78 mg/m^3	0,52 mg/m^3
South	1,27 mg/m^3	1,29 mg/m^3	0,01 mg/m^3
West	4,56 mg/m^3	0,9 mg/m^3	0,02 mg/m^3
The maximum admitted limit GR no. 128/2002	0,5 mg/m^3		

It is noticed that the higher the distance from the pollution source the lower lead concentration values. Thus in South and West, at 1500 m distance, the obtained values ranged within the normal limits.

Table 2: Average lead values in soil samples - Acumulatorul Area

Sampling area	Sampling depth (cm)	Obtained values depending on the distance from the pollution source (ppm)			
		200 m	500 m	1000 m	2000 m
In the East of the polluting source	0-5	750	250	450	200
	5-10	1000	-	-	-
	10-20	75000	-	-	-
	20-50	150	-	-	-
	50-100	150	-	-	-
In the West of the polluting source	0-5	700	450	200	150
	5-10	400	-	-	-
	10-20	1800	-	-	-
	20-50	300	-	-	-
	50-100	250	-	-	-
The admitted limit (GR no. 128/2002)	100 ppm				

Soil analysis (table no. 2) in the neighborhood of the industrial area confirmed lead presence in surface soil (0 – 5 cm), with values exceeding by 2.5 to 7 times the admitted limit. The concentrations decrease with the distance from the polluting source.

The presence of lead in the soil samples in concentrations that exceed both the warning and intervention limits implies long and continuous soil pollution with this metal.

In table no. 3 there are presented the values of pH and lead concentrations in drinking and surface waters.

Table 3: Average lead values in water samples - Acumulatorul Area

Source type	Sampling point	No. of samples	Obtained values mg/l	Water pH
Surface water	Lake 1	10	0,004	7,9
	Lake 2	12	0,039	7,9
	Lake 3	12	0,038	7,7
The maximum admitted limit: 161/2006 Order	0,05 mg/l			-
Drinking water	Well 1	12	0,09	7,6
	Well 2	10	0,027	8,0
	Well 3	12	0,031	7,8
The maximum admitted limit: 311/2004 Law	0,01 mg/l			6,5-7,4

Analyzing the results, it can be noticed that lead concentrations in the surface waters did not exceed the maximum admitted limit in any sample, while in drinking water there were recorded overvalues in all sampling points, by 3 to 9 times. Regarding water pH, it is slightly alkaline.

Lead's high concentrations detected in the soil and water had a negative effect upon the cultivated plants in this area, used as forages. By radicular absorption, the plants stored lead in concentrations that exceed the maximum limit by 2.4 times in the alfalfa hay and by 5.48 times in the fodder flour (table no. 4).

Table 5: Average lead values in forage samples - Acumulatorul Area

Sample type	No. of samples	Lead level mg/kg	The maximum admitted limit Reg. 1881/2006 mg/kg
Alfalfa hay	10	94,65	40
Wheat flour	12	54,81	10

Lead concentration in animal organs (table no. 5) recorded exceeding of the maximum admitted limits by 16 times in liver and by 24 times in kidneys.

Table 5: Average lead values in dead or slaughtered animals - Acumulatorul Area

Sample type	No. of samples	Lead values mg/kg	The maximum admitted limit Reg. 1881/2006 mg/kg
Liver	11	8,18	0,5
Kidney	11	11,86	0,5

The clinical examination of the animals in "Acumulatorul" area revealed loss of appetite, incoordination and equilibrium disorders, violent psychomotor signs, digestive troubles with saturnine colic, head against the manger posture, dromomania.

The histopathological examination revealed: hepatic cells degeneration caused by fat infiltration in the peripheral areas of the hepatic lobules, bile duct hyperplasia, degenerative kidney modifications, glomerulo-nephritis.

The high lead concentrations in air, water, soil, plants and organs samples were correlated with the clinical symptomatology of the animals, dominated by psychomotor and digestive signs and with severe hepatic and renal lesions.

DISCUSSION

In relation with the results obtained by other authors [1, 4, 7], the study shows alarming values which point a major lead pollution in the area near Bucharest. The pollution level is close to that in the well-known critical point in Romania – Copsa Mica [3], the lead values being similar for water, soil, fodder concentrations and just a little lower (by 1.4-1.5 times) for liver and kidney concentrations.

CONCLUSIONS

1) Lead concentrations exceed the maximum admitted level by 2,5-16 times in air samples collected from the eastside of "Acumulatorul" area, at 50 m distances. It was observed a decreasing of obtained values with the distance from the polluting source.

2) The average lead content in "Acumulatorul" area show overvalues of maximum admitted limits by 2-7.5 times in water samples, by 2-5 times in soil and by 16-24 times in organs.

3) The high lead concentrations found in air, water, soil, plants and organs, are correlated with clinical signs and pathological changes, which confirm the fact that there is present a chronic lead poisoning in the East side of Bucharest.

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RESEARCHES CONCERNING THE PHYSICAL AND CHEMICAL PARAMETERS OF SURFACE WATERS IN PRAHOVA COUNTY

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Keywords: surface waters quality, physical-chemical parameters, quality category

Abstract: Within the natural ecosystem, water has an essential role in maintaining natural ecological balance and in human society development. Surface waters quality in Prahova County (Prahova and Teleajen) was established by harvesting samples from six sections (Adancata, Floresti, Predeal, Moara Domneasca, Valeni and Cheia), located at different distances from the rivers mouth. From the harvested samples were assessed physical-chemical parameters: temperature, pH, suspensions, dissolved oxygen, oxygen biochemical and chemical consumption, ammonia, nitrites, nitrates, ortophosphates, total phosphor, chlorides, sulphates, filterable residue, general ions, toxic pollutants (copper, zinc, chrome), phenols and anionic detergents.
The methods for establishing the above parameters and the results interpretation were those provided by 161/2006 Order regulations regarding surface waters quality categories. The study led to the following conclusions:

- Nutrients group ($N\text{-}NH_4^+$, $N\text{-}NO^{2-}$, $N\text{-}NO^{3-}$, $P\text{-}PO_4^{3-}$, P) has recorded values which ranged water from Prahova - Adancata section in the third quality category and from the other sections in the second one;
- The water from Teleajen River was classified as follows: Moara Domneasca section in the fourth category and both Cheia and Valeni sections in the first quality cathegory;
- Water salinity and general ions indicators (filterable residue, chlorides, sulphates, calcium, magnesium and sodium) are classifying the water in the two rivers in the 3rd quality category for most of the sections, except Predeal (Prahova) and Cheia (Teleajen), which belong to the second category;
- Dissolved oxygen, oxygen biochemical and chemical consumption have recorded values which framed the water from Prahova and Teleajen Rivers within the first quality category, except the water samples in Adancata section (Prahova) – second category and Moara Domneasca section (Teleajen) – fourth category;
- Specific toxic pollutants (copper, zinc, chrome) framed both of the rivers' water into the first quality category;
- Relevant chemical indicators (phenols, anionic detergents) have recorded values which framed the waters from the both rivers in the second quality category for most of the sections;
- Prahova River in Adancata section and Teleajen River in Moara Domneasca section are the most polluted, as most of the quality parameters values have revealed. The high level of polluted elements in these two sections is correlated with the small distances from the rivers' mouth, respectively 18 km (Adancata) and 16 km (Moara Domneasca).

INTRODUCTION

Within Earth natural ecosystem, water has an essential role in maintaining natural ecological balance and in human society development. Due to the water importance, it appeared the need of water management and sources protection.

Water quality protection represents a part of the environment protection measures and mainly aims to preserve and improve waters' both physical-chemical and biological features.

There are known in our country some areas with a high level of pollution, fact which has consequences on air, soil and water quality.

Establishing the main surface waters quality in Ploiesti area is important in order to draw the public attention and to take serious measures for environmental protection, preservation and improvement.

MATERIALS AND METHODS

Surface waters quality in Prahova area was established based on the water samples harvested from Prahova and Teleajen Rivers, in three sections for each, at different distances from the rivers mouth.

More precisely, for Prahova River, the sections were: Adancata – at 18 km distance from the river mouth; Floresti – at 124 km distance and Predeal – at 191 km distance. For Teleajen Rriver, the sampling sections were: Moara Domneasca – 16 km distance; Valeni – 72 km distance and Cheia – 113 km distance from the river mouth,

From the harvested samples there were assessed the physical-chemical indicators, which are ranged, according Order no. 161/2006, into six categories:

1. Thermal conditions (temperature, pH and suspensions);
2. Oxygen condition (dissolved oxygen, oxygen biochemical consumption OBC, oxygen chemical consumption OCC-Mn);
3. Nutrients (ammonia -N-NH_4^+, nitrites NO_2^-, nitrates -NO_3^-, orthophosphates -P-PO_4^{3-} and total phosphorus -P);
4. Salt concentration (filterable residue, chlorides – Cl^-, sulphates –SO_4^{2-}, calcium – Ca^{2+}, magnesium –Mg^{2+}, sodium –Na^+);
5. Specific natural toxic pollutants (copper –Cu^{2+}, total iron – Fe, total mangan –Mn, zinc –Zn, hexavalent chrome –Cr^{6+});
6. Chemical indicators (phenol and anionic detergents).

The methods for assessing the above indicators and for interpreting the results were conformingly to the regulations provided by Order no. 161/2006 regarding surface waters quality categories.

RESULTS AND DISCUSSIONS

Average physical-chemical indicators values of surface waters (Teleajen and Prahova) from Prahova County are shown in 6 tables.

Based on the results for these parameters, certain sections from the two rivers' waters have been classified into one of the 5 quality category provided by Order no. 161/2006.

In table 1 there are presented physical indicators average values: temperature, pH, suspensions.

Analyzing the results, it can be noticed that the water of Prahova and Teleajen rivers in all three sections of each of them belongs to first quality category according Order no.161/2006.

Average values and standard deviations for the physical-chemical indicators which reflect the oxygen condition (dissolved oxygen, oxygen biochemical and chemical consumption) are shown in table 2.

Regarding dissolved oxygen, most of the harvested samples recorded values belonging to the first quality category, except the samples harvested in Prahova – Adancata section, where the quality category is the second one and in Teleajen –Moara Domneasca section: 4^{th} quality category.

Oxygen biochemical consumption (OBC_5) framed the two rivers' water in most of the sections in the first quality category except section Adancata (Prahova River) where, due to

the values recorded, the water belongs to the third category, as well as the water from Moara Domneasca section (Teleajen River), which belongs to the fifth category.

The obtained values of the oxygen chemical consumption are classifying the water from the two rivers in most of the sections as first category waters except Teleajen – Moara Domneasca water, which belongs to the second category.

Table 1

Average values and standard deviations of physical indicators: thermal condition, pH and suspensions in surface waters – Prahova area

Surface water	Sampling section	Number of samples	Assessed indicators					
			Temperature (^{0}C)		pH		Suspensions (mg/dm^3)	
			Average value	Standard deviation	Average value	Standard deviation	Average value	Standard deviation
Prahova	Adancata 18 km distance	12	13.7	8.70	7.9	0.26	89.5	97.14
	Floresti 124 km distance	12	12.3	8.44	7.8	0.57	56.9	79.78
	Predeal 191 km distance	8	8.5	6.95	8.2	0.28	55.1	60.03
Teleajen	Moara Domneasca 16 km distance	12	14.3	7.37	7.5	0.43	118.9	115.52
	Valeni 72 km distance	12	11.2	6.66	7.6	0.87	28.5	28.42
	Cheia 113 km distance	9	8.8	6.18	8.2	0.18	23.7	17.93
Admitted limits according Order no. 161/2006			No regulations		6.5 – 8.5			

Table 2

Average values of physical-chemical indicators: oxygen condition in surface waters – Prahova area

Surface water	Sampling section		Number of samples	Assessed indicators					
				Dissolved O_2 (mg O_2/l)		OBC_5 (mg/l)		OCC-Mn (mg/l)	
				Average value	Standard deviation	Average value	Standard deviation	Average value	Standard deviation
Prahova	Adancata		12	7.49	1.573	7.92	8.276	3.09	1.987
	Floresti		12	9.87	7.449	2.62	1.428	1.69	1.513
	Predeal		8	10.45	1.201	2.30	1.370	1.81	0.912
Teleajen	Moara Domneasca		12	4.56	2.850	26.47	18.682	6.42	4.888
	Valeni		12	16.18	22.371	1.85	1.999	2.20	3.532
	Cheia		9	10.17	1.467	1.92	1.666	1.52	1.012
Admitted limits according Order no. 161/2006		Quality categories	I	9		3		5	
			II	7		5		10	
			III	5		7		20	
			IV	4		20		50	
			V	< 4		> 20		> 50	

Average values of the nutrients from the surface waters in Prahova area are presented in table 3.

In water samples harvested from Prahova River – Adancata section all indicators in nutrients class (ammonia, nitrites, nitrates, orthophosphates and total phosphorus) have recorded values specific to the third water quality category.

In the water from Teleajen River – Moara Domneasca section, the obtained values are specific to the fourth water quality category.

In the other sections, the recorded values classify the water from the two rivers in first category (Teleajen, Cheia and Valeni sections) and in the second category (Prahova, Predeal and Floresti sections).

In table 4 are presented the average values of physical-chemical indicators included into the general ions and salinity group: filterable residue, chlorides, sulphates, calcium, magnesium and sodium. Analyzing the results it can be noticed that for the most of water samples the indicators' values belong to the third quality category, except the water from Prahova River – Predeal section and Teleajen – Cheia section where the values belong to the second quality category.

Table 3

Average values of physical-chemical indicators: nutrients in surface waters – Prahova area

Surface water	Sampling section		Assessed indicators										Global quality categories
			$N\text{-}NO_4^+$ (mg N/l)		$N\text{-}NO_2^-$ (mg /l)		$N\text{-}NO_3^-$ (mg/l)		$P\text{-}PO_4^{3-}$ (mg P/l)		P (mg P/l)		
			Average value	Standard deviation	Average value	Standard deviation	Average value	Standard deviation	Average value	Standard deviation	Average value	Standard deviation	
Prahova	Adancata		1.097	0.6030	0.094	0.0303	2.740	1.8984	0.3556	0.67672	0.7180	1.07035	III
Prahova	Floresti		0.165	0.1570	0.031	0.0179	2.087	1.4292	0.0412	0.03648	0.2998	0.58202	II
Prahova	Predeal		0.360	0.2238	0.027	0.0145	2.173	0.8622	0.1110	0.08901	0.2506	0.11881	II
Teleajen	Moara Domneasca		2.458	2.2284	0.128	0.1628	2.044	1.5589	0.2178	0.24375	0.6724	0.46539	IV
Teleajen	Valeni		0.079	0.0677	0.019	0.0304	2.433	1.1373	0.0279	0.04607	0.0793	0.11919	I
Teleajen	Cheia		0.088	0.0740	0.006	0.0030	1.416	0.6829	0.0261	0.03636	0.1203	0.25946	I
Admitted limits according Order no. 161/2006		I	0.4		0.01		1		0.1		0.15		
		II	0.8		0.03		3		0.2		0.4		
		III	1.2		0.06		5.6		0.4		0.75		
		IV	3.2		0.3		11.2		0.9		1.2		
		V	> 3.2		> 0.3		> 11.2		> 0.9		> 1.2		

Table 4

Average values of physical-chemical indicators: salinity in surface waters – Prahova area

Surface water	Sampling section	Quality categories	Assessed indicators (mg/l)											
			Filterable residue		Cl^-		SO_4^{2-}		Ca^{2+}		Mg^{2+}		Na^+	
			AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Prahova	Adancata	III	1039.4	221.63	302.8	103.19	76.5	8.24	114.9	27.60	37.6	22.42	196.0	0,0
Prahova	Floresti	III	537.2	143.37	138.2	49.89	49.5	1.67	90.9	12.22	22.4	7.98	120	0,0
Prahova	Predeal	II	280.8	45.86	48.9	33.23	31.6	34.96	70.5	12.74	25.9	19.84	45.0	0,0
Teleajen	Moara Domneasca	III	949.0	234.47	282.1	109.28	67.6	41.15	114.4	22.47	54.2	31.12	20.0	0,0
Teleajen	Valeni	III	695.0	172.61	197.9	88.05	75.6	61.65	98.3	20.88	39.6	5.96	5.0	0,0
Teleajen	Cheia	II	237.6	31.03	37.1	34.94	46.7	56.77	68.9	13.72	24.6	18.66	20.0	0,0
Admitted limits according Order 161/2006	I		500		25		60		50		12		25	
	II		750		50		120		100		50		50	
	III		1000		250		250		200		100		100	
	IV		1300		300		300		300		200		200	
	V		> 1300		> 300		> 300		> 300		> 200		> 200	

AV = average value
SD = standard deviation

Average values of specific toxic pollutants (copper, total iron, manganum, zinc and chrome) are shown in table 5. In the samples harvested from the two rivers, in the six sections and regarding all indicators, the values obtained following the assessment classify the water in the first quality category.

In table 6 are presented the average values of phenols and anionic detergents – chemical relevant indicators.

Phenols have recorded values corresponding to the second quality category in the case

of the samples harvested from Prahova River, in all the three sections (Adancata, Predeal and Floresti) and from Teleajen River – Moara Domneasca section.

Concerning the anionic detergents value, it belongs to the second quality category in samples harvested from Prahova River – Predeal section and from Teleajen River – Moara Domneasca section.

Table 5

Average values of physical-chemical indicators: specific natural toxic pollutants in surface waters – Prahova area

Surface water	Sampling section	Global quality categories	Assessed indicators (mg/l)									
			Cu^{2+}		Total Fe		Total Mn		Zinc		Cr^{6+}	
			AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Prahova	Adancata	I	5.0	1.886	0.10	0.044	0.028	0.0233	9.40	10.710	3.78	9.2980
Prahova	Floresti	I	4.0	0.00	0.09	0.040	0.023	0.0148	6.00	3.162	1.40	2.951
Prahova	Predeal	I	4.0	0.00	0.04	0.016	0.016	0.0072	5.38	6.632	0.0	0.0
Teleajen	Moara Domneasca	I	5.0	2.000	0.13	0.076	0.095	0.0675	7.82	3.027	2.33	3.615
Teleajen	Valeni	I	4.18	1.401	0.07	0.034	0.030	0.0343	4.91	3.833	0.0	0.0
Teleajen	Cheia	I	4.0	0.00	0.06	0.069	0.009	0.0695	2.89	1.616	0.0	0.0
Admitted limits according Order 161/2006		I	Background		0.3		0.05		Background		Background	
		II	2		0.5		0.1		5.		2	
		III	4		1.0		0.3		10		4	
		IV	8		2.0		1		25		10	
		V	> 8		> 2.0		> 1		> 25		> 10	

AV = average value
SD = standard deviation

Table 6

Average values of chemical indicators in surface waters – Prahova area

Surface water	Sampling section	Number of samples	Global quality categories	Assessed indicators			
				Phenols (mg/l)		Anionic detergents (mg/l)	
				Average value	Standard deviation	Average value	Standard deviation
Prahova	Adancata	12	II	6.10	8.987	62.4	57.64
Prahova	Floresti	12	II	5.0	11.614	67.3	54.10
Prahova	Predeal	8	II	5.83	9.109	104.9	94.94
Teleajen	Moara Domneasca	12	III	14.38	20.688	126.3	213.62
Teleajen	Valeni	12	II	2.00	1.764	37.1	26.80
Teleajen	Cheia	9	II	2.29	1.604	31.4	25.74
Admitted limits according Order no. 161/2006		Quality categories	I	1		100	
			II	5		200	
			III	20		300	
			IV	50		500	
			V	> 50		> 500	

On the whole, regarding the surface water quality in Prahova area it can be noticed that Prahova River has recorded, at most of the assessed parameters in Predeal and Floresti sections, values corresponding to the first quality category and the highly polluted water was the one in Adancata section.

Concerning Teleajen River, the highest pollution level was recorded in Moara Domneasca section.

Studying the distances from the river mouth for the different sampling sections, it was noticed that there is a correlation between the pollution levels, meaning also waters quality category, and the distances. Thus, Adancata section is located at 18 km from the Prahova River mouth and Moara Domneasca section at 16 km on Teleajen River, unlike the other sections which are located at higher distances: on Prahova Rivers – Floresti section at 124 km and Predeal section at 191 km, on Teleajen River – Valeni section at 72 km and Cheia section

at 113 km.

Therefore, the high load of pollutant elements in the above two sections (Adancata and Moara Domneasca) is explained by the pollutant accumulation along the rivers stream.

CONCLUSIONS

Following the research we can conclude:

- Nutrients group ($N\text{-}NH_4^+$, $N\text{-}NO^{2-}$, $N\text{-}NO^{3-}$, $P\text{-}PO_4^{3-}$, P) has recorded values which ranged water from Prahova - Adancata section in the third quality category and from the other sections in the second one;
- The water from Teleajen River was classified as follows: Moara Domneasca section in the fourth category and both Cheia and Valeni sections in the first quality cathegory;
- Water salinity and general ions indicators (filterable residue, chlorides, sulphates, calcium, magnesium and sodium) are classifying the water in the two rivers in the 3rd quality category for most of the sections, except Predeal (Prahova) and Cheia (Teleajen), which belong to the second category;
- Dissolved oxygen, oxygen biochemical and chemical consumption have recorded values which framed the water from Prahova and Teleajen Rivers within the first quality category, except the water samples in Adancata section (Prahova) – second category and Moara Domneasca section (Teleajen) – fourth category;
- Specific toxic pollutants (copper, zinc, chrome) framed both of the rivers' water into the first quality category;
- Relevant chemical indicators (phenols, anionic detergents) have recorded values which framed the waters from the both rivers in the second quality category for most of the sections;
- Prahova River in Adancata section and Teleajen River in Moara Domneasca section are the most polluted, as most of the quality parameters values have revealed. The high level of polluted elements in these two sections is correlated with the small distances from the rivers' mouth, respectively 18 km (Adancata) and 16 km (Moara Domneasca).

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OBSERVATIONS CONCERNING THE AIR QUALITY IN THE INFLUENCE AREA OF A CHEMICAL UNIT

OBSERVAȚII PRIVIND CALITATEA AERULUI ÎN ZONA DE INFLUENȚĂ A UNUI COMBINAT PETROCHIMIC

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In the area of influence of Arpechim Pitesti Chemical Unit there was watched the air quality from samples harvested from five checkpoints: Pitesti, Bradu, Oarja, Prundu and Topoloveni.

In these samples, the following parameters were measured: HCl, CO, H_2CO, NO_2, SO_2, NH_3 and phenols. Analyzes and the results interpretation were done on the basis of up-to-date standards.

The measurements reach to the following conclusions:

- *all the assessed parameters have recorded smaller values than maximum admitted limits stipulated in STAS 12574/87, in all samples and all checkpoints;*
- *the phenols concentrations was constant in all the watched area, which proves that these chemical compounds are not generated by the industrial activity of oil processing;*
- *ammonium and HCl variation of concentrations indicates that there are others pollutants generators beside the industrial activity of oil processing.*

Industrial activity of Arpechim Pitesti was planed in such manner for reducing the environmental pollution and its objective is mostly accomplished.

Pollution became a reality for the most industrial areas and in the condition of lacking some strong measures of reducing it could produce dangers to the whole society.

During the campaign for diminishing the pollution, information has got an essential role. Thus, the pollutant sources must be identified, described and monitored and also the main pollutants elements, their action, the interdependence between nixes, the permitted doses, their testing and measuring methods.

The mediating of the pollutant phenomena led to the understanding of the phenomena and the drafting of their diminishing methods.

The chemical unit Arpechim Piteşti, being considered a pollutant source of the environment, has a permanent activity of monitoring the influence of the oil industrial processing activity on the environment, conformingly the aspects had in view by GEMS-Ro. The up-to-date study is a specific one to the environment protection activity and followed the pollutants elements impact on their air quality

in the area. The monitoring of the air quality in the influence area of Arpechim Piteşti Chemical Unit is necessary to underline easily and in time of some non-function during the technologic processes, of some negative phenomena in the specific substances evolution, the sources and correction modality of these deficiencies, for avoiding the negative influence on the environment.

MATERIALS AND METHODS

In the influence area of Arpechim Piteşti Chemical unit there was followed the air quality by sampling in five points: Piteşti (Environmental Protection Agency), Bradu, Oarja, Prundu and Topoloveni.

By sampling there were carried out the next chemical parameters: HCl, CO, SO_2, NO_2, NH_3, phenols and aldehyds. There were chosen the pollutants mentioned above because they cold have as emit tent the industrial activity of oil processing and they have major negative effects on plant development.

The determination were carried out conformingly the law stipulated standards: for NH_3 – STAS 10712/76; NO_2 – STAS 10329/75; SO_2 – STAS 10194/75; HCl – STAS 10943/77; fenoli – STAS 11027/76 and H_2CO – STAS 11332/79.

Analyze of the obtained date was made according to the limits stipulated in 12574/85 STAS.

RESULTS AND DISCUSSIONS

The results of the determination regarding the chemical parameters of the air are shown in *table 1*.

Table 1

Air quality in the influence area of a Chemical Unit

Sampling point	No. of samples	Average values of the indicators (mg/dm^3)						
		HCl	H_2CO	NH_3	NO_2	SO_2	CO	fenoli
Piteşti – Environmental Protection Agency	199	0,0087	0,0025	0,0022	0,048	0,044	1,576	0,0008
Bradu	167	0,013	0,0033	0,028	0,0147	0,040	1,603	0,0011
Oarja	167	0,014	0,0022	0,030	0,0192	0,054	1,582	0,0017
Prundu	178	0,014	0,0014	0,020	0,0142	0,046	1,628	0,0010
Topoloveni	178	0,012	0,0010	0,0193	0,0101	0,049	1,421	0,0010
CMA STAS 12574-87	-	0,1	0,012	0,1	0,1	0,25	2,0	0,03

According to the data in the table, HCl recorded values under the limit of 0,1 mg/dm^3 in all samples and all sampling points. Being into the atmosphere in concentrations under the limits of toxicity, with a maximum admitted

concentration of 0,1 ppm, HCl has a great effects on plant development, but in high concentration induce the chlorosis of the leaves and even the appearance of some necrotic points.

According to the obtained results, the aldehyds didn't exceed the stipulated limit by STAS 12574/87 in all samples and also all sampling points.

Aldehyds (photochemical oxidant pollutants) results into the air by oil catalytic transformation in explosive engines and exhausted gases.

Ammonium, even it is not a specific toxic substance for the oil industrial processing activity and it could react with some specific compounds, constituting smog. The ammonium content in the atmosphere, being uniform spreaded in the tested area (*table 1*) situated more under the maximum admitted limit, along the whole monitored period.

Nitrogen dioxide recorded values under the maximum admitted limit in all samples and sampling points.

A nitrogen oxide into the air over the maximum admitted limit provoke chronic poisoning and leaves scale, and over 10 ppm appear general scales.

Sulph dioxides, according to the tabular data touched concentrations under the maximum admitted limits by standards in all sampling points. It has a priority place in atmospheric pollutant group; they could come from different sources, the petrochemical industry and waste scale and also transportation.

Sulph dioxide concentrations always vary due to the different moving of the air mass. People and animals, and also plants are sensible to its action, changing their enzyme systems and essential physiologic processes.

Carbon monoxide, according to the obtained results recorded values under the maximum admitted limit in all samples.

The amount of phenols in the air were situated at very low levels, more under the maximum admitted limit in all sampling points.

CONCLUSIONS

The concentration of the carried out parameters were place dat low levels according to the admitted limit (STAS 12574/87).

The amount of phenols, along the monitored space, shows that they don't come from the oil industrial processing activity.

The variation of ammonium and hydrogen chloride shows the existence of other generators than the oil industrial processing activity.

The Chemical Unit oriented itself as the environmental pollution, be more reduce recording a permanent qualitative evolution of the environment.

REFERENCES

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